

VITAMIN REQUIREMENT OF
HUMAN ADULT

By E. M. Hume Etc.

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**VITAMIN A REQUIREMENT OF
HUMAN ADULTS**

**AN EXPERIMENTAL STUDY
OF VITAMIN A DEPRIVATION IN MAN**

**A Report of the Vitamin A Sub-Committee
of the
Accessory Food Factors Committee**

(Compiled by E. M. HUME and H. A. KREBS)

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PREFACE

ONE of the surprises of the early months of war was the revelation of a disconcerting prevalence of scabies among the inhabitants of our larger cities. As a result, towards the end of 1940, the Ministry of Health approved proposals for an experimental study of the natural history and mode of transmission of the disease. The programme had as its central feature a plan for the deliberate introduction of the infestation among a small community of volunteers, upon whom it would be possible to make day-to-day observations over a long period, and for this purpose it was decided to seek the cooperation of conscientious objectors to military service. The scabies investigation, which was initiated and directed by Dr. Kenneth Mellanby, proved a success and the "Sorby Research Institute," as the converted villa in Sheffield where it took place was renamed, became the home of a series of experiments in human biology, which continued, at first under the Ministry of Health and then from the spring of 1942 under the Medical Research Council, for the next five years.

The study described in the present Report was made in response to a request from the Ministry of Food for information on the adult requirement of vitamin A or its precursor, carotene, and was planned and controlled by a team of workers, of which the nucleus was formed by members of the Vitamin A Sub-Committee of the Accessory Food Factors Committee. The investigation was made on volunteer conscientious objectors at the Sorby Research Institute, twenty men and three women, mostly between the ages of 20 and 30, cooperating in it. War conditions made it doubtful how long the inquiry could be continued, and for this and other reasons it was considered necessary to start the volunteers on the experimental diet with no preliminary period of observation. The plan adopted was to give sixteen of them a diet virtually devoid of vitamin A and carotene until unmistakable signs of deficiency appeared, and then to determine what dose of vitamin A or carotene was needed to ensure recovery to normal. The remaining seven volunteers served as positive controls, receiving the same diet as the main group, but with a prophylactic supplement of either vitamin A or carotene from various sources. The experiment began in July, 1942, and was expected to be complete in six to eight months.

In fact it lasted two years and even at the end not all the original expectations had been realized. After eight months of deprivation there was still no discernible change in the volunteers beyond a lowering of the blood carotenoids; this, though useless as a criterion of depletion, was at least evidence to the sceptical that the diet was not being evaded. Gradually there began to appear a drop in the vitamin A level of the plasma and a deterioration in the capacity for dark adaptation, the two changes that proved to be the only reliable criteria of depletion. By these criteria no more than three men could be judged "unmistakably" deficient and hence suitable for therapeutic tests, although several of the volunteers persisted with the diet for more than 18 months and one for over two years. No other unequivocal signs of deficiency were found in any of the sixteen deprived subjects at any time during the whole course of the experiment. Such abnormalities as follicular hyperkeratosis, conjunctival degenerations or undue fatigue—all commonly associated or thought to be associated with

Vitamin A deficiency—were either absent or equally present in the deprived and the non-deprived or present in the same subjects both before and after depletion. Two cases of tuberculosis occurred among the volunteers but it was not possible to determine whether their occurrence during the experimental period was more than a coincidence.

The prolonged delay before the onset of nutritional changes, their sparse incidence and undramatic character aroused in the investigators a feeling of surprise which is in itself a measure of the novelty and value of the results achieved. It is clear that vitamin A deficiency is much less easily or rapidly induced in hitherto well-fed adults than has previously been supposed. This is a conclusion of practical importance, but, it must not be overlooked that the scope of the present investigation was restricted and that its results are not applicable to the needs of children, pregnant or nursing mothers, or the chronically undernourished.

It is the negative conclusions of the experiment and a sense of the complexity inseparable from all such studies on human beings that are the dominant impressions left by a reading of this Report. The positive findings were too scanty to justify a precise estimate of the requirement of vitamin A or carotene, and the tentative recommendations subsequently made by the Sub-Committee allow a generous margin of safety and do not conflict with previous authoritative opinion. Emphasis is laid on the wide differences in the availability of carotene from various sources and the consequent undesirability of fixing any single figure for the carotene requirement.

Much of the interest of this long and elaborate investigation is to be found in the detailed descriptions of experimental technique and apparatus, and of the modifications and improvements introduced as a result of experience. Among the new developments may be mentioned Livingston's method of rod scotometry, which promises to provide a sensitive gauge of impaired night vision.

The volunteers were free to abandon this experiment whenever they chose. That they preferred to persevere month after month with the insipid monotony of their diet and the disagreeable routine of clinical and laboratory examinations is no small tribute to their tenacity and public spirit.

MEDICAL RESEARCH COUNCIL,
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16th November 1948.

CONTENTS

	Page
INTRODUCTION	7
Organization of the Research Team	9
Acknowledgments	10
 I.—CONCISE ACCOUNT OF THE EXPERIMENT	 11
A.—General Plan	11
B.—Effects of the Vitamin A Deficient Diet	12
<i>Criteria of deficiency</i>	13
C.—Therapeutic Tests with Vitamin A and Carotene	24
<i>Vitamin A</i>	24
<i>Carotene</i>	26
<i>Summary of the effects of dosing</i>	28
D.—Prophylactic Tests with Vitamin A and Carotene	29
<i>Vitamin A</i>	29
<i>Carotene</i>	31
E.—Requirement of Vitamin A and Carotene	39
<i>Requirement in terms of vitamin A</i>	40
<i>Requirement in terms of β-carotene</i>	41
<i>Requirement of carotene in relation to its availability from different sources</i>	42
<i>Effect of tocopherol or other stabilizers on the carotene requirement</i>	43
<i>Amount of β-carotene equivalent to vitamin A</i>	43
<i>Discussion of the literature</i>	44
 II.—ELABORATION OF SPECIAL ASPECTS	 46
F.—Measurements with the Adaptometer of Craik	46
<i>Final rod threshold</i>	46
<i>Cone-rod transition time</i>	46
G.—Comparison of Wald's and Craik's Adaptometers	46
H.—Time Required for Maximum Dark Adaptation	47
I.—" Seasonal " Changes in the Final Rod Threshold	50
J.—Rod Scotometry	54
<i>Procedure</i>	54
<i>Effects of the deficient diet</i>	56
<i>Effects of dosing</i>	57
<i>Conclusion</i>	58
K.—Effect of Consumption of Alcohol on Capacity for Dark Adaptation and Plasma Values for Vitamin A	58
L.—An Examination of the Vitamin A Reserves in the Liver of a Sample of the Population of Great Britain, Made by T. Moore with the Assistance of A. C. Cooper	62
M.—A Study of the Normal Value for the Vitamin A and Carotenoid Content of Human Blood Plasma, Made by T. Moore and Z. A. Leitner	65
N.—Is Carotene Synthesized in the Human Gut?	71

	<i>Page</i>
III.—DETAILS OF THE EVIDENCE	74
O.—Particulars and Management of the Volunteers	74
P.—Diet	77
<i>Composition of the basal diet</i>	77
<i>Adequacy of the diet</i>	81
<i>Vitamin A potency of the basal diet</i>	84
<i>Supplements of vitamin A and carotene</i>	86
Q.—Methods Used for the Estimation of Vitamin A, Total Carotenoids and Carotene	90
<i>Blood plasma</i>	90
<i>Faeces</i>	97
<i>Foodstuffs</i>	99
R.—Measurement of the Capacity for Dark Adaptation	100
<i>Meaning and characteristics of the dark adaptation curve</i>	100
<i>Adaptometer of Wald</i>	102
<i>Adaptometer of Craik</i>	110
<i>Technique of rod scotometry</i>	114
S.—Particulars of Medical Examinations	120
<i>Enlargement and hyperkeratosis of the hair follicles</i>	120
<i>Summary of the report by W. J. Wellwood Ferguson on the slit-lamp examination of the eye</i>	123
<i>Summary of the report by J. L. Burn on audiometry measurements</i>	124
<i>Summary of the report by D. Russell Davis on the psychological state of the volunteers</i>	124
T.—Statistical Report on the Values for the Contents of Vitamin A and Total Carotenoids in the Blood Plasma, by J. O. Irwin	127
U.—Monthly Average Bodyweight for Each Volunteer (<i>Table</i>)	136
GENERAL SUMMARY	138
REFERENCES	143

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INTRODUCTION

In 1942, the Ministry of Food asked the Medical Research Council for information, more accurate than was then available, about the vitamin A or carotene requirement of human adults.

The question was referred to the Vitamin A Sub-Committee of the Accessory Food Factors Committee. It could only be answered by an experiment on human beings and it was therefore decided to study a number of conscientious objectors who volunteered for the purpose. It was planned to give the volunteers a diet deficient in vitamin A and carotene, but otherwise adequate, until unmistakable signs of vitamin A deficiency appeared, and then to ascertain the amount of vitamin A or carotene which caused the signs of deficiency to disappear.

A few similar experiments had been attempted before. At the time when the present experiment was planned the most comprehensive had been that of Wagner (1940) who reported that unmistakable signs of deficiency developed within 6 months, and were cured by administration of graded doses of vitamin A or carotene. He concluded that the minimum human daily requirement of vitamin A is 2,000 I.U. as a vitamin A concentrate, or 5,000 I.U. as β -carotene. Booher, Callison, and Hewston (1939) recorded unmistakable signs of impaired dark adaptation in five subjects after 16, 27, 29, 39, and 124 days of deprivation. From therapeutic tests they concluded that amounts of vitamin A between 25 and 55 I.U. per kg. bodyweight, or double this as β -carotene, maintained normal dark adaptation.

Both these and other observations suggested that well-defined signs of vitamin A deficiency would appear within 6 months but, about the time when the present research was begun, a very short account was received of the experiment made by Brenner and Roberts (1942), who found no signs of deficiency in three subjects deprived for $7\frac{1}{2}$ months. The depletion times in the present experiment proved to be even longer.

Though the need to ascertain the human requirement of vitamin A and carotene was the prime motive of the present experiment, studies of the signs of deficiency and their measurement were of equal importance. Thus, investigation of the capacity for dark adaptation, of the values for vitamin A and carotene in the plasma, and of various other possible criteria of early vitamin A deficiency, provided an extensive series of results, often with importance of their own.

For the presentation of such a variety of results, it seemed best to begin with a description of the general plan of the experiment and to follow this with a comparatively brief narrative of the course of the depletion, of the curative tests, and of the prophylactic tests which supplied the positive control. In the course of the narrative, only such references are made to technique and to the results of the collateral studies as are indispensable to the understanding of the main theme. This brief account is contained in

Section I. Section II (p. 46) elaborates some special aspects which may be of interest to the general reader but are not an essential part of the main theme. Section III (p. 74) contains the description of the technique and some of the full particulars of the experiment.

It has proved impracticable to include in the present Report all the detailed results of the various special investigations which were undertaken during the experiment; these have been deposited at the Library of the National Institute for Medical Research, Hampstead, London, N.W.3, where they are available for consultation. A limited number of copies of the following protocols can be supplied on request:

Protocol A.—Report on General Medical Examinations, by J. Pemberton.

Protocol B.—Table giving Full Data for Each Volunteer of (1) Values for Capacity for Dark Adaptation (Final Rod Threshold and Cone-Rod Transition Time, Measured with the Adaptometer of Wald), (2) Changes in the Vitamin A Potency of the Diet, and (3) Values for Vitamin A and Total Carotenoids in the Blood Plasma.

Protocol C.—Table giving Results of Rod Scotometry Measurements Made on 18 of the Volunteers, Deprived and Non-deprived, between August, 1943, and September, 1944.

Protocol D.—Tables giving Vitamin A Content of Liver and other Details of Individual Subjects, on which the Report by T. Moore and A. C. Cooper was Based.

Protocol E.—Report on Slit-Lamp Examination of the Eye, by W. J. Wellwood Ferguson.

Protocol F.—Report on Audiometry, by J. L. Burn.

A preliminary report of the experiment has been published in *Nature* (Medical Research Council: Vitamin A Sub-Committee and Associates, 1945).

Certain conventions have been adopted for the presentation of the results. Throughout the Report "carotene" refers to the biologically active fraction, mainly β -carotene, whilst the term "total carotenoids" is used to include also inactive carotenoid pigments. Values for carotene are expressed in I.U. on the basis of the colour equivalent of 0.6 μ g. β -carotene being equal to one I.U. The values for total carotenoids also are expressed in I.U. but inverted commas are used ("I.U.") to indicate that, although the colour equivalent is the same, there is no fixed biological value since a biologically inactive fraction of varying magnitude may be included.

In connection with analytical determinations,

"Cambridge" refers to analyses carried out by T. Moore, with technical assistance by A. W. Davies, at the Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council.

"Liverpool" refers to analyses carried out by R. A. Morton and T. W. Goodwin at the Department of Biochemistry, University of Liverpool.

"Oxford" refers to analyses carried out by H. M. Sinclair and C. M. Wood at the Oxford Nutrition Survey.

"Reading" refers to analyses carried out by S. K. Kon, E. H. Mawson, S. Y. Thompson, and K. M. Henry at the National Institute for Research in Dairying, University of Reading.

"Sheffield" refers to analyses carried out by H. A. Krebs, Department of Biochemistry, University of Sheffield.

Organization of the Research Team

The organization of the research team was as follows :

General Clinical Examination

J. Pemberton, M.D., M.R.C.P.

H. M. Sinclair, D.M., B.Sc.

Ophthalmological Examination, including Use of the Slit Lamp

W. J. Wellwood Ferguson, M.B., Ch.B.

H. M. Sinclair, D.M., B.Sc., and other members of the Oxford Nutrition Survey.

Estimation of Blood Vitamin A and Carotenoids

S. K. Kon, D.Sc., Ph.D., F.R.I.C., Mrs. E. H. Mawson, Ph.D., M.Sc.

T. Moore, D.Sc., Ph.D., A. W. Davies, J. Tosic, Ph.D., M.Sc., and Miss A. C. Cooper.

R. A. Morton, D.Sc., Ph.D., F.R.I.C., and T. W. Goodwin, M.Sc., A.R.I.C.

H. M. Sinclair, D.M., B.Sc., and Miss C. M. Wood, B.Sc.

Blood Cell Count including Platelets, Haemoglobin Estimation

H. J. Barrie, B.M., B.Ch.

Fractional Test Meal

J. Pemberton, M.D., M.R.C.P.

Skin Biopsy

J. Pemberton, M.D., M.R.C.P., and H. J. Barrie, B.M., B.Ch.

Measurement of Capacity for Dark Adaptation

Miss C. M. Wood, B.Sc. (Adaptometer of Wald).

W. Bartley (Adaptometer of Wald and Rod Scotometry).

The late K. J. W. Craik, Ph.D., and Mrs. S. MacPherson, B.Sc. (Adaptometer of Craik).

Night Vision Scotometry

P. C. Livingston, C.B.E., A.F.C., F.R.C.S.

W. Bartley.

Estimation of Carotene in Faeces

S. K. Kon, D.Sc., Ph.D., F.R.I.C., and Mrs. E. H. Mawson, M.Sc., Ph.D.

T. Moore, D.Sc., Ph.D., A. W. Davies, J. Wierzychowski (Polish Army Medical Corps), and Miss A. C. Cooper.

R. A. Morton, D.Sc., Ph.D., F.R.I.C., and T. W. Goodwin, M.Sc., A.R.I.C.

Audiometry

J. L. Burn, M.D., B.Sc., D.P.H.

Psychological Examination

D. Russell Davis, M.D., M.R.C.P., D.P.M., and W. Bartley.

Chemical Analysis of Foods

H. A. Krebs, M.D., F.R.S.

T. F. Macrae, O.B.E., D.Sc., Ph.D., and the Royal Air Force Nutrition Team.

T. Moore, D.Sc., A. W. Davies and Miss A. C. Cooper.

R. A. Morton, D.Sc., Ph.D., F.R.I.C., and T. W. Goodwin, M.Sc., A.R.I.C.

S. K. Kon, D.Sc., Ph.D., F.R.I.C., S. Y. Thompson, Ph.D., and Miss K. M. Henry, Ph.D., A.R.I.C.

Sampling of Specimens (Blood, Faeces)

H. A. Krebs, M.D., F.R.S.

Biological Test of Vitamin A Potency of the Diet

W. A. Broom, B.Sc.

Statistical Analysis

Miss K. H. Coward, D.Sc.

J. O. Irwin, Sc.D., D.Sc. (Results of Blood Determinations and Audiometry).

Charge of Records

H. A. Garling.

Supervision and Organization of Arrangements in Sheffield

H. A. Krebs, M.D., F.R.S.

K. Mellanby, O.B.E., D.Sc.

General Organizer and Secretary to the Team

Miss E. M. Hume, M.A.

The writing of the Report was undertaken by E. M. Hume and H. A. Krebs, who received invaluable help in the preparation of graphs and tables from the professional experience of one of the volunteers, H. A. Garling.

Acknowledgments

The Team owes grateful acknowledgment for help from a number of sources :

Dr. V. H. Booth and Miss B. L. Norden, Dunn Nutritional Laboratory, Cambridge, for carrying out carotene and vitamin A determinations.

Sir Percival Hartley, National Institute for Medical Research, for supplying a preparation of carotene in arachis oil.

Dr. A. C. Thaysen, National Chemical Laboratory, Teddington, for supplying food yeast (*Torulopsis utilis*).

Messrs. Roche Products, Ltd., for supplying ascorbic acid tablets.

Messrs. Lever Bros., for supplying carotene in arachis oil and special margarine.

Messrs. Chivers and Sons, Ltd., for supplying special jams and canned carrots.

Messrs. Rowntree and Co., Ltd., for supplying special chocolate and clear gums.

Messrs. Nestlé's Milk Products, Ltd., for supplying homogenized spinach and homogenized carrots.

Messrs. Cranfield Bros., Ltd., Ipswich, for supplying Canadian G.R. white flour, specially bleached with agene.

City of Sheffield Lighting Engineer and Staff, for providing facilities for photometric measurements.

Dr. H. Droller, Sheffield Royal Hospital Laboratory, for taking electrocardiograms and measuring basal metabolic rates.

Dr. Rupert Hallam, Sheffield Royal Infirmary, for advice on dermatological problems.

Professor G. L. Roberts, University of Sheffield, for dental examinations.

Dr. J. L. Grout, Sheffield Royal Hospital, for X-ray examinations.

Sir Franklin Kidd, F.R.S., Low Temperature Research Station, Cambridge, for help in food analysis and in obtaining specially dehydrated vegetables.

The Director of the Meteorological Office, Air Ministry, for records of sunshine and temperature.

The United States of America, for crystalline β -carotene supplied under Lease-Lend.

Dr. K. Tansley, Physiology Department, Hospitals Centre, Birmingham, for reading the draft and making helpful suggestions about the physiology of vision.

After the experiment was finished, but before the Report was written, the team of workers suffered an irreparable loss by the death in a traffic accident of Dr. K. J. W. Craik, on 7th May, 1945. He had made a provisional report on his own and Mrs. Macpherson's contribution but the results as a whole had never been submitted to him, and the team was deprived of his wise criticism of the dark adaptation results obtained by the various techniques, and of the interpretation to be put upon them in relation to one another.

I. CONCISE ACCOUNT OF THE EXPERIMENT

A.—General Plan

The experiment began at the end of July, 1942. Twenty men and three women, between 19 and 34 years of age, volunteered to live on a diet deficient in vitamin A for periods ranging from 6½ to 25 months (Table I). Particulars of the volunteers and their management are set out on p. 74.

On an average, the daily diet contained probably less than 70 I.U. of α - and β -carotene and negligible amounts of vitamin A, but was adequate in all other respects (*see* p. 77 and p. 81).

Sixteen subjects received the diet unsupplemented, with the intention that they should be dosed with vitamin A or carotene when signs of depletion had developed. Seven subjects served as positive controls, of whom two received a daily supplement of about 2,500 I.U. of vitamin A as distilled natural esters in oil, and the other five about 5,000 I.U. daily of carotene as β -carotene in oil or margarine, or as carrots, spinach, or cabbage.

TABLE I

Names of Volunteers, Duration of Deficient Diet, and Main Experimental Data on Supplements
(For details of first 7 volunteers, *see* Table VIII, p. 30)

Name	Duration of deficient diet (months)	Data on supplements
Bartley, M. (Mrs.) ..	8½	} Received supplement of carotene prophylactically.
Bartley, W. ...	23	
Broderick, J. ...	19	
Drake, G. ...	15	
Garnett, C. ...	6½	
Drabble, H. ...	18	} Received supplement of vitamin A prophylactically.
Tridgell, L. ...	22½	
Bloomfield, H. ...	25	Received supplement of carotene after 24½ months.
Brown, L. ...	13	Received single large dose of vitamin A one week before end of deficient diet.
Garling, H. ...	22½	Received supplement of vitamin A after 21½ months.
Golding, A. ...	22	Became seriously deficient during thirteenth month; dosed with vitamin A after 14 months.
Hicken, B. ...	17	Relinquished diet on medical advice owing to onset of pleurisy.
Proctor, N. ...	24	Became seriously deficient after fifteenth month; dosed with carotene after 17½ months.
Russell, E. (Miss) ..	11½	Received supplement of carotene after 10½ months.
Saunders, P. ...	12½	Received large dose of vitamin A 10 days before end of deficient diet.
Stuart, S. ...	11½	Received supplement of vitamin A after 10½ months.
Thompson, W. ...	20	Received supplement of vitamin A after 18 months.
Watson, C. ...	23	Received supplement of carotene after 22 months.
Whitehead, A. ...	18½	Received supplement of carotene after 14½ months.
Whitehead, D. (Mrs.)	13½	Received supplement of vitamin A during last week but one of deficient diet.
Williams, D. ...	20	Received supplement of vitamin A after 18 months.
Wodeman, R. ...	20½	Received supplement of vitamin A after 18 months.
Woodhouse, J. ...	21½	Received supplement of carotene after 20 months.

It was planned to carry out the greatest possible variety of examinations which might be relevant. Those which proved most useful in assessing the course of the depletion, because they gave positive results, were the determination of the vitamin A and carotenoid content of the blood plasma, and the measurement of the capacity for dark adaptation.

For the interpretation of the results, especially from the positive controls receiving carotene supplements, estimation of carotene in the faeces was required.

Numerous other important investigations were made but they failed to reveal major deviations from the normal. These included measurement of bodyweight, general clinical examination, examination of the eye with the slit-lamp microscope, skin biopsy, haemoglobin estimation, counts of the blood cells including the platelets, fractional test meals, exercise tolerance tests, and audiometry and psychological tests, including those for memory and for mental and physical fatigue.

Many of the investigations were repeated independently by two, three, or even four different workers.

B.—Effects of the Vitamin A Deficient Diet

In all those who did not receive a supplement of carotene, the first change resulting from the deficient diet was a rapid drop in the carotenoid content of the blood, as previous authors had observed. Within three months the initial average value of 150 "I.U." per 100 ml. of plasma fell to a fairly stable level varying from 12 to 40 "I.U.". Very little, if any, of this residual carotenoid content was due to carotene (*see* Table XII, p. 37).

No other consistent changes which could be ascribed to the deficient diet were observed during the first 8 months.

After about 8 months the plasma value for vitamin A slowly began to decline in most subjects. The capacity for dark adaptation, measured by the rod threshold, deteriorated in all of them during the months December, 1942, and January, February, and March, 1943, but improved without any apparent cause in April, 1943, 9 months from the start of the experiment. This improvement was shown by all, though it was only slight in two whose plasma value for vitamin A was declining fairly fast.

After 12 months volunteers began to leave the team for reasons unconnected with the experiment. Some of these showed no signs of depletion, but in others the values for vitamin A in the plasma and for the rod threshold suggested the beginning of a decline. Twelve volunteers began a second year of the deficient diet and two of them showed signs of deficiency in the blood value for vitamin A and in the level of the rod threshold, which deteriorated steadily until they were dosed, one after 14 and the other after 17½ months. Besides a rise in the rod threshold these two men showed an increase in the time of transition from cone to rod vision, the initial value being about 8 minutes in each, and the final value about 18 minutes in one and 25 in the other. No other recognized signs of depletion or definite abnormality occurred in these or any of the other subjects at any time.

After 18½ months only four subjects were still receiving the basal diet un-supplemented and, of these, one was beginning to show definite deterioration in rod threshold, cone-rod transition time, and plasma value for vitamin A, which progressed until therapeutic dosing was begun after 22 months. Of the other three, one continued for 20, one for 21½, and

one for 24½ months, without showing any signs of depletion beyond a moderate lowering of the plasma value for vitamin A in two of them ; one did not show even this.

The thirteen subjects who completed the experiment without showing unmistakable depletion all received therapeutic doses of vitamin A or carotene, whichever seemed more suitable. In this way as much information as possible was gleaned, though the number of genuinely depleted subjects was far less than had been expected.

CRITERIA OF DEFICIENCY

The plasma values for vitamin A and total carotenoids, and the capacity for dark adaptation, being the only criteria of moderate deficiency on which reliance could be placed, are now considered separately.

Plasma Values for Vitamin A and Total Carotenoids

Samples of blood were examined in three laboratories : in Cambridge, in Liverpool, and in Oxford. Cambridge and Oxford received samples drawn about every fortnight ; Liverpool, whose technique necessitated a larger amount of blood, received samples only from alternate drawings about every 4 weeks. The procedure of collecting and sending the blood, and the methods of testing it, are described on p. 90. All the plasma values for vitamin A and total carotenoids for each volunteer are recorded in Protocol B.

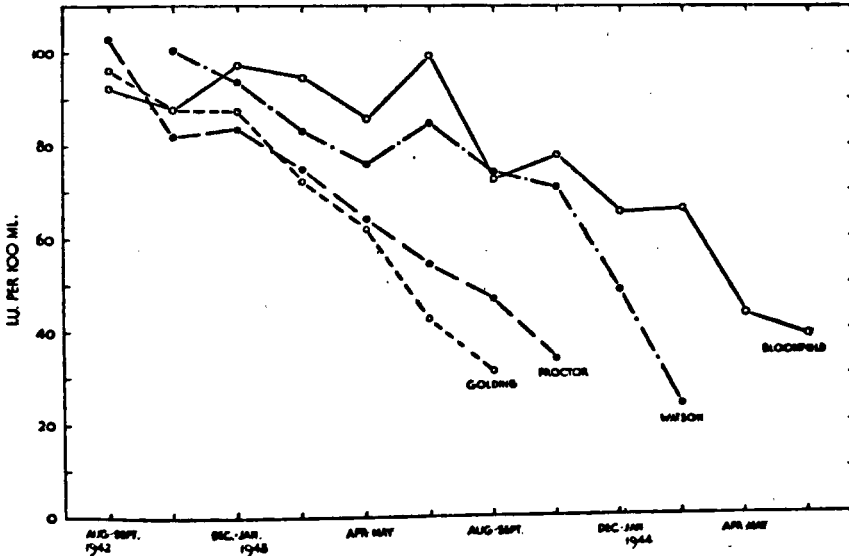


FIG. 1.—Behaviour of the vitamin A content of the blood plasma of the four deprived subjects showing the most marked fall. The points represent two-monthly averages.

Vitamin A.—The plasma values showed great variation. At first they ranged from about 65 to about 120 I.U. per 100 ml., with an average between 80 and 90 ; after eight months the values for ten out of the sixteen volunteers showed a definite downward trend (see Table II). The fall became especially marked in four men, Golding, Proctor, Watson, and Bloomfield, as can be seen from Fig. 1. One man, Garling, showed no appreciable drop even after

TABLE II

Summary of the Values for the Vitamin A Content of the Blood Plasma of the 16 Volunteers who Received the Deficient Diet Unsupplemented
(The values represent the average of all the data from the three laboratories, collected within successive periods of two months, expressed as I.U. per 100 ml. plasma : for a statistical examination, see p. 127)

Name	Aug.- Sept., 1942	Oct.- Nov.	Dec., 1942- Jan., 1943	Feb.- March	April- May	June- July	Aug.- Sept.	Oct.- Nov.	Dec., 1943- Jan., 1944	Feb.- March	April- May	June- July
Bloomfield	93	88	98	95	86	101	74	79	66	67	44	40
Brown	96	84	102	83	74	81						
Garling	93	118	121	113	94	108	94	106	88	93		
Golding	96	88	88	73	63	43	31					
Hicken	87	85	79	65	75	69	63	70				
Proctor	103	82	84	75	65	54	48	34				
Russell	83	88	71	72	66							
Saunders	81	76	72	54	57	58						
Stuart	73	67	51	56	52							
Thompson	86	76	82	72	85	80	82	67	59			
Watson	91	101	94	83	76	86	75	71	49	24		
Whitehead, A. ..	121	100	106	79	85	91	66					
Whitehead, D. ..	71	77	73	62	56	59						
Williams	67	72	76	54	65	71	59	64	53			
Wodeman	91	82	91	88	85	78	72	76	63			
Woodhouse	69	83	67	72	61	57	57	53	46			
<i>Average of 16 subjects..</i>	88	85	85	75	72							
<i>Average of 14 subjects..</i>					73	74						
<i>Average of 11 subjects..</i>						76	66					
<i>Average of 9 subjects..</i>							69	69				
<i>Average of 7 subjects..</i>								74	61			
<i>Average of 3 subjects..</i>									68	61		
<i>Remaining subject ..</i>										67	44	40

22 months. The data for the remaining subjects have been combined in a composite curve, shown in Fig. 2. This curve shows a downward trend after 8 months, with irregularities whenever the number of subjects decreased. These results are in agreement with, but go further than, those

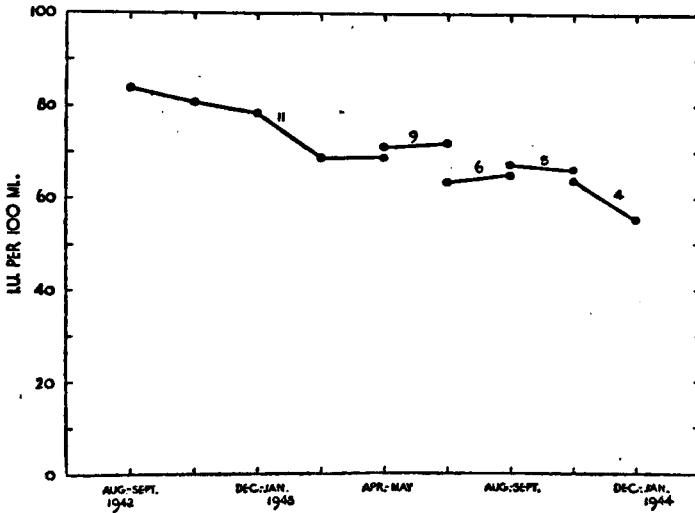


FIG. 2.—Behaviour of the vitamin A content of the blood plasma. Composite curve for all the deprived subjects except the four shown in Fig. 1, and Garling who showed no fall even after 22 months. The points represent two-monthly averages for the number of subjects indicated by the numerals.

obtained in the depletion experiments of Wald, Brouha, and Johnson (1942) and of Brenner and Roberts (1943). The experiments of these two groups of authors did not last more than $6\frac{1}{2}$ and $7\frac{1}{2}$ months, respectively; during these periods they did not observe any downward trend in the blood values for vitamin A, which accords with the behaviour of most of the subjects in the present experiment. This shows that the British and American subjects had substantial liver reserves. On the other hand Wagner (1940) found no trace of vitamin A in the blood after 6 months' depletion (*but see* personal communication, p. 45), and Steven (1943) found a considerable fall within from 38 to 87 days of deprivation; these discrepancies may be due to differences in technique or in liver reserves.

Total carotenoids.—The routine estimations made on blood plasma were for total carotenoids and the results are expressed in "I.U." (*see* p. 8).

Those who have previously studied the effect of a diet low in carotenoids on the carotenoid content of the blood have all observed an early fall (Wald, Brouha, and Johnson, 1942; Steven, 1943; Brenner and Roberts, 1943). This was confirmed in the present experiment.

In the present experiment the initial average value for plasma carotenoids was about 150 "I.U." per 100 ml. and it then fell rapidly until it reached a

level of between 30 and 40 "I.U." per 100 ml. after eight weeks (Table III and Fig. 3). A further slight and irregular fall to between 20 and 30 "I.U." per 100 ml. occurred when, in the summer of 1943, National Wheatmeal containing about 170 "I.U." total carotenoids per 100 g. was replaced by a specially bleached white flour containing about 65 "I.U." per 100 g. A composite curve of the average value for all the volunteers receiving the deficient diet is shown in Fig. 3; no individual curves are given, since none of the volunteers showed any marked deviation from the average.

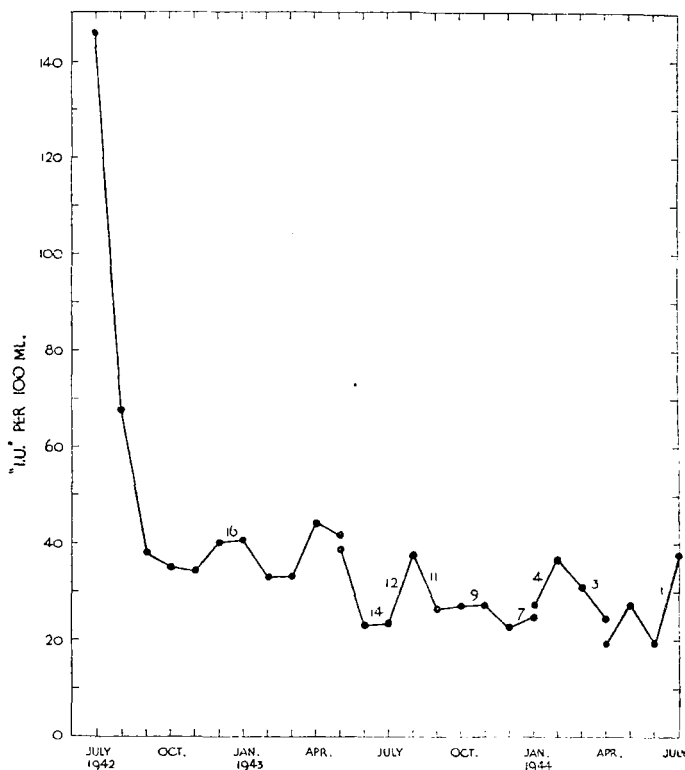


FIG. 3.—Behaviour of the total carotenoid content of the blood plasma. Composite curve for all the deprived subjects. The points represent monthly averages for the number of subjects indicated by the numerals.

After 9 months' depletion a sample, large enough for chromatographic analysis, was obtained by pooling blood from twelve volunteers. No α - or β -carotene could be detected in this, although the value for total carotenoids was 30 "I.U." per 100 ml.; on other occasions only traces were detected (Table XII, p. 37).

The value for carotenoids in the blood cannot be used as a criterion of the state of depletion because it has no simple relation to the intake of vitamin A or to the level of vitamin A in the blood or liver (*see* p. 29), but it does reflect the amount of carotenoids in the diet. The behaviour of the blood carotenoids, especially the absence of α - and β -carotene from the plasma, is evidence of the poverty in carotenoids of the basal diet and of the steadfastness of the volunteers in adhering to it.

Capacity for Dark Adaptation

The chief measurements were made with the portable adaptometer of Wald (1941), which is based on principles elaborated by Hecht (1937). It would probably have been preferable to use the instrument of Hecht and Shlaer (1938), but this was not available for the experiment. Additional measurements were made with the adaptometer of Craik and the rod scotometer of Livingston (1944), but for the purpose of this concise account the results obtained with the apparatus of Craik and of Livingston are omitted ; they are presented in Section II (pp. 46, 54).

Readers unfamiliar with the physiology of dark adaptation are referred to p. 100, where the relevant points are briefly set out. The technique of measurement is fully described on p. 102. All the values for the final rod threshold and the cone-rod transition time obtained with the adaptometer of Wald are given in Protocol B.

Since Wald, Jeghers, and Arminio (1938), Dow and Steven (1941), and Hecht and Mandelbaum (1939) expressed the view that the final rod threshold is the most suitable single index for detecting vitamin A deficiency, attention at the beginning of the present experiment was focused on the measurement of the final rod threshold, and the "short method" (see p. 105), which requires from the operator about one-quarter of the time needed for the "full method", was adopted as the routine procedure. It was applied at approximately weekly intervals, while the full adaptation curve was measured about once a month. When the experiment had been in progress for about a year and the first subject was beginning to show signs of deprivation, it was realised that, contrary to previous belief, postponement of the change from cone to rod vision was also taking place as a result of nutritional deprivation ; more frequent measurements of the cone-rod transition time under standardized conditions were therefore taken subsequently.

Final rod threshold.—The final rod threshold values obtained with the Wald adaptometer showed considerable variations for the same subjects tested at short intervals. "Normal" values were between 1.37 and 2.30 log. μm lamberts (Table XLI, p. 106). Changes up to 0.5 log. unit occurred within a few hours and up to 0.7 log. unit within three days (Table XLII, p. 107). This variability obscured any but the most definite trends. However, when values were averaged for one or two months, less pronounced trends became apparent.

For the first four months of depletion no change took place ; the average value in log. μm lamberts for all sixteen deprived subjects was 1.85 for August, 1942, and 1.87 for November, 1942 (Table IV). During the following five months there was a definite deterioration which was shown by every one of the deprived subjects ; the average was 2.03 in January, 1943, and 2.06 in February. In April and May, 1943, the average rod threshold returned to the initial value. These changes are discussed elsewhere (p. 50), and reasons are given for believing that the temporary deterioration was a sign of nutritional deficiency.

During the remaining 16 months of the experiment only three subjects, Golding, Proctor, and Watson, showed a marked rise in threshold, though all those who continued into a second winter again showed the slight deterioration in January. All the deprived subjects improved about April and May, 1943, except Golding and Proctor who only improved a little

TABLE IV

e Values for all Tests Made with the Wald Adaptometer during Successive Periods of 4 Weeks, for the Final Rod Threshold of the Sixteen Volunteers Who Received the Deficient Diet Unsupplemented
(The values are expressed in log. μm lamberts)

name	11 July to 9 Aug. 1942	10 Aug. to 5 Sept.	6 Sept. to 3 Oct.	4 Oct. to 31 Oct.	1 Nov. to 28 Nov.	29 Nov. to 26 Dec.	27 Dec. to 23 Jan. 1943	24 Jan. to 20 Feb.	21 Feb. to 20 Mar.	21 Mar. to 17 April	18 April to 15 May	16 May to 12 June	13 June to 10 July	11 July to 7 Aug.	8 Aug. to 4 Sept.	5 Sept. to 2 Oct.	3 Oct. to 30 Oct.	31 Oct. to 27 Nov.	28 Nov. to 25 Dec.	26 Dec. to 22 Jan. 1944	23 Jan. to 19 Feb.	20 Feb. to 18 Mar.	19 Mar. to 15 April	16 April to 13 May	14 May to 10 June	11 June to 8 July	9 July to 5 Aug.	10 Aug.
..	1.77	1.65	1.80	1.70	1.69	1.80	2.06	1.87	1.77	1.84	1.75	1.49	1.35	1.43	1.59	1.72	1.58	1.60	1.73	1.55	1.69	1.49	1.49	1.57	1.64	1.67	1.71	1.61
..	1.71	1.64	1.80	1.65	1.67	1.72	1.90	1.98	1.84	1.77	1.71	1.70	1.74	1.60	1.45	1.72	1.83	1.84	1.95	1.99	1.92	1.81	1.85	1.95	2.18			
..	—	1.88	1.87	1.92	1.85	1.88	2.04	—	2.10	1.87	1.98	1.87	1.70	1.59	1.69	1.72	1.71	1.83	1.84	1.95	1.99	1.92	1.81	1.85	1.95	2.18		
..	—	2.06	1.86	2.12	2.14	2.22	—	2.30	2.10	2.30	1.88	2.16	1.99	2.27	2.25	2.81	—	—	—	—	—	—	—	—	—	—	—	—
..	1.78	1.79	1.85	1.80	1.71	1.86	1.96	2.02	1.84	1.76	1.87	1.58	1.55	1.55	1.60	1.58	1.59	1.60	1.74	—	—	—	—	—	—	—	—	
..	1.79	1.84	2.07	1.82	2.00	2.17	2.22	2.38	2.24	2.33	2.18	2.02	2.16	2.15	2.42	—	—	—	—	—	—	—	—	—	—	—	—	
..	1.81	1.89	1.93	1.92	1.79	1.88	1.99	1.79	1.87	1.86	1.86	1.98	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91	1.91
..	1.71	1.78	1.87	1.93	1.90	2.01	2.07	2.24	2.13	2.01	1.80	1.73	1.73	1.70	—	—	—	—	—	—	—	—	—	—	—	—	—	
..	2.04	2.01	2.18	2.11	2.08	2.03	2.07	2.28	2.17	2.19	1.91	1.61	1.49	1.52	1.42	1.59	1.57	1.55	1.58	1.55	1.83	1.72	2.15	2.41	2.28			
n	1.55	1.95	1.86	1.84	1.76	1.84	2.01	1.87	1.95	1.79	1.77	1.70	1.77	1.68	1.68	1.86	1.64	1.62	1.53	1.71	1.80	1.72	2.15	2.41	2.28			
d, A.	1.72	1.71	1.89	1.96	1.89	1.99	2.00	2.10	1.95	1.95	1.98	1.70	1.74	1.74	1.74	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
d, D.	1.75	1.87	1.79	1.80	1.80	1.97	1.86	1.92	2.10	—	1.62	1.73	1.74	1.81	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68
n	1.76	1.86	1.83	2.03	1.96	2.03	2.05	2.07	2.01	—	1.77	1.80	1.81	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68	1.68
n	1.86	1.80	1.98	1.97	1.83	1.88	1.95	1.95	1.90	2.01	1.71	1.59	1.57	1.56	1.56	1.66	1.52	1.62	1.75	1.73	1.77	1.77	1.77	1.77	1.77	1.77	1.77	1.77
n	—	2.07	1.82	2.02	1.99	1.99	2.22	2.09	1.98	2.07	1.91	1.97	1.77	1.68	1.80	1.88	1.80	1.95	1.93	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98	1.98
10c	1.74	1.83	1.87	1.80	1.79	1.83	1.99	2.07	1.88	1.86	1.76	1.60	1.60	1.48	1.57	1.55	1.82	1.66	1.98	1.84	1.91	1.70						
of 13 subjects	1.77																											
of 16 subjects		1.85	1.88	1.90	1.87	1.94		2.06	1.97		1.85																	
of 15 subjects							2.03			1.98	1.84	1.74	1.72	1.70														
of 14 subjects														1.70														
of 13 subjects														1.71														
of 11 subjects														1.73														
of 9 subjects															1.88													
of 8 subjects															1.79		1.79	1.78	1.90									
of 7 subjects																			1.92									
of 4 subjects																				1.76	1.84							
of 3 subjects																					1.83	1.68	1.83	1.98	2.03	1.64	1.67	1.71
of 2 subjects																						1.67	1.83	1.98	2.03	1.64	1.67	1.71
of 1 subject																												1.61

I.—CONCISE ACCOUNT OF THE EXPERIMENT

(Table IV). During July, August, and September, 1943, Golding deteriorated rapidly, his average value in September, 1943 being 2.81 log. units ; at this stage he was dosed with vitamin A owing to distressing attacks of migraine (*see p. 23*).

Proctor's rod threshold rose gradually from August, 1943 onwards until it reached an average of 3.12 in January, 1944.

Watson's rod threshold remained fairly constant for about 20 months until March, 1944. It then began to rise and reached an average value of 2.41 in May, 1944. His highest value at any time was 3.65, but the value just before dosing with carotene was 2.61. Towards the end of the period of deprivation his range of variations was considerably greater than is normally seen. He was dosed after 22 months.

The average rod threshold of the remaining deprived volunteers (Table IV) did not deviate significantly from the initial value except during the winter season, as already mentioned.

Cone-rod transition time.—The cone-rod transition time of the volunteers at the beginning of the experiment and of a number of normal people, measured under the standardized conditions described on p. 105, was found to vary between 5 and 12 minutes (Table XLIII, p. 109). Throughout the whole experiment it remained almost completely within this range in all subjects, except in the three, Golding, Proctor, and Watson, whose rod threshold rose as a result of the depletion, and possibly in one other, Woodhouse. The highest values recorded were 25 minutes for Golding, 19 minutes for Proctor, and 33½ minutes for Watson. In Golding the first definite lengthening, to 14 minutes, was noted on 1st July, 1943, at a time when the final rod threshold was only just beginning to rise. In Proctor the changes in the cone-rod transition time and final rod threshold were noted simultaneously in September, 1943, while in Watson the changes in cone-rod transition time definitely preceded those in the rod threshold. In February, 1944, his cone-rod transition time rose to 14 minutes, in March to 20 minutes ; a major rise in the rod threshold began only in April, 1944.

It is clear, therefore, that in vitamin A deficiency lengthening of the cone-rod transition time may occur at the same time as, or precede, the deterioration of the rod threshold, contrary to the findings of Hecht and Mandelbaum (1939).

An example to illustrate the effects of vitamin A deprivation on the dark adaptation curve is given in Fig. 4, which shows a number of curves for Proctor at different stages of depletion.

Among the previous authors who have examined the effects of vitamin A deprivation on the capacity for dark adaptation, Hecht and Mandelbaum (1939, 1940), and Wald, Jeghers, and Arminio (1938) reported very prompt deterioration on the deficient diet. Of seventeen subjects studied by Hecht and Mandelbaum (1940), fourteen showed an "unmistakable" rise in the rod threshold within a few days of the start of the diet. In the other three a "clear and continuous rise" occurred after 22, 55, and 60 days, respectively. Booher, Callison, and Hewston (1939) depleted five subjects and recorded a rise after 16, 27, 29, 39, and 124 days, respectively. Steven (1943) reports deterioration after 38 and 87 days, and Wagner (1940) after about 6 months. Steffens, Bair, and Sheard (1939), and Brenner and Roberts (1943), on the other hand, failed to observe significant changes after 6 months. It is at

present impossible to say whether these contradictory results of different authors are really due to extreme variations in the magnitude of the liver reserves of vitamin A, or whether the raised threshold values recorded do not always represent corresponding degrees of deficiency.

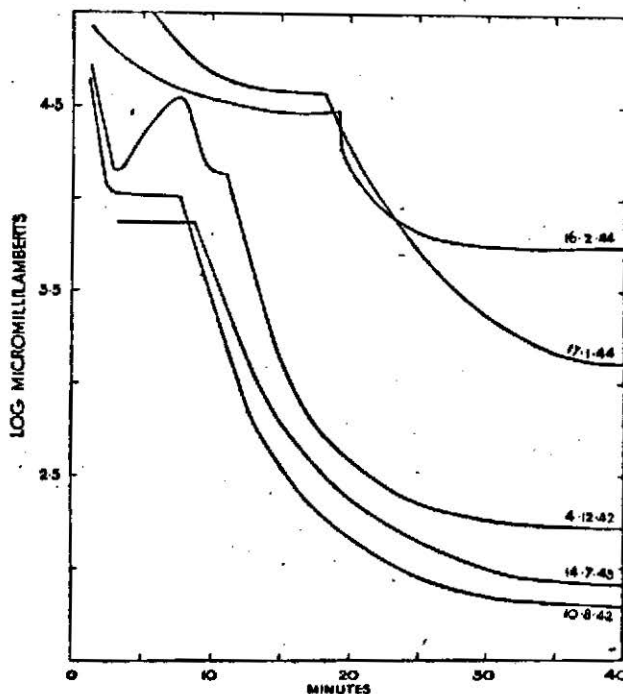


Fig. 4.—Curves of dark adaptation, measured with the Wald apparatus, for the deprived subject, Proctor, at different stages of depletion. The differences in the cone threshold, in particular the fluctuations in the curve of 4.12.42, are of doubtful significance (see p. 108). In other cases (e.g. Garling) there was no rise in the cone threshold as the result of deprivation.

Correlation between plasma vitamin A level and capacity for dark adaptation.—It can be seen from Fig. 1 (p. 13) and Table II (p. 14) that all three subjects (Golding, Proctor, and Watson) whose dark adaptation deteriorated significantly had low average plasma vitamin A levels at the critical time. A fall of the average plasma level to below 50 I.U. per 100 ml. in every case preceded the deterioration of dark adaptation by a few weeks, and no abnormal values for dark adaptation were noted in any subject as long as the plasma level of vitamin A remained above 50 I.U. per 100 ml. By the time the deterioration of night vision became pronounced, the average blood level was below 40 I.U. per 100 ml. On the other hand none of the deprived subjects whose dark adaptation, as measured by Wald's adaptometer, remained normal, had average plasma vitamin A levels below 40 I.U. per 100 ml.

It must be emphasized that throughout this discussion the *average* vitamin A values, as shown in Table II, p. 14, were considered. In view of the considerable scatter of the results the above statements do not apply to each individual vitamin A value observed.

Results of General Medical Examinations

Bodyweight (see Table LIII, p. 136).—There was no consistent loss such as was described by Wagner (1940).

Minor abnormalities.—At the routine examinations special attention was directed to the skin, particularly the hair follicles, to the external appearance of the eyes, to the buccal cavity, and the respiratory system. Reflexes, blood pressure, and pulse rates also were examined. Special investigations included blood examination (haemoglobin, red cells, white cells, and platelets); urine analysis (sugar, protein, sediment, and epithelial cell counts); fractional test meals, electrocardiograms, measurement of basal metabolic rate, chest radiography, dental examination, skin biopsy (hair follicles), slit-lamp examination of the conjunctiva and cornea, and psychological tests for memory and for mental and physical fatigue. The volunteers were asked to report subjective sensations of impaired health, such as tiredness, irritability, headaches, dyspeptic pains, and eyestrain, and to record the incidence and durations of minor illnesses such as coughs or colds. Only in the later stages of the experiment did the examining clinicians learn which volunteers were deprived.

The great majority of signs, symptoms, and results of special tests were not markedly different in the deprived and non-deprived groups, or in the same person before, during, and after deprivation of vitamin A. The only signs and symptoms which seemed to be commoner in the deprived group, though their interpretation was doubtful, were dryness of the skin, which was noted in ten of this group and in none of the prophylactic group, and eye discomfort, variously described as soreness, streaming, pricking, or a feeling of sand in the eyes, which occurred in eleven of the deprived group and in only one of those not deprived.

Hearing.—Audiometry tests (see p. 124) were made on some of the volunteers and it was possible to obtain a continuous series of results for seven of them. These showed a definite deterioration in three out of five of the deprived subjects but no deterioration in the two non-deprived.

Blood platelets.—Wagner's (1940) report that the number of platelets in the blood dropped as a result of vitamin A deprivation was not confirmed. The platelet counts showed considerable variations but there were no deviations from the normal and no trend related to the vitamin A intake.

Follicular hyperkeratosis.—This condition, which has often been ascribed to vitamin A deficiency, was present at the start and varied during the experiment in several of the volunteers, but these variations bore no relation to the vitamin A intake, so that the conclusion was reached that the tendency to develop keratinized hair follicles was not specifically related to the state of vitamin A nutrition. This agrees with the conclusion reached by Stannus (1945). A fuller account of the observations, which include inspections by the binocular dissecting microscope and histological examination of excised skin from three subjects, is given on page 120.

Conjunctivae.—The various microscopic changes which Kruse (1941) attributed to vitamin A deficiency (opacity, wrinkling, thickening, xerosis of the conjunctiva, "spots") were seen with the slit-lamp in the deficient group and in the controls. Their occurrence did not vary with the vitamin A intake. There were no Bitot's spots.

“Clinical” night blindness.—Only two subjects, Golding and Proctor, thought that their night vision was somewhat impaired when the state of deficiency was at its height. They thought they had greater difficulty in finding their way in the black-out on dark nights, but Proctor was not very definite about it. It is noteworthy that they were so little conscious of their defective night vision, although the final rod threshold had deteriorated to one-tenth of the starting value in Golding and to one-hundredth of the starting value in Proctor.

Fatigue.—This has often been recorded as an early sign of nutritional deficiency. In the present experiment neither the subjective statements of the volunteers nor the psychological tests produced evidence of abnormal fatigue as a result of vitamin A deprivation.

General comment.—The non-occurrence of the various signs and symptoms ascribed by previous workers to slight vitamin A deficiency should not necessarily be taken as invalidating their statements. Chronic deprivation caused by intakes below the level of requirement over many years, or environmental factors such as temperature and occupational exertions, or age, or additional shortcomings in the diet, all may modify the clinical manifestations of a low vitamin A intake. The present experiment, however, leaves little doubt that, in uncomplicated vitamin A deprivation abruptly initiated in young adults with good liver reserves, the earliest definite signs of depletion are defective night vision, with a raised rod threshold and prolonged cone-rod transition time, and a fall of the value for vitamin A in the blood plasma.

Major illnesses.—Four of the volunteers contracted major illnesses, three during, and one after, the period of deprivation :

<i>Name</i>	<i>Illness</i>	<i>Date of onset</i>
Tridgell	Impetigo contagiosa	November, 1942, after 3 months of deprivation.
Golding	Migraine	September, 1943, after 14 months of deprivation.
Hicken	Tuberculous pleurisy	About November, 1943, after 16 months of deprivation.
Williams	Tuberculous disease of the spine	Before November, 1944 ; 20-month period of deprivation finished on April 2nd, 1944.

With regard to the two cases of tuberculosis, while the men affected were very fit at the beginning of the experiment and there was no family history of tuberculosis, it is impossible to say whether the simple deprivation of vitamin A played any part in the aetiology of their illnesses. In neither instance was the condition “open”, and X-ray examination of all the remaining volunteers failed to reveal any who had pulmonary tuberculosis. It may at least be concluded, therefore, that case to case infection among the volunteers was ruled out.

Golding’s migraine was coincident with the period of maximum deficiency. He had five severe attacks during the last 16 days of the period of deprivation. He had never had such attacks before, nor did they recur after treatment with vitamin A. Full details of the case histories of these men are included in Protocol A.

Carotenoid Content of Faeces

Total carotenoids and α - and β -carotene were estimated in the faeces at three different stages of depletion. The techniques of sampling and analysis are described on p. 97 and the data are set out in Tables XXVII (p. 70) and XXVIII (p. 72). Most samples were analysed at both Cambridge and Liverpool, but the Cambridge results for α - and β -carotene were probably too high through the inclusion of xanthophyllic esters derived mainly from flour which, in the first period (*see* p. 80), was National Wheatmeal and, in the second and third periods, white flour. Because of this probable error the Cambridge data, though included in Tables XXVII and XXVIII, are omitted in discussing the results.

The carotene values for the faeces, like those for the plasma, bore no relation to the general state of vitamin A nutrition as measured by the values for the rod threshold and for vitamin A in the plasma, but they were a valuable index of the poverty of the diet in carotene. The daily excretion of α - and β -carotene varied between 31 and 264 I.U. (average 122 I.U.) when National Wheatmeal was eaten, and between 27 and 178 I.U. (average 81 I.U.) when white flour was eaten.

C.—Therapeutic Tests with Vitamin A and Carotene

When the experiment was planned it was hoped that all, or most, of the sixteen subjects who received the basal diet without supplements would develop unmistakable signs of deficiency, which could be used as criteria in testing the effectiveness of graded doses of vitamin A and carotene. Unfortunately, only three (Golding, Proctor, and Watson), to whom frequent reference has already been made, behaved in this way. Eight others, who for various reasons had to be dosed with vitamin A or carotene, failed to develop unmistakable signs of deficiency, and the conclusions which can be drawn from observations on them are therefore limited. The remaining five left the experiment prematurely and at such short notice that they could be used only for examining the effects of very large doses of the vitamin.

Of the three subjects showing unmistakable signs of deficiency, two were dosed with carotene and one with vitamin A; of the eight imperfectly depleted subjects, four were dosed with carotene and four with vitamin A. When practicable a small dose was given first and, if no improvement was observed, the dose was increased.

VITAMIN A

Vitamin A was given as a solution of distilled natural esters in arachis oil.

Dose about 1,300 I.U.—The smallest daily dose was approximately 1,300 I.U. It was given to Golding who was adequately depleted, and to Thompson, Williams, and Wodeman who were inadequately depleted.

Golding received the dose for 199 days after a period of depletion of 425 days. He showed a response by all three criteria—final rod threshold, cone-rod transition time, and blood vitamin A level (Table V). The first signs of improvement appeared within 2 weeks, but, in general, improvement was slow, developing gradually over the course of 6 months. The final rod threshold reached and maintained its best level, which could not be improved by higher doses of vitamin A, after about 6 months.

Golding's plasma vitamin A values showed a considerable rise, from an average of 22 I.U. per 100 ml. before dosing, to monthly average values between 50 and 88 (Table V), but the blood level was further raised when larger doses, together with an unrestricted diet, were given at a later date (Fig. 5, and Protocol B).

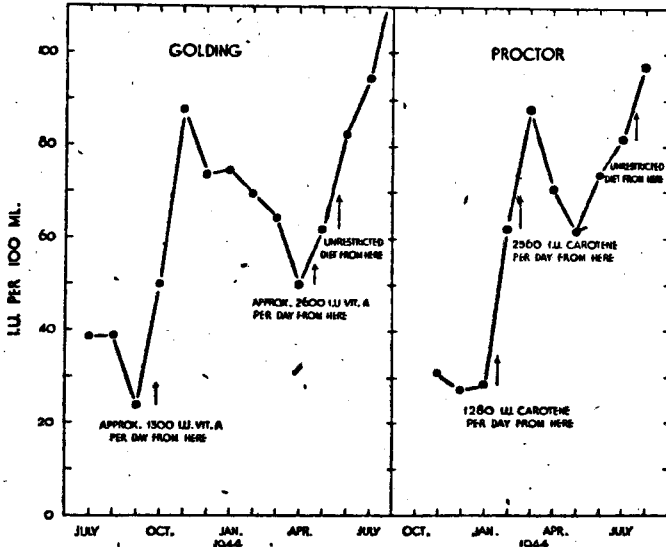


FIG. 5.—Response to dosing of the values for vitamin A in the blood plasma of two depleted subjects (Golding and Proctor). The points represent monthly averages.

Thompson, Williams, and Wodeman received 1,250 I.U. vitamin A for 64, 63, and 78 days, respectively, after periods of depletion of 555, 552, and 546 days, respectively. The three criteria showed little change after dosing but it should be noted that the plasma vitamin A levels of these three, though somewhat depressed, were much higher than Golding's when the dosing started (Protocol B), and that the capacity for dark adaptation was within the normal range. Very large doses of vitamin A, and unrestricted diet, later raised the vitamin A plasma level but had no definite effect on the criteria of the capacity for dark adaptation.

TABLE V
Golding: Effects of Giving about 1,300 I.U. Vitamin A Daily

Period	Final rod threshold 4-weekly average* (log. μ m lamberts)	Cone-rod transition time (minutes)	Plasma vitamin A monthly average* (I.U. per 100 ml.)
Before dosing	2.81	25	22
First month after dosing .. .	2.38	—	50
Second month after dosing .. .	2.20	9½	88
Sixth month after dosing .. .	1.81	10	65

* Note.—The 4-weekly average refers to precisely 4-week periods, whilst the monthly average refers to periods of one calendar month.

Dose of 2,600 I.U.—This dose was given to Golding after treatment for 199 days with 1,300 I.U. vitamin A, and to Stuart after 325 days of depletion. Neither subject showed any signs of deficiency at the start of the dose. Golding received the dose for 45 days and Stuart for 25 days. No effects of the dosing were noted; especially noteworthy was the absence of any significant rise in the vitamin A plasma level from the average value for the preceding period, of 65 I.U. for Golding and 52 I.U. for Stuart.

Large doses of vitamin A.—Towards the end of the experiment most volunteers were given an unrestricted diet and large doses of vitamin A in order to replenish their reserves. The supplementary dose for most of them was 24,000 I.U. per day for about 42 days, so that the total supplement was about 1,000,000 I.U. Measurements were continued during and after the period of replenishment. The blood vitamin A level rose in all of them, usually to values of 100 I.U. or more per 100 ml. (Protocol B), but no other effects of these large doses were detected. The capacity for dark adaptation did not improve beyond the range attained with small doses.

CAROTENE

Carotene was given in the form of a solution in arachis oil. It was β -carotene with a small admixture of α -carotene. As a considerable portion of the dose was excreted unchanged in the faeces it is useful to introduce the term "maximum effective dose" to represent the difference between the amount administered and the amount excreted. How much of the carotene that was not excreted was actually absorbed, and how much was destroyed in the gut, remained uncertain. The maximum effective dose was determined in a number of subjects (see Table XXVIII, p. 72); it varied for carotene in oil between 63 and 82 per cent. of the amount ingested.

Dose of 1,250 I.U.—The smallest daily dose of carotene was about 1,250 I.U. This dose was given to Proctor, who showed unmistakable signs of deficiency, and to Bloomfield, A. Whitehead, and Woodhouse, who were inadequately depleted.

Proctor received the dose for 30 days after a period of depletion of 539 days. During the period of treatment the final rod threshold deteriorated from 3.12 to 3.49 log. units, although the plasma levels for vitamin A and

TABLE VI
Proctor: Effects of Giving about 1,250 and about 2,500 I.U. Carotene Daily

<i>Period</i>	<i>Final rod threshold 4-weekly average* (log. μm lamberts)</i>	<i>Cone-rod transition time (minutes)</i>	<i>Plasma vitamin A monthly average* (I.U. per 100 ml.)</i>	<i>Plasma carotenoids monthly average* ("I.U." per 100 ml.)</i>
Before dosing	2.87	—	28	19
	3.12	17½	29	19
First month on 1,250 I.U. ..	3.49	17	63	39
First month on 2,500 I.U. ..	3.27	13½	89	58
Fourth month on 2,500 I.U. ..	3.33	10	66	68
Sixth month on 2,500 I.U. ..	2.56	8	83	74

* *Note.*—The 4-weekly average refers to precisely 4-week periods, whilst the monthly average refers to periods of one calendar month.

carotenoids both showed a rise (Table VI). In view of the deterioration of the final rod threshold the dose could not be regarded as effective, and it was therefore doubled (*see below*).

Bloomfield, A. Whitehead, and Woodhouse received 1,250 I.U. carotene for 15, 35, and 50 days, respectively, after periods of depletion of 755, 448, and 605 days, respectively. No effects on the plasma levels for vitamin A or carotenoids, or on dark adaptation, were noted. Except for a slightly prolonged cone-rod transition time in Woodhouse their capacity for dark adaptation was within the normal range, and lack of response to dosing is therefore of limited significance. It is, however, of interest that Woodhouse's cone-rod transition time returned to its normal figure later, when an unrestricted diet and large doses of vitamin A were given (Protocol B).

Dose of 2,500 I.U.—Proctor received this dose for 165 days, after the smaller dose had failed to arrest the deterioration of his capacity for dark adaptation. It was given to Watson, who also showed unmistakable signs of deficiency, for 40 days after a depletion period of 667 days, and to A. Whitehead who was inadequately depleted and had failed, like Proctor, to respond to a dose of 1,250 I.U.

With this dose Proctor slowly improved by all criteria (Table VI). The improvement became definite during the first month but further progress was slow. By the sixth month the cone-rod transition time had returned to the normal range (*see p.108 and Table XLIII, p.109*), while the final rod threshold had much improved (from 3.49 to 2.56 log. units) though still considerably above its starting value of about 1.80 log. units. When, after 165 days of treatment with 2,500 I.U. carotene, an unrestricted diet was given, the rod threshold quickly reached its starting value (Protocol B).

Watson showed an extremely quick response of the final rod threshold ; within 7½ hours of dosing the rod threshold fell from 2.61 (the reading immediately before dosing) to 1.68 log. units and was maintained at this level (Table VII and Protocol B). The cone-rod transition time was first recorded 48 hours after dosing and had by then returned from 23 to 9 minutes. The plasma vitamin A level rose within one week to 59, and within three weeks to 96, I.U. per 100 ml, whilst the value for plasma carotenoids rose rather more slowly (Table VII). The capacity for dark adaptation was not further improved by an unrestricted diet with large vitamin A supplements.

TABLE VII
Watson : Effects of Giving about 2,500 I.U. Carotene Daily

<i>Period</i>	<i>Final rod threshold (log. μm lamberts)</i>	<i>Cone-rod transition time (minutes)</i>	<i>Plasma vitamin A (I.U. per 100 ml.)</i>	<i>Plasma carotenoids ("I.U." per 100 ml.)</i>
Before dosing	2.28*	19½ to 33½	19	28
One week after dosing ..	1.68	6	59	27
Three weeks after dosing ..	1.67	8	96	53

* Average of 3 readings.

A. Whitehead who, when dosing started, had no signs of deficiency apart from a moderately lowered plasma vitamin A value, showed no important changes except a slight rise of the average plasma carotenoid level.

Larger doses of carotene.—A. Whitehead was given 4,800 I.U. β -carotene and Russell 6,000 I.U. β -carotene towards the end of the controlled experimental period (Protocol B). Both showed some rise of the plasma carotenoid values and A. Whitehead showed a rise also of the plasma vitamin A value, but no other effects were noted.

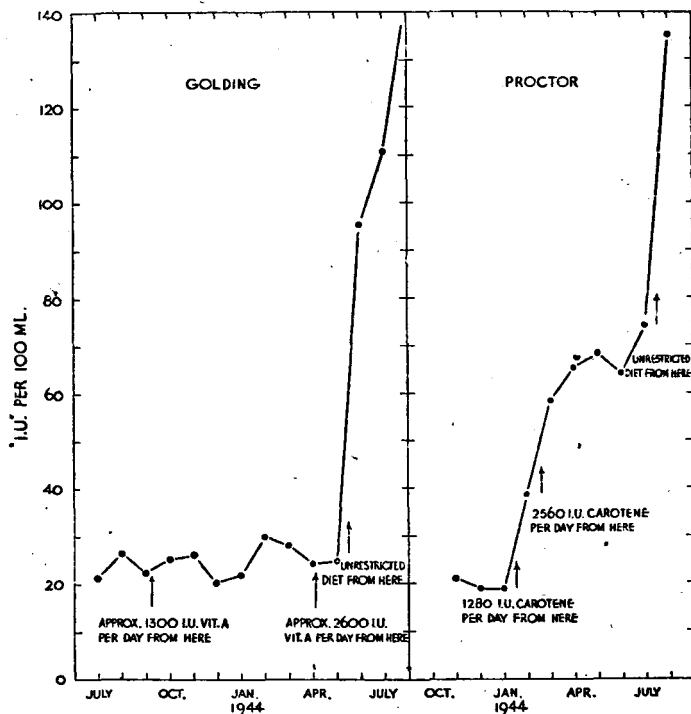


FIG. 6.—Response to dosing of the values for total carotenoids in the blood plasma of two depleted subjects (Golding and Proctor). The points represent monthly averages.

SUMMARY OF THE EFFECTS OF DOSING

With a daily dose of about 1,300 I.U. vitamin A in oil one adequately deprived subject, Golding, showed a gradual restoration of the capacity for dark adaptation to a level which could not be subsequently improved with larger doses. His plasma value for vitamin A rose also, but it was further increased by very large doses of vitamin A. With the same dose of about 1,300 I.U. the somewhat lowered plasma values for vitamin A of three inadequately depleted subjects (Thompson, Williams, and Wodeman), were not significantly improved, and the small changes in the normal, and almost normal, values of the rod threshold of all three were probably not significant.

With a daily dose of about 1,250 I.U. carotene in oil, one adequately depleted subject, Proctor, showed an increase in the value for plasma vitamin A, but a deterioration of the rod threshold. Three inadequately depleted subjects (Bloomfield, A. Whitehead, and Woodhouse), showed no response by either criterion.

With a daily dose of about 2,500 I.U. carotene in oil, two adequately depleted subjects, Proctor and Watson, showed improvement by all the criteria. Watson, whose state of depletion was less advanced than Proctor's

when dosing began, recovered promptly and completely within 3 weeks. With Proctor recovery was slow and not quite complete when, after 5½ months, the dose was replaced by an unrestricted diet.

D.—Prophylactic Tests with Vitamin A and Carotene

Prophylactic tests were planned to run concurrently with the depletion tests. The main object was to provide "positive controls" to test whether the basal diet was adequate in all respects except vitamin A and carotene. The magnitude of the protective doses of vitamin A and carotene was, however, selected to be as far as was known marginal, so that the results might give additional evidence on the amount of the requirement. With carotene this object could be more fully attained if a simultaneous study was made of the faecal excretion of carotene from a number of different foods. The source of carotene was therefore changed at intervals and, when no food was under test, β -carotene in oil was usually given.

For varying periods five volunteers received the basal diet with daily supplements of carotene from various sources, and two with a daily supplement of vitamin A (see Table VIII; also Tables XI, p. 34 and XXVIII, p. 72).

VITAMIN A

Vitamin A was given as the same solution of distilled natural esters in oil which was used for the therapeutic tests. The two subjects, Drabble and Tridgell, did not go straight from a normal diet to the basal diet supplemented with vitamin A. Tridgell started to receive the basal diet unsupplemented but contracted impetigo after 3½ months. Since this did not respond to treatment with merthiolate, it was thought necessary to end his depletion, and he was therefore dosed with 24,000 I.U. vitamin A daily for 19 days. After this he was given the basal diet without supplements for 35 days, and then a daily dose of about 2,500 I.U. vitamin A in oil was added for about 17 months. This was increased for 14 days at the end of the period to 5,800 I.U. daily, after which the diet was unrestricted. Drabble entered the experiment 3 months later than most of the volunteers. He received the unsupplemented basal diet for 70 days but it was then decided that he should become a companion to Tridgell, whose impetigo had disappeared by this time, and receive a regular supplement of vitamin A. He was given a daily dose of about 2,500 I.U. vitamin A for 14 months. This was increased to 5,000 I.U. daily for 55 days, followed by an unrestricted diet to which 96,000 I.U. vitamin A was added for 5 days.

Plasma values for vitamin A.—The level of vitamin A in the blood plasma remained fairly constant throughout their respective experimental periods of 14 and 17 months (Protocol B). The average values at the beginning and end, respectively, of the experiment were for Drabble 85 and 90, and for Tridgell 71 and 77, I.U. per 100 ml. No significant increase occurred when the dose was raised to 5,800 I.U. but, on an unrestricted diet without supplements, the average values were 140 for Drabble and 117 for Tridgell.

Plasma values for total carotenoids.—The plasma values for carotenoids were entirely unaffected by dosing with vitamin A, and behaved in the same way as in the subjects undergoing depletion. They fell rapidly to a level of about 30 "I.U." per 100 ml. and then slowly decreased still further to an average of 21 for Drabble and 22 for Tridgell at the end of the experimental period.

TABLE VIII

Period of Administration and Nature of Supplements Given to Subjects Receiving the Deficient Basal Diet with Regular Daily Additions of Vitamin A or Carotene

<i>Drabble</i>	<i>Tridgell</i>	<i>Bartley, M.</i>	<i>Bartley, W.</i>	<i>Broderick</i>	<i>Drake</i>	<i>Garnett</i>
Deficient diet, 17.11.42 to 27.1.43	Deficient diet, 13.8.42 to 2.12.42	β -Carotene c. 5,000 I.U. daily in various forms, 3.8.42 to 14.4.43	β -Carotene c. 5,000 I.U. daily in various forms, 16.8.42 to 15.6.44	β -Carotene c. 5,000 I.U. daily in various forms, 28.7.42 to 9.9.43	Vitamin A 2,500 I.U. I.U. daily, 23.3.43 to 12.4.43	β -Carotene c. 5,000 I.U. daily, 27.7.42 to 12.2.43
Vitamin A c. 2,500 I.U. daily, 28.1.43 to 16.3.44	Vitamin A 24,000 I.U. daily, 3.12.42 to 22.12.42		Deficient diet, 16.6.44 to 10.7.44	Deficient diet, 10.9.43 to 25.1.44	β -Carotene c. 5,000 I.U. daily, 13.4.43 to 15.6.44	
Vitamin A c. 5,000 I.U. daily, 17.3.44 to 11.5.44	Deficient diet, 23.12.42 to 27.1.43			Vitamin A 24,000 I.U. daily, 26.1.44 to 7.2.44	Deficient diet, 16.6.44 to 30.6.44	
	Vitamin A c. 2,500 I.U. daily, 28.1.43 to 14.6.44			Vitamin A 48,000 I.U. daily, 8.2.44 to 14.2.44		
	Vitamin A 5,800 I.U. daily, 15.6.44 to 30.6.44			Vitamin A 72,000 I.U. daily, 15.2.44 to 18.2.44		
				Deficient diet, 19.2.44 to 25.2.44		

Capacity for dark adaptation.—When the two men began to serve as positive controls in January, 1943, the temporary deterioration of the rod threshold shown by all volunteers was approaching its maximum. Like the other volunteers, both Drabble and Tridgell showed some rise of the rod threshold during the following month; after this it improved in both men, and remained fairly constant in Drabble, showing no deterioration in the winter 1943–44. Tridgell always tended to give higher values than the majority of normal subjects; his rod threshold remained under observation until early in 1946 and always continued to give values between 2.1 and 2.3 log μm lamberts (Table XVI, p. 53).

Neither subject showed a cone-rod transition time outside the normal range at any period of the experiment (*see* Table XLIII, p. 109).

Summary.—A daily dose of 2,500 I.U. vitamin A appeared to maintain both Drabble and Tridgell. The value for vitamin A in the plasma, however, did not on this treatment reach the level subsequently attained by them on an unrestricted diet.

CAROTENE

The periods of time for which the five subjects served as positive controls receiving about 5,000 I.U. daily of carotene ranged from 6½ to 22 months (Tables I, p. 11 and VIII, p. 30).

Carotene was given in the form of :

- sliced canned carrots ;
- the same carrots made into purée by passage through a “Latapie” grinder ;
- canned homogenized carrots ;
- β -carotene in arachis oil ;
- β -carotene in margarine ;
- canned spinach purée ;
- canned homogenized spinach ;
- reconstituted dehydrated cabbage.

Particulars of these preparations and of their carotene content are given on pp. 88–90. Canned materials of one batch, rather than fresh vegetables, were used in order that the supply might be uniform.

Faecal excretion of carotene.—Before the effects of carotene on the body can be considered quantitatively it is necessary to discuss the faecal excretion of the carotene ingested. Although vitamin A is known to disappear entirely from the gastro-intestinal tract as long as, according to Wald, Carroll, and Sciarra (1941), the amount given in a single dose is below about 25,000 I.U., considerable amounts of ingested carotene invariably appear in the faeces (*see also* With, 1941).

In order to determine the faecal excretion of carotene from various sources, seven-day samples of the faeces were analysed at Cambridge and Liverpool. The period of collection began after the subject had received the supplement under investigation for at least three days, usually much longer. Details of the technique are given on p. 97. The full results are set out in Tables XXVII and XXVIII (pp. 70, 72) and a summary in Table IX where the average percentage of carotene excreted by the group on each of the test materials is shown. Table XXVIII also gives the “maximum effective dose”, an expression used to describe that part of a given dose of carotene which is not excreted; whether the whole of this non-excreted portion is actually

TABLE IX

Prophylactic Tests with Carotene: Average Values for the Vitamin A and Total-Carotenoid Content of the Blood Plasma and for the Final Rod Threshold of the Groups of Subjects Receiving Carotene Supplements, Arranged in Ascending Order of the Maximum Effective Dose (see also Tables XXVII and XXVIII, pp. 70, 72)

Supplement	Average amount of carotene excreted in the faeces (per cent. of intake)	Vitamin A potency (I.U. per day)		Plasma value (average for three laboratories)		Final rod threshold (log. μ m lamberts)
		Intake	Maximum effective dose	Vitamin A (I.U. per 100 ml.)	Total carotenoids ("I.U." per 100 ml.)	
Carrots, canned, sliced	76	5,000	1,250	83	66	1.90
Carrots, canned, sliced, purée ..	75	5,000	1,250	86	80	2.03
Cabbage, dried, outer leaves :						
Small dose	59	3,225	1,325	85	122	1.57
Large dose	73	6,450	1,775	98	104	1.55
Spinach, canned, purée	59	4,300	1,775	92	173	1.55
Carrots, canned, homogenized ..	44	5,100	2,850	112	158	1.52
Spinach, canned, homogenized ..	57	6,700	2,875	106	217	1.56
Carotene in margarine	29	5,200	3,700	97	125	1.52
Carotene in oil	26	5,100 to 6,400	3,775 to 4,725	81	133	1.94

absorbed, or whether some of it is destroyed in the intestines, remains uncertain.

It will be seen that there are considerable variations between the values for different persons receiving the same supplement under apparently the same conditions. There are considerable variations also for the same person receiving the same supplement in different test weeks. Moreover, the agreement between the two laboratories is not always good. Therefore, only major differences can be considered of any significance.

Of the supplements tested, excretion was highest with sliced carrots, of which an average percentage of 76 was excreted. Adding 10 grammes of margarine or mincing the carrots in a "Latapie" grinder had no effect on the amount excreted, but commercially homogenized carrots showed a considerably lower average excretion of 44 per cent. The diameter of the holes in the "Latapie" grinder and, therefore, the maximum diameter of the particles was 1 mm.; according to information from the manufacturers the maximum diameter of the particles in the homogenized carrots was 0.05 mm. and the average was rather less than 0.02 mm.

The percentage excretion of carotene from green vegetables was, on the average, between 57 and 73. There was no appreciable difference in the excretion of carotene from canned spinach, whether homogenized or not, and from the rather coarse and tough reconstituted outer leaves of cabbage.

The lowest excretion, on the average 26 and 29 per cent., occurred when carotene was dissolved in arachis oil and in margarine.

These values are in fair agreement with those to be found in the literature, which contains only a few data for human adults comparable with the present results. Table X summarizes previous findings for comparison with the present results. Data from infants (Heymann, 1936; van Zeven, 1945) are not included, since they are not comparable.

TABLE X

Percentage of Ingested Carotene from Various Sources Excreted in the Faeces of Human Adults

(In part quoted from van Zeven, 1945. The ranges shown represent varying numbers of observations)

Authors	Source of carotene ingested		
	Carotene in oil	Carrots	Spinach
Van Eekelen and Pannevis (1938)	41	99 (cooked)	94 (cooked)
Wagner (1940)	44 to 54	—	—
With (1940; 1941) ..	25 to 70	40 to 70 (purée, cooked)	65 to 70 (cooked)
Eriksen and Höygaard (1941)	—	81 (cooked) 99 (raw)	42 (cooked) 55 (raw)
Virtanen and Kreula (1941)	—	80 (raw, grated) 96 (cooked)	—
Wald, Carroll and Sciarra (1941)	60	70 to 80	—
Present investigation ..	26 (18 to 34)	76 (canned) (43 to 100) 75 (canned purée) (43 to 100) 44 (canned, homogenized) (37 to 54)	59 (canned purée) (25 to 77) — 57 (canned, homogenized) (51 to 73)

TABLE XI

Prophylactic Tests : Values for the Final Rod Threshold and for the Content of Vitamin A and Total Carotenoids in the Blood Plasma in Relation to Intake of Carotene and Vitamin A (the periods with carotene are arranged in chronological sequence except for that with carotene in oil which is made up from a number of scattered short periods. For further details, see Table XXVIII, p.72)

Supplement	Average amount of carotene excreted in the faeces (per cent. of intake)	Vitamin A potency (I.U. per day)		Name	Test period (days)	Plasma value (average Cambridge, Liverpool, Oxford)		Final rod threshold Wald (log. μ m lamberts)
		Intake	Maximum effective dose*			Vitamin A (I.U. per 100 ml.)	Total carotenoids ("I.U." per 100 ml.)	
Carrots, canned, sliced	76	5,000	1,200	Bartley, Mrs.	144	77	59	1.94
				Bartley	149	90	87	1.78
				Broderick	155	76	59	1.98
				Garnett	144	88	59	1.91
				<i>Average</i>		83	66	1.90
Carrots, canned, sliced, purée ..	75	5,000	1,250	Bartley, Mrs.	71	80	83	2.16
				Bartley	69	110	90	1.91
				Broderick	64	72	79	1.93
				Garnett	27	83	68	2.13
				<i>Average</i>		86	80	2.03
β -Carotene in arachis oil with hydroquinone	26	5,100 to 6,400	3,775 to 4,725	Bartley, Mrs.	24	77	99	2.35
				Bartley	90	82	146	1.67
				Broderick	51	65	131	2.08
				Drake	72	100	155	1.64
				<i>Average</i>		81	133	1.94
Spinach, canned purée	59	4,300	1,775	Bartley	74	98	170	1.48
				Broderick	73	83	175	1.63

				Drake	74	95	173	1.55
				<i>Average</i>		92	173	1.55
dried, outer leaves ..	59	3,225	1,325	Bartley	57	82	101	1.43
				Broderick	37	69	149	1.63
				Drake	57	105	117	1.64
				<i>Average</i>		85	122	1.57
	73	6,450	1,775	Bartley	20	85	103	1.44
				Drake	20	110	105	1.65
				<i>Average</i>		98	104	1.55
in margarine	29	5,200	3,700	Bartley	63	94	124	1.38
				Drake	63	100	125	1.65
				<i>Average</i>		97	125	1.52
canned, homogenized ..	44	5,100	2,850	Bartley	52	106	149	1.47
				Drake	53	117	166	1.56
				<i>Average</i>		112	158	1.52
canned, homogenized ..	57	6,700	2,875	Bartley	46	105	211	1.50
				Drake	46	107	223	1.61
				<i>Average</i>		106	217	1.56
A ester concentrate ..	—	2,500 to 2,900	—	Drabble	414	73	27	1.65
				Tridgell	504	71	29	1.94
				<i>Average</i>		72	28	1.80
		4,700 to 5,800	—	Drabble	56	92	24	1.60
				Tridgell	16	79	27	2.25
				<i>Average</i>		86	26	1.93

* Calculated as percentage of intake not excreted.

In view of the high percentage excretion of carotene with the various supplements, although the aim was to maintain the dose of β -carotene at about 5,000 I.U. daily, the average maximum amount which in fact could have been absorbed ranged from 1,250 to 4,725 I.U.

The intake and maximum effective dose of the various supplements are set out in Table XI in relation to the plasma values for vitamin A and total carotenoids and to the values for the final rod threshold for each individual receiving the different supplements. In this Table the results are arranged in the chronological sequence in which the subjects received the various supplements, so that the course of behaviour of the criteria may be followed throughout the experiment as a whole, and particularly the behaviour of the final rod threshold can be compared with that of the subjects undergoing depletion (Table IV, p.19).

In the summary Table IX, the same data are set out, but only as the averages for the groups, and the data are arranged in increasing order of magnitude of the average maximum effective dose, in order to make plain any corresponding trend in the plasma values or final rod threshold.

Plasma values for vitamin A.—The average plasma values for vitamin A for the groups receiving the various supplements ranged from 81 to 112 I.U. ; the average values for the individuals within the groups are sufficiently consistent to suggest that at least the differences between the extremes in this range have some significance. Table IX shows that an increase in the average maximum effective dose is accompanied by a corresponding increase in the plasma values for vitamin A, except in the case of carotene in oil and carotene in margarine, when the blood values are low relative to the maximum effective dose. It is not surprising that this should be so in spite of the low proportion of carotene which the body can extract from natural foodstuffs, since these are likely to contain, in close association with the carotene, vitamin E and other natural protective substances which might well be more effective than the hydroquinone added to the carotene solution in oil. Such an effect has been demonstrated by Guggenheim (1944) with rats.

When the values for the different groups are considered in relation to their chronological sequence, it is found that the highest values are reached towards the end of the whole experiment ; this could hardly have been so if the maximum effective dose of carotene had throughout been less than the requirement. In general, the plasma values for vitamin A suggest that whether a daily intake of 5,000 I.U. β -carotene is enough for an ordinary human adult or not depends on the proportion that the body can extract from the material in which it is presented.

Plasma values for carotenoids.—Since the values are for total carotenoids they do not exactly reflect the amounts of vitamin A precursors in the plasma. Larger amounts of blood than were usually available were necessary for a chromatographic separation of the pigments into α - and β -carotene on the one hand and inactive carotenoids on the other, but this was done on a few occasions for those receiving as carotene supplements carotene in oil, homogenized carrots, or homogenized spinach (Table XII). With carotene in oil or homogenized carrots the difference between the values for total carotenoids and for α - and β -carotene was roughly 30 "I.U." per 100 ml., which was about the value found in subjects receiving the basal diet with no carotene supplement. In the two instances where the supplement was homogenized spinach, the removal of the inactive pigments from the blood

TABLE XII
s of Chromatographic Analyses of Carotenoids in Blood Plasma at Various Times in Relation to Dietary Supplements

Time of daily dietary supplement at time of blood collection (I.U.)	Origin of plasma	Analysis		Plasma content	
		Date	Laboratory	Total carotenoids ("I.U." per 100 ml.)	α- and β-Carotene (I.U. per 100 ml.)
	Mixed sample from 12 subjects (see footnote*)	22.3.43	Liverpool	30.0	0
	Mixed sample from 7 subjects (see footnote †)	8.2.44	Reading	17.5	3.5
	Bloomfield	3.3.44	Reading	30.0	1.5
	Garling	3.3.44	Reading	21.7	—
	Watson	3.3.44	Reading	43.3	—
	Woodhouse	3.3.44	Reading	18.3	2.0
	Garling	30.3.44	Reading	14.5	3.1
	Garling	21.4.44	Reading	13.8	2.2
	Bloomfield	4.5.44	Reading	15.0	2.2
in oil (6,400)	Mixed sample from Bartley, Mrs. Bartley, Broderick	13.4.43	Liverpool	69.0	38.3
nized carrot (5,100)	Bartley	8.2.44	Reading	128.3	95.0
in oil (1,280)	Proctor	8.2.44	Reading	27.5	11.0
in oil (4,800)	Whitehead	20.2.44	Reading	88.3	45.2
nized carrot (5,100)	Bartley	3.3.44	Reading	116.7	81.7
nized carrot (5,100)	Drake	3.3.44	Reading	156.7	120.8
in oil (2,560)	Proctor	3.3.44	Reading	50.0	26.3
in oil (5,120)	Bartley	30.3.44	Reading	121.7	84.2
in oil (5,120)	Drake	30.3.44	Reading	143.3	117.5
nized spinach (6,700)	Bartley	21.4.44	Reading	170.0	61.7
in oil (2,560)	Proctor	21.4.44	Reading	54.2	28.3
nized spinach (6,700)	Drake	4.5.44	Reading	223.3	78.3
in oil (1,320)	Woodhouse	4.5.44	Reading	51.7	37.7
A (1,275)	Mixed sample from Golding	8.2.44	Reading	13.5 } 40	2.2
A (2,550)	Mixed sample from Tridgell	8.2.44	Reading		
A (2,550)	Mixed sample from Drabble	3.3.44	Reading		
A (2,550)	Mixed sample from Tridgell	3.3.44	Reading		
A (1,275)	Golding	3.3.44	Reading	21.7	3.5
A (1,275)	Thompson	3.3.44	Reading	20.0	2.7
A (1,275)	Williams	3.3.44	Reading	20.0	
A (1,275)	Wodeman	3.3.44	Reading	28.3	

* Mixed sample: Wodeman 6 ml., Garling 6 ml., Hicken 7.5 ml., Williams 11.5 ml., Whitehead 11.5 ml. Bloomfield 13 ml., Proctor 13.5 ml., 4 ml., Mrs. Whitehead 19 ml., Woodhouse 21 ml., Watson 23 ml., Thompson 27 ml.

† Mixed sample: Thompson, Bloomfield, Watson, Broderick, Garling, Wodeman, Woodhouse.

by chromatography reduced the value per 100 ml. plasma by a much larger amount, from 170 and 223 "I.U." to 62 and 78 I.U. Thus, when green vegetables are eaten, a relatively large proportion of the total carotenoids in the plasma has no vitamin A potency.

The different supplements of carotene all prevented the rapid fall of blood carotenoids which occurred in the deprived group and in those receiving vitamin A prophylactically. There were considerable differences in the levels of carotenoids in the plasma according to the source and, on the whole, there was a direct correspondence between the size of the maximum effective dose and of the average carotenoid level in the plasma, as Table XI shows. The correspondence was, however, not perfect, possibly because the effect was partly obscured by the varying amounts of inactive carotenoids contributed by the different sources of carotene.

Capacity for dark adaptation.—At the beginning of the experiment during the winter of 1942-43, those receiving carotene supplements showed a temporary deterioration of the final rod threshold in the same way as those undergoing depletion (see p. 52); the supplements given at this time were 5,000 I.U. β -carotene as canned carrots, sliced or as purée, of which the average maximum effective dose was only about 1,300 I.U. daily and, for short periods only, β -carotene in oil for which the maximum effective dose was much higher.

In the winter of 1943-44 the deprived subjects, still undergoing depletion, again showed deterioration of the final rod threshold, but the subjects receiving carotene supplements did not do so; the daily supplements at that time were of carotene margarine and of homogenized carrots, with the much higher average maximum effective doses of 3,700 and 2,850 I.U., respectively.

Apart from the temporary deterioration in the first winter, the final rod threshold showed no significant variations throughout the period during which the supplements were taken. The cone-rod transition time showed no deterioration at any time. The occurrence, however, of the temporary deterioration in the deprived subjects as well as in those receiving carotene supplements is not easy to interpret and makes it difficult to compare the value of the different carotene supplements for their effects on dark adaptation. It is suggested elsewhere (p. 54) that the temporarily raised rod thresholds found in winter might be attributable to insufficient intake, even though in summer the rod threshold values of the deprived subjects returned to normal without any increase of intake.

Even if the above interpretation is not accepted, it is at least certain that, with a daily intake of about 5,000 I.U. β -carotene throughout, but with an average maximum effective dose ranging from 1,250 to 3,700 I.U. daily and rising as the experiment progressed, the final rod threshold was improved to the subject's optimum level and was fully maintained there.

The behaviour of the final rod threshold does, therefore, lend support to the conclusion drawn from the behaviour of the plasma values for vitamin A, that the requirement is well met by a daily intake of 5,000 I.U. β -carotene, if the body can extract a sufficiently large proportion of it.

Basal diet only.—Three volunteers, W. Bartley, Broderick, and Drake, who had received carotene supplements for 22, 13, and 14 months, respectively, received the basal diet unsupplemented for respective periods

of 24, 137, and 14 days. The value for the plasma carotenoids immediately began to fall. In Broderick, the only one of the three thus deprived for any length of time, there was a fall also in the vitamin A content of the plasma, and a small rise of the final rod threshold (Protocol B).

For the greater part (330 days) of the prophylactic period, Broderick was receiving an average maximum effective dose of carotene of 1,775 I.U. daily or at times much less; the early appearance of signs of depletion suggests that he was getting less than his requirement and that his liver reserves of vitamin A were relatively low.

E.—Requirement of Vitamin A and Carotene

In this Report the term "requirement" is used to mean the smallest amount of a dietary essential which will maintain health. The requirement will therefore vary according to the availability of the dietary essential from the food and also according to the degree of "health" aimed at. Experience with animals has shown that there is usually a wide difference between the smallest dose necessary to prevent obvious symptoms of deficiency, and the dose necessary for a maximum rate of growth. For successful reproduction and for the maintenance of sleek skin, bright eyes, and other outward signs of perfect health, even larger doses may be necessary. For instance, in rats deprived of vitamin A, Moore (1943) found that the yellow pigment of the incisor teeth was not normally developed until the intake of vitamin A was increased well beyond that which restored fairly rapid growth; and Sherman, Campbell, Udiljak, and Yarmolinsky (1945) showed that doubling the intake of vitamin A, regarded as adequate, "deferred old age and increased the length of life".

The only criteria of depletion which were available in this study were the plasma value for vitamin A and the behaviour of the capacity for dark adaptation. It is on the maintenance at, or restoration to, normal of these criteria, particularly the second, that reliance has been placed in assessing the requirements.

Vitamin A and its precursors present an unusual complexity of problems owing to their varying availability from different foods. Apart from the varied needs of different individuals, the principal problems of this kind in trying to assess the vitamin A requirement of a normal human adult are:

- (1) The amount required when the source is vitamin A;
- (2) The amount required when the source is β -carotene; the availability of β -carotene from different foods; the equivalence of β -carotene and vitamin A;
- (3) The value of other active carotenoids in terms of vitamin A;
- (4) The effect of tocopherols or other stabilizers on the requirement.

In the present experiment no attempt was made to answer the third and fourth questions, and no information was obtained about them. From a fundamental scientific standpoint the relative biological value of the different carotenoids is of great interest, but from the practical point of view it is not so important because β -carotene greatly preponderates over the other forms in most diets. Some information was obtained about the first two questions and the nature of this information will be reviewed before it is considered in relation to the results of other workers.

If an experiment of the kind discussed here had been made on animals, it would have been repeated with much larger numbers in order to widen the factual basis before conclusions were drawn. Since a repetition of the human experiment was impracticable, it is necessary to try to extract the maximum amount of information from the data even if, in view of the narrow basis, the conclusions can only be regarded as provisional.

Apart from forming a basis for generalizations, each result on a human being has its value, if the result is reliable. If, for instance, a daily dose of 1,300 I.U. vitamin A restored the impaired capacity for dark adaptation of one deprived subject, this fact gives information about some members at least of the community. What remains uncertain is whether the person is a representative one and what is the scatter for the remainder.

The problem is one in which the human individuals and the different foods offered to them are capable of combining in a pattern so complex that it is difficult to formulate a standard value for the requirement of vitamin A, and almost impossible to deduce accurately from dietary surveys the effective intake of carotene.

REQUIREMENT IN TERMS OF VITAMIN A

The evidence obtained about the magnitude of the requirement in terms of vitamin A is as follows :

(1) In a therapeutic test on one adequately depleted subject, receiving a daily dose of 1,300 I.U. of a vitamin A concentrate, the deteriorated capacity for dark adaptation was restored slowly to normal. The lowered plasma value for vitamin A also was considerably raised, but not to the level between 100 and 120 I.U. per 100 ml. which is prevalent among the normal members of the population (p. 67). With large doses of vitamin A and an unrestricted diet it subsequently rose to this level.

(2) In prophylactic tests with a daily dose of 2,600 I.U. of vitamin A concentrate, the capacity for dark adaptation of two subjects was maintained for more than a year. The plasma values for vitamin A were maintained throughout, but, as in the above case, they reached levels between 100 and 120 I.U. per 100 ml. only when larger doses of vitamin A and an unrestricted diet were given. The genuineness of the protection in the prophylactic tests would have been better demonstrated if unequivocal signs of deficiency had been less rare in the depleted group. Evidence, though of a rather debatable kind, is however given elsewhere (p. 52) to show that slight signs of deficiency were shown at certain times by all the depleted subjects, but not by those treated prophylactically with vitamin A.

(3) Investigation of the liver reserves of the population of Great Britain in the years 1941-44 yielded a value of 500,000 I.U. for the whole liver, as possibly representative (p. 63). By the simplest method of calculation this should last, at the rate of 2,600 I.U. daily, for about 200 days and, at the rate of 1,300 I.U. daily, for about 400 days. In fact, no subject in the present experiment was markedly depleted in less than 400 days.

All the facts just marshalled are consistent with a value not greater than 1,300 I.U. daily for the minimum protective dose. Such a dose proved curative for a depleted subject and would therefore in all probability suffice for undepleted subjects. The dose given prophylactically was twice this

amount, no dose smaller than 2,600 I.U. having been tested, but the values for plasma vitamin A and capacity for dark adaptation of the two non-depleted subjects were nearly the same as those which were ultimately found with the dose of 1,300 I.U. in the subjects who had been depleted. None of the three subjects with these doses attained the plasma values for vitamin A of 100 to 120 I.U. per 100 ml., which are prevalent among the general population (p. 67), until after they had been given an unrestricted diet and larger doses of vitamin A ; but it is not known whether values as high as these are necessary for optimum health.

In assessing the minimum protective dose it is certainly relevant to consider the magnitude of the liver reserves in the population of Great Britain and to compare them with the time needed for depleting the reserves in the present experiment. When this is done, the daily expenditure, obtained by the rough expedient of dividing the former value by the latter, is nearer 1,300 than 2,600 I.U.

What evidence there is thus points to a value of about 1,300 I.U. daily as the minimum protective dose of vitamin A. With this figure as a basis it is necessary to try to fix the larger figure that shall cover the requirement of normal adults with their own inherent variability, enhanced by the variety of their activities and environment, and that shall ensure for them the margin of protection at which it is decided to aim. In the absence of adequate experimental evidence, which could be obtained only by intricate experiments on very large numbers of volunteers, it is difficult to decide whether doubling the dose would confer the maximum improvement in health or whether the dose should be increased fourfold, eightfold, or even more. To satisfy these ill-defined, additional needs and allow a margin of safety, it does not therefore seem too generous approximately to double the minimum protective dose and recommend a round figure of 2,500 I.U. vitamin A daily for the requirement of a normal human adult. Whether this amount is sufficient to allow the accumulation of liver reserves cannot be stated with certainty, but the very adequate liver reserves of the subjects of this experiment cannot have been built up in the past on daily intakes much larger than 2,500 I.U. vitamin A or an equivalent mixture of carotene and vitamin A.

REQUIREMENT IN TERMS OF β -CAROTENE

The evidence obtained about the magnitude of the requirement of β -carotene is as follows :

(1) In a therapeutic test on one adequately depleted subject a daily intake of 1,250 I.U. β -carotene in oil failed to halt the deterioration of the capacity for dark adaptation.

In therapeutic tests on the same, and another, adequately depleted subject a daily dose of 2,500 I.U. β -carotene in oil was followed by improvement as judged by all criteria.

Since about one-quarter of the carotene in oil was excreted unchanged, the maximum effective doses of carotene were probably about 950 and 1,900 I.U., respectively.

(2) In prophylactic tests with five subjects a daily intake of about 5,000 I.U. carotene from various sources appeared on the whole to prevent deterioration in the criteria of deficiency, but there were certain consistent differences according to the maximum effective dose of the

supplement. The capacity for dark adaptation and the plasma values for vitamin A were lower during the earlier stages of the experiment, especially during the cold season, when canned carrots with an average maximum effective dose of 1,250 I.U. formed the chief supplement. Subsequently, with maximum effective doses of more than 2,000 I.U., the capacity for dark adaptation and the plasma values for vitamin A rose and remained on a higher level irrespective of season.

Any estimate of the minimum protective dose of carotene must be based on the maximum effective dose and not on the intake. The facts presented above suggest that the minimum protective dose *in these terms* must be more than 950 or 1,250 I.U. daily and less than 1,900 I.U. daily. A maximum effective dose of 1,500 I.U. β -carotene daily is therefore proposed as the minimum protective dose and it is suggested that the human requirement be assessed on this basis.

The reasons for doubling the value of the minimum protective dose in order to arrive at the requirement have been set out in discussing the requirement in terms of vitamin A. For the same reasons a maximum effective dose of 3,000 I.U. is put forward as the daily requirement of β -carotene. To provide this the intake of β -carotene must vary according to its availability from the various foods.

REQUIREMENT OF CAROTENE IN RELATION TO ITS AVAILABILITY FROM DIFFERENT SOURCES

If an average value of 3,000 I.U. daily is accepted on the above basis as the desirable maximum effective dose for a normal adult, the following amounts of carotene represent the corresponding necessary intakes, that is to say the amounts which must be offered to the body, from the various sources. These amounts are calculated on the basis of the data set out in Table IX (p. 32).

	<i>Requirement I.U. daily</i>	<i>Divisor for calculating available potency in terms of vitamin A</i>
Carrots, boiled, sliced	12,000	4.0
Carrots, domestic purée	12,000	4.0
Carrots, homogenized	5,500	1.8
Cabbage, spinach	7,500	2.5
β -Carotene in fat	4,000	1.3

In the absence of data for the availability of carotene in other foods, it is suggested that the carotene requirement from other root vegetables should be calculated as for carrots, and from other leafy vegetables as for cabbage and spinach. Further data on the availability for human beings of carotene from various sources are much needed.

This assessment can in no way be regarded as final but it is perhaps the safest which can be made at present. There are certain circumstances which might tend to make the estimate somewhat too high. As already pointed out (p. 26) it is doubtful how much of the maximum effective dose is actually absorbed and how much is destroyed in the gut, and it is possible that the

proportion destroyed is larger with carotene in oil than with carotene from natural sources. The figure of 3,000 I.U. daily, adopted as desirable for the requirement in terms of the maximum effective dose, is mainly based on observations with carotene in oil, which was the only material used in the therapeutic tests. With carotene in oil the difference between the maximum and actual effective doses may be greater than with natural sources of carotene, as is suggested by a comparison of the plasma values for vitamin A with the respective maximum effective doses (p. 38 and Table IX, p. 32; *see also* Nylund and With (1942)). If this is so, the estimate of the requirements from natural sources would be too high, but the evidence is as yet too scanty to justify modification of the estimate.

In the Medical Research Council's War Memorandum on the Nutritive Values of Wartime Foods (Medical Research Council, 1945a), it is recommended that, for a whole diet, the total carotene value expressed in I.U. (1 I.U. being taken as equivalent to 0.6 μ g. β -carotene) should be divided by 3, after which the figure could be added to that for preformed vitamin A. The divisors obtained in the present experiment range from 1.3 to 4 for the individual foods tested, and thus support the use of a compromise value of 3, but it is suggested that individual divisors should be used whenever possible.

In the preliminary report of the present experiment (Medical Research Council: Vitamin A Sub-Committee and Associates, 1945) it was tentatively suggested that the values for the daily requirement should, with an allowance for safety, be set at 2,500 I.U. of vitamin A or 5,000 I.U. of β -carotene. The further analysis of the results, which has been made since then, makes it clear how undesirable it is to fix any single figure for carotene, the values for the daily requirement ranging for the few foods tested from 4,000 to 12,000 I.U. If there is any urgent reason for using a single figure for the requirement, the consistent course is to adopt 7,500 I.U. daily, thus making the value three times that for the requirement expressed in terms of vitamin A.

EFFECT OF TOCOPHEROL OR OTHER STABILIZERS ON THE CAROTENE REQUIREMENT

Lack of information about the tocopherol content of foodstuffs made it impossible to control the amount of tocopherol in the diet, and no attempt was made to regulate the content of anti-oxidants in the diet beyond the inclusion of hydroquinone in the β -carotene solution.

It was probable that during the first 10 months of the experiment, when the bread was made from National Wheatmeal, the tocopherol content was optimal.

AMOUNT OF β -CAROTENE EQUIVALENT TO VITAMIN A

For the rat, by established usage, one I.U. of β -carotene is equivalent to one I.U. of vitamin A, when both of these are given in oil. It has never been determined whether the relationship is the same for man.

Owing to the complexity of the conditions, and especially to lack of knowledge about the efficiency of absorption of very small doses of carotene by the rat, no very definite verdict can be pronounced from the present experiment. When allowance had been made for the fact that only about three-quarters of the β -carotene was absorbed from oil by man, it was roughly estimated that 1,500 I.U. of β -carotene and 1,300 I.U. of vitamin A were the equivalent minimum protective doses. If it is assumed that minimum doses of carotene are completely absorbed by the rat, then it would appear that the relationship for man may not differ greatly from that for the rat.

DISCUSSION OF THE LITERATURE

The Technical Commission on Nutrition of the Health Organization of the League of Nations (League of Nations, 1938) recommended 2,000 to 4,000 I.U. vitamin A daily as the amount for an adult. It is clearly laid down that this should be partly from animal and partly from vegetable sources.

Booher (1938) discussed the magnitude of the requirement on the basis of the relevant data in the literature and from studies of her own. She concluded that the physiological minimum daily requirement of vitamin A for a man weighing 70 kg. would appear to be about 1,400 to 2,000 U.S.P. units*. To provide a margin of safety and to allow for part of the supply to be in the form of carotene, she suggested that the intake of a normal adult should be around 3,000 U.S.P. units, of which "a large proportion" should be vitamin A rather than β -carotene.

In Booher's own studies on five young adults, with the capacity for dark adaptation as criterion of depletion, the daily doses which restored and maintained this criterion were for a 70 kg. man 1,750 to 3,850 U.S.P. units of vitamin A as cod liver oil or 3,010 to 7,201 U.S.P. units of carotene in oil. (Booher, Callison, and Hewston, 1939). Similar studies with five other young adults, two dosed with cooked peas and three with cooked spinach, showed that the amount of carotene which had to be offered daily to restore and maintain the dark adaptation was, for the various individuals in terms of a 70 kg. man, 3,290 and 3,990 U.S.P. units from peas, and 5,390, 6,090, and 7,070 U.S.P. units from spinach (Booher and Callison, 1939)†.

These results are not in disagreement with those of the present experiment in spite of the fact that Booher's studies differ in the very much shorter times, mostly less than 40 days, needed for depletion.

In another study, previously mentioned in this Report, that of Wagner (1940) in Germany, depletion by a variety of criteria was brought about in a comparatively short time also. The values at which Wagner arrived for the requirement were 2,500 I.U. as vitamin A or 5,000 I.U. as β -carotene. The vitamin A concentrate used in this experiment was the German commercial preparation, "Vogan"; it is now generally accepted that "Vogan" was over-valued, owing to the adoption of too high a conversion factor (Grab and Moll, 1939), but it has been ascertained recently (personal communication, Wagner, 1947; Scheunert, 1947) that the "Vogan" used in Wagner's experiment was retested in Scheunert's laboratory and that the correct figure was used in this experiment.

Nylund and With (1942), on the basis of the above data and of studies of their own, estimated the optimum requirement for a man of 70 kg. at 1,750 to 2,800 I.U. as vitamin A, and at about one-and-a-half times to twice this amount as carotene in oil.

* When the U.S.P. Reference Cod Liver Oil was first adopted as a subsidiary International Standard in 1935, it was accepted that the international unit defined in terms of β -carotene and the U.S.P. unit defined in terms of the Reference Cod Liver Oil were identical in value. Subsequently this was found not to be so and a collaborative test by the Vitamin A Sub-Committee of the Medical Research Council's Accessory Food Factors Committee reported that the U.S.P. unit was equivalent to 0.873 I.U. (Hume, 1939).

† In a continuation of the same studies, Callison and Orent-Keiles (1947) found that the daily dose of carotene, estimated chemically as α - and β -carotene, from raw and cooked carrots that would restore deteriorated capacity for dark adaptation in their 4 partially depleted subjects ranged from 4,450 to 8,110 I.U. (85 to 115 I.U. per kg. bodyweight). No studies of faecal excretion were made.

There does not seem to be any obvious explanation of the comparatively short period of depletion needed to produce deterioration in the capacity for dark adaptation, which is reported by Booher, and also by other workers whose results are not otherwise relevant. Differences in the liver reserves do not seem to afford a satisfactory explanation, unless it is assumed that the experimental subjects in the U.S.A. were not representative of the general population but selected as likely to be deficient in vitamin A. In the experiment of Wagner, however, smallness of liver reserves is the probable explanation; his subjects were under detention in an institute where they had been receiving a diet nearly devoid of vitamin A for periods of up to two years before the beginning of the experiment (Wagner, personal communication, 1947).

Finally, it remains to compare the values for the requirement obtained in the present experiment with those laid down by the Committee on Food and Nutrition of the National Research Council, which have been widely accepted. In the National Research Council's recommended dietary allowances the amount of vitamin A allotted to a male or female adult is 5,000 I.U. In the original version of the schedule (National Research Council: Committee on Food and Nutrition, 1941) a footnote was added stating "Requirements may be less if provided as vitamin A, greater if provided chiefly as the pro-vitamin carotene". In the most recent revision (National Research Council: Committee on Food and Nutrition, 1945) the footnote is changed and explains that the allowance depends on the relative amounts of vitamin A and carotene, and that it is based on the premise that "approximately two-thirds of the vitamin A value of the average diet is contributed by carotene and that carotene has half, or less than half, the value of vitamin A".

This is a less precise method of stating the requirement than to express it all as β -carotene or all as vitamin A, but the values of 2,500 I.U. all as vitamin A, or 7,500 I.U. all as carotene, recommended in this Report can be brought roughly within the National Research Council's latest estimate if the value attributed to carotene is "less than half." that for vitamin A.

II. ELABORATION OF SPECIAL ASPECTS

F.—Measurements with the Adaptometer of Craik

The adaptometer of Craik, like that of Wald, is essentially built on the basis of the specifications worked out by Hecht. The main differences are in the presentation of the test light and in the fixation of the retina. The test light is an intermittent flash in Wald's, and a continuous light in Craik's, apparatus. The part of the retina tested by Wald's method is rigidly fixed, but with Craik's method a larger area of the retina is tested in order to avoid local fatigue. In the Craik apparatus a continuous record is obtained, and the adjustment of the brightness is made by the subject and not by the investigator.

The technique is fully described on p. 110. The results are recorded in Tables XLIV (p. 112) and XLV (p. 113).

FINAL ROD THRESHOLD

It should be understood that the units in which the brightness of the test light is expressed are log. equivalent foot candles for the Craik apparatus, whilst log. μm lamberts are used for the Wald adaptometer. Since one equivalent foot candle equals $1.076 \times 10^6 \mu\text{m}$ lamberts, the logarithms of the two systems differ by about 6 units. In actual fact the difference between the normal range as measured by the two adaptometers is about 7 log. units, the figures for the Wald apparatus being of the order of +2, those of the Craik adaptometer of the order of -5. The different presentation of the test light, and other factors, account for this. Owing to the opposite signs of the log. units, a deterioration of rod vision is indicated by a rise of the absolute figure in the Wald method and by a lowering of the absolute figure in the Craik method.

With Craik's method, the range in threshold values shown by the subjects receiving supplements was between -5.3 and -6.5 log. units, that shown by the deprived subjects throughout the period of depletion was between -4.5 and -6.4. The variations for most individuals were considerable, up to 1.05 log. units (Bartley) and with one exception (Golding) bore no definite relation to the state of deprivation. Golding's threshold values deteriorated from initial values of -5.65 and -6.0 to -5.2 and -5.3 towards the end of the period of depletion, from August to October, 1943. In Watson and Proctor there is perhaps a suggestion of a deteriorated threshold before dosing but in view of the smallness of the change and the small number of measurements this cannot be regarded as definite. There was no general "seasonal" change during the spring of 1943 of the kind found with Wald's apparatus.

CONE-ROD TRANSITION TIME

The data fully confirmed those obtained with the Wald adaptometer. Abnormally long times were observed in Golding, Proctor, and Watson: *i.e.*, the same subjects who showed abnormalities with the Wald instrument.

G.—Comparison of Wald's and Craik's Adaptometers

It might be expected that agreement on the length of the cone-rod transition time would be good whichever instrument was used, as long as the "bleaching" lights were of the same order and the apparatus was suitable

for recording the dark adaptation curve (Hecht, 1937 ; Hecht, Haigh, and Chase, 1937). On the other hand it is not unexpected that the effects of vitamin A depletion on the final rod threshold should differ according to the procedure employed. It is striking that, in all three cases where the Wald adaptometer recorded changes after depletion, the Craik apparatus registered a much smaller change, if any. This is particularly obvious with Proctor ; as measured by the Wald adaptometer the change was from an initial average of 1.79 log. units to an average of 3.12 log. units at the height of depletion, while the change in individual values recorded by the Craik adaptometer was from -5.9 to -5.4 log. units, a change of doubtful significance. It would thus seem that the Wald method is more sensitive in detecting the effect of vitamin A depletion on the final rod threshold. This may be connected with the different presentation of the test light or, more probably, with the differences in the fixation of the retina. It may be a disadvantage of Craik's method, from the point of view of detecting early stages of failing function, that the retina is not rigidly fixed. Observations by Mandelbaum (1941) and the results of rod scotometry (p. 54) indicate that deterioration of night vision is not uniform throughout the field and that, if the retina is not fixed, the instrument will record the threshold of the most efficient part of the retina and will overlook defects limited to certain areas.

If different instruments agree on the values for the cone-rod transition time, but differ in their sensitivity towards changes of the final rod threshold, the sequence of the changes as recorded by adaptometers will obviously vary with the type of instrument used. With Craik's apparatus the cone-rod transition time changed first in all cases ; with Wald's apparatus the cone-rod transition time changed first in Watson, but almost simultaneously with the rod threshold in Golding and Proctor. The importance of the procedure in influencing the sequence of events is of special interest with reference to the findings of Hecht and Mandelbaum already quoted. It remains to be explored whether the findings of these authors, which apparently conflict with those of the present experiment, can be explained through differences in technique.

H.—Time Required for Maximum Dark Adaptation

In normal persons who have been exposed to a bleaching light, as used in the Wald adaptometer, the rod threshold reaches a constant or "final" value after 20 to 30 minutes. This has been noted by many observers, and is confirmed in the present investigations. The following data, however, show that this does not apply to vitamin A deficient persons. Three subjects—Proctor showing definite signs of defective dark adaptation after 18 months of vitamin A free diet ; Garling showing slight and doubtful signs of defective dark adaptation (Protocol C) after 18 months of vitamin A free diet ; and Drabble showing normal dark adaptation, having received a vitamin A supplement of 2,500 I.U. daily for 14 months—were kept in a dark room continuously for 44 hours and their rod threshold was measured periodically. They lived and slept during this period in a completely darkened room. A covered electric radiator supplied heating, an electric fan ventilation, and a wireless set entertainment. The experiment began at 3 p.m. on 24th January, 1944, and was continued until 11 a.m. on

26th January. The results of the measurements with the Wald adaptometer are shown in Table XIII.

TABLE XIII

Behaviour of the Rod Threshold after Varying Periods up to 44 Hours in the Dark.

Time in the dark (hours) (minutes)		Rod threshold (log. μm lamberts)		
		Drabble	Garling	Proctor
0	30	1.58	1.88	3.36
0	45	1.52	1.80	3.40
1	—	1.52	1.62	3.12
1	30	1.52	1.55	3.03
3	—	1.70	1.62	2.85
4	50	1.52	1.66	2.78
8	—	1.62	1.70	2.63
12	—	1.48	1.48	2.63
16	—	1.59	1.52	2.42
20	—	1.55	1.48	2.70
24	—	1.59	1.62	2.63
28	—	1.52	1.77	2.67
32	—	1.52	1.55	2.38
36	—	1.48	1.57	2.38
40	—	1.52	1.55	2.34
44	—	1.66	1.62	2.56

The normal subject, Drabble, reached an almost constant level within 30 minutes, with subsequent slight fluctuations around this value. The second subject, Garling, probably slightly deficient, reached the minimum range after 60 to 90 minutes; his final threshold was of the same order as that of Drabble, but it was attained more slowly. The definitely deficient subject, Proctor, improved steadily over a period of 8 hours. He showed a small further improvement after 16 hours, but this was not consistently maintained and his final threshold was still considerably above the normal range. The differences in the speed of dark adaptation are further illustrated by the following comparison:

After 30 minutes Drabble's threshold differed from his best by 0.10 log. units.

After 30 minutes Garling's threshold differed from his best by 0.40 log. units.

After 30 minutes Proctor's threshold differed from his best by 1.02 log. units.

A number of further measurements with the same and other subjects are recorded in Table XIV, which shows the rod threshold after adaptation periods of 20 minutes and $7\frac{1}{2}$ to $9\frac{3}{4}$ hours (overnight). These confirmed the results in Table XIII.

The experiment recorded in Table XIII was repeated with Garling and Proctor on 11th September, 1944, after the depleted stores of vitamin A had been replenished by oral administration of about one million I.U. It will be seen from Table XV that the dark adaptation time of both men had by then returned to the normal range.

These observations indicate that the time required for reaching the true final threshold, "the dark adaptation time", may show considerable

variation according to the state of vitamin A nutrition. Whilst in normal subjects the value after 30 minutes represents very nearly the true final rod threshold, this is not the case in vitamin A deficient persons.

TABLE XIV

Comparison of the Rod Threshold after 20 Minutes, and Overnight (after 7½ to 9½ Hours) in the Dark with no Preliminary Bleaching

Name	Date	Rod threshold (log. μm lamberts)		Remarks
		After 20 minutes	Overnight	
Golding ..	25.9.43	2.95	2.13	After 14 months on deficient diet. After treatment with about 1,250 I.U. vitamin A daily for 6 months.
	13.4.44	1.76	1.48	
Proctor ..	8.1.44	3.19	2.13	After 17 months on deficient diet. After one month with 1,250 I.U. caro- tene daily, then 5 months with 2,500 I.U. carotene daily.
	17.4.44	3.21	2.34	
Watson ..	18.4.44	2.44	1.44	After 23 months on deficient diet.
Garling ..	19.5.44	2.06	1.52	After 22 months on deficient diet.
Drabble ..	15.1.44	1.59	1.34	After 14 months on deficient diet supplemented with 2,500 I.U. vitamin A daily.
Drake ..	10.4.44	1.55	1.34	After 12 months on deficient diet supplemented with about 5,000 I.U. carotene daily.

TABLE XV

Time Required for Maximum Dark Adaptation: Rod Threshold after Prolonged Exposure to Darkness of Two of the Same Subjects as in Table XIII, after Treatment with Vitamin A

Time in the dark (minutes)	Rod threshold (log. μm lamberts)	
	Garling	Proctor
0	3.83	3.88
15	1.96	2.05
30	1.70	1.84
45	1.51	1.67
60	1.58	1.70
75	1.61	1.64
90	1.61	1.64
105	1.58	1.61
120	1.58	1.61
135	—	1.54
150	—	1.54
165	—	1.58

Professor J. R. Marrack has drawn attention to a passage in a paper by de Gouvea (1883) bearing on these observations. The paper deals with nutritional night blindness among workers on agricultural estates in Brazil.

The author states that the retina of persons suffering from night blindness may partially recover overnight.

“Auf unseren Fazenden stehen die Arbeiter sehr früh auf und gehen zu ihrer Arbeit vor dem Erscheinen der Sonne, wenn die Helligkeit noch viel geringer ist als Abends nach Sonnenuntergang, und doch beschwert sich kein Hemeralop, dass er um diese Zeit schlecht sehe, die Fälle ausgenommen, wo schon eine bedeutende Amblyopie besteht. Die Ruhe während einer einzigen Nacht ist hinreichend, um die Sensibilität der Retina wiederherzustellen”*.

Though the lengthening of the dark adaptation time may be of considerable theoretical interest, its bearing on the problem of diagnosing defective night vision remains to be investigated. For distinguishing between deficient and non-deficient persons, the measurement of the value, say after 30 minutes, is obviously more suitable than the true final rod threshold. Differences that are pronounced at 30 minutes largely disappear as the time allowed for dark adaptation lengthens.

I.—“Seasonal” Changes in the Final Rod Threshold

The values for the final rod threshold as measured with the Wald apparatus exhibit a peculiar phenomenon which, if it cannot be satisfactorily explained, can equally well not be ignored. If all the values for the subjects undergoing depletion are averaged month by month, the corresponding graph shows a gradual deterioration of the threshold from November, 1942 to January, 1943, the depletion having begun in August, 1942. This deterioration of the threshold is shown in the monthly averages of all the subjects undergoing depletion, though some show the peak in January and others in February; the difference in the values for these two months is, however, small. After February the average threshold improves gradually through March, April, and May and then very suddenly in June. Not all of the subjects continued their depletion after June, 1943; a graph has therefore been constructed of the average values for the seven subjects who were undergoing depletion continuously from August, 1942 to January, 1944 (Fig. 7). From this it is apparent that the threshold remained low through July and August, 1943 and then gradually rose again until January, 1944, after which the number of available subjects became too small for comparison. The whole amount of the deterioration in mid-winter was not great, only about 0.2 log. unit, and for a single individual would be disregarded, but it is shown by every individual and certainly the graph of their average readings suggests a rise to a peak in January, 1942 and then a fairly steady fall, each of these movements being spread over some months.

The existence of the two trends and the fact that the peak, though occurring in January or February for all the individuals, occurred for some in January and for others in February, makes it improbable that these changes in the threshold were due to undetected changes in the strength of the test light.

The bulb of the test light was replaced from time to time by a newly calibrated one, and the relation of the changes in the threshold to changes of

* On our plantations the labourers rise early and start work before sunrise, when the light is much dimmer than in the evening after sunset, and yet none of the hemeralopes complains that he cannot see well in the morning, unless a considerable degree of amblyopia is already present. A single night's rest is enough to restore the sensitivity of the retina.

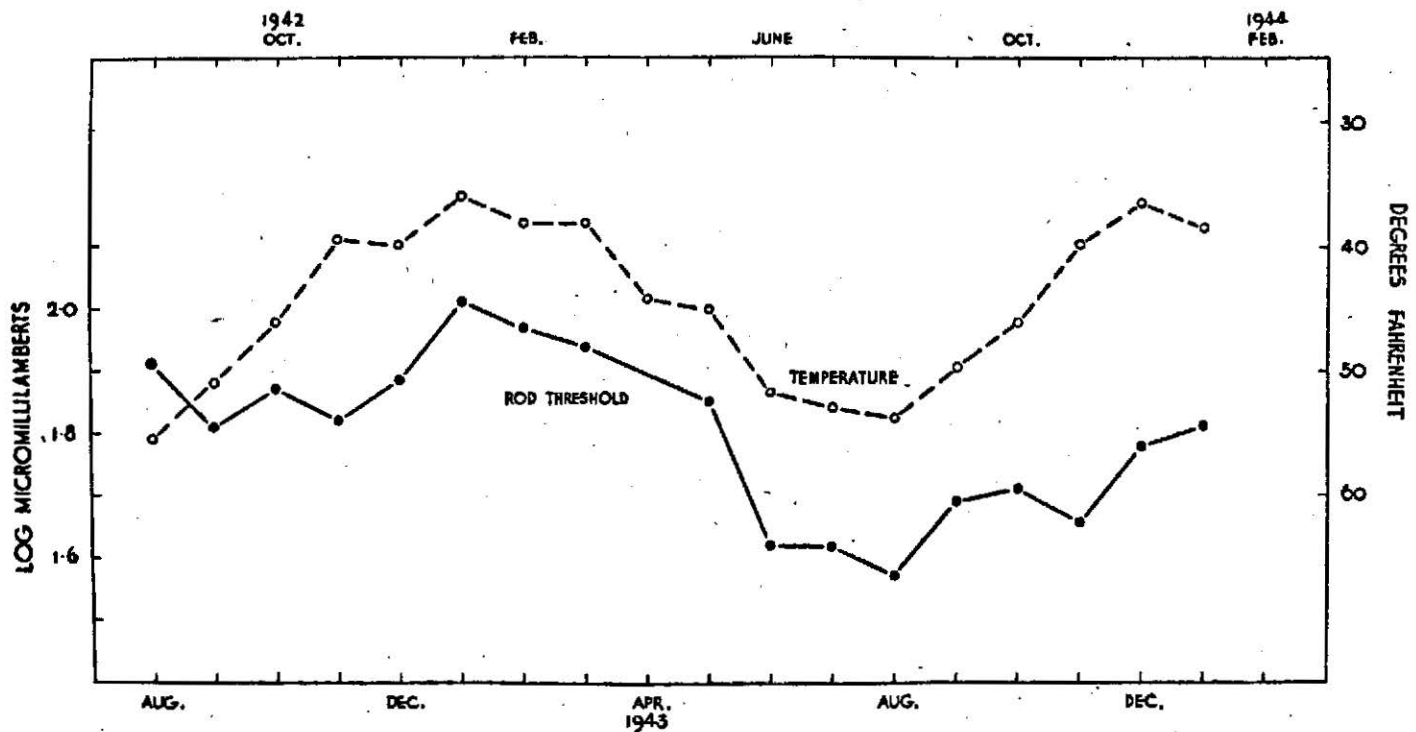


FIG. 7.—Monthly averages of values for the final rod threshold, measured with the Wald adaptometer, for seven deprived subjects (Bloomfield, Garling, Thompson, Watson, Williams, Wodeman, Woodhouse) and monthly averages of daily minimum temperature.

the bulb was examined statistically over the period of the peak and decline ; they were found to be unrelated, but the marked and sudden improvement shown by most subjects in June, 1943 may have been connected with faulty standardization. From this time onwards the threshold values were always at a lower level but they again showed the same trend upwards to January, 1944, as had been shown to January, 1943.

It is important in connection with this "seasonal" phenomenon to consider the behaviour of the non-deprived subjects, and of any normal individuals on whom such a series of observations of the rod threshold have been made. Unfortunately, the data for the non-deprived subjects are not sufficient for the construction of an average curve and the only possibility is to consider each person separately. W. Bartley was the only one of them who continued the experiment over both the winters, 1942-43 and 1943-44. He showed deterioration of the rod threshold in the first winter when he was receiving a very small supplement of carotene, but showed none in the second winter when the dose was larger. Broderick took part in the experiment in 1942-43 when the carotene dose was small, and showed deterioration ; in the winter of 1943-44, he was not receiving any supplement, and then also he showed deterioration. Drake took part only in the second winter when the dose of carotene was larger, and showed no deterioration. Drabble and Tridgell who received vitamin A, not carotene, in a dose which was considered to be adequate, took part over the whole only of the second winter. Drabble showed no deterioration. Tridgell's final rod threshold was always poor ; it deteriorated in the winter of 1943-44, but never recovered subsequently, either in the summer or with normal diet and large doses of vitamin A, so that it cannot be used in evidence.

A group of 20 persons having a normal intake of vitamin A, on whom serial readings of the rod threshold were taken over more than one year, was observed in another human experiment which followed the present one. In it the subjects were deprived of vitamin C, but received more than 5,000 I.U. preformed vitamin A daily. These subjects, of whom some, including Tridgell, were the same as in the present experiment, did not show the rise in the final rod threshold during the winter, 1944-45 (Table XVI).

The phenomenon was not shown by the readings with the Craik adaptometer but it has been shown that this instrument is less sensitive to minor degrees of deterioration of the rod threshold (p. 47).

In attempting to explain the phenomenon it is necessary to examine its relation to diet, to other criteria of depletion, and to weather.

Diet.—The phenomenon was shown by all those undergoing depletion of vitamin A but not by the subjects of the vitamin C experiment who were receiving a diet definitely sufficient in vitamin A. As to the behaviour of the subjects who were receiving the deficient diet supplemented, those whose maximum effective dose of carotene was low at the critical time (Bartley and Broderick) showed the phenomenon in 1942-43. In 1943-44, when the maximum effective dose was considerably higher, Drake and Bartley did not show the phenomenon, nor did Drabble who was receiving 2,500 I.U. vitamin A daily.

It might be thought that the summer improvement was caused by slight changes in the vitamin A potency of the diet due to the inclusion, from the permitted list (Table XXX, p. 77), of rather more vegetables with a small

TABLE XVI

Monthly Values for the Final Rod Threshold (in log. μm lamberts) Determined with the Wald Apparatus on 20 Volunteers Taking Part in a Subsequent Experiment at Sheffield in which they were Deprived of Vitamin C but given a Diet Containing Vitamin A and Carotene and with a Daily Supplement of 4,500 I.U. Vitamin A Concentrate

Name	October, 1944	November	December	January, 1945	February	March	April	May	July	August	September	December	January, 1946
Bartley ..	—	1.35; 1.60	—	1.45	1.45	1.48	1.45	1.45	1.50	1.72	1.65	1.40	1.47
Drake ..	—	—	—	1.68	1.73	1.70	1.78	1.72	—	1.89	1.82	1.76	1.80
Garling ..	—	—	—	1.90	2.15	2.00	2.02	2.04	1.96	2.10	2.05	1.95	1.95
Golding ..	—	—	—	—	1.93	1.85	1.90	1.80	1.81	—	1.90	2.00	1.95
Hill ..	—	1.70; 1.80	1.89	1.82	2.00	2.17	2.10	2.20	2.36	—	—	2.00	2.22
Hudson ..	—	1.85; 1.85; 1.86	—	1.82	1.83	1.85	1.93	1.87	1.94	2.05	1.90	1.85	1.89
Jackson ..	—	1.98	—	1.87; 1.85	1.85	1.95	1.82	1.82	1.95	1.85	1.88	1.70	1.41
Milburn ..	—	1.53; 1.45; 1.49	—	1.47	1.50	1.58	1.65	1.45	1.50	—	—	1.60	—
Parry ..	—	—	1.83	1.82; 1.85	1.90	1.98	1.88	2.00	2.00	2.02	—	—	—
Proctor ..	—	—	—	2.09	1.95	2.05	2.07	1.96	2.26	2.20	2.15	2.15	2.35
Robinson ..	1.87	—	1.93	1.98	1.75	1.70	1.85	1.77	1.74	1.98	1.85	1.90	1.80
Sanderson ..	—	1.53; 1.55; 1.53	—	1.61	1.60	1.50	1.54	1.75	1.55	1.72	1.75	1.60	1.69
Tridgell ..	2.20; 2.10	2.30	—	2.29	2.15	2.14	2.15	2.25	2.19	2.20	2.20	2.10	2.25
Way ..	—	—	2.19; 2.12	2.10; 2.16	2.05	1.85	1.90	2.10	1.91	2.25	2.18	2.10	1.97
Whinfield ..	—	1.87	1.84	1.87; 1.85	1.85	1.74	1.80	1.75	2.12	1.80	1.85	1.90	1.95
Williams ..	—	—	—	1.54	1.50	1.63	1.24	—	—	—	—	—	—
Wodeman ..	—	—	—	1.71	1.75	2.00	1.91	1.75	2.18	1.90	2.00	1.70	1.80
Woodhouse ..	—	—	—	—	1.83	1.70	1.85	1.77	1.80	1.90	1.98	1.75	1.85
"X" ..	1.50	1.60; 1.44	—	1.58	1.65	1.40	1.53	1.53	1.60	1.60	1.60	—	—
Mrs. "Y" ..	—	1.84; 1.75	—	1.56	1.45	1.70	1.65	1.65	1.74	—	—	—	—
<i>Average*</i>	1.84	1.76	1.93	1.79	1.79	1.80	1.80	1.82	1.90	1.95	1.92	1.84	1.89

* In calculating the group average one figure only is used for each subject, the average of the results being taken in any month in which more than one determination was made on the same subject.

carotene contribution. This, however, seems improbable, since deterioration of the threshold was not accelerated when the vegetables were taken away completely from some of the subjects for 4 months in the summer.

Other criteria.—The only other criteria which are available for comparison with the behaviour of the rod threshold values are the plasma values for vitamin A and carotenoids. These did not show any trends corresponding with those of the rod threshold values.

Weather.—Meteorological data for the hours of sunshine, and the maximum and minimum temperatures, at Sheffield have been examined and it will be seen from Fig. 7 that the curve of the final rod threshold values runs parallel with that of the average monthly minimum temperature. The two curves are certainly similar, though it is difficult to see any causal connection between the deterioration of the rod threshold and exposure to wintry conditions. Chevallier (1945) has reported verbally that he found raised final rod thresholds in a group of soldiers re-examined during a period of exposure to intense cold but the soldiers were at the time also much fatigued.

Discussion

The period of observation was too short to decide whether or not there was any causal link between the changes of the average monthly minimum temperature and those of the final rod threshold. If a causal link is assumed, the interpretation is tentatively offered that, in human subjects receiving a sub-optimal amount of vitamin A, wintry conditions cause a slight rise in the final rod threshold which is abolished in the spring without any increase in the intake of vitamin A. Whatever the cause, "seasonal" changes of this kind must be borne in mind as a possibly disturbing factor in depletion experiments, in therapeutic trials, and in comparative assessments of nutritional states at different times of the year.

J.—Rod Scotometry

During the 13th month of the experiment P. C. Livingston (1944) applied his new technique of "rod scotometry" to the examination of thirteen of the volunteers, including two of those receiving protective supplements (Bartley, Drake, Bloomfield, Brown, Garling, Golding, Hicken, Proctor, Watson, Whitehead, A., Whitehead, D., Williams, and Wodeman). Considerably reduced fields of vision were found in five (Golding, Hicken, Whitehead, D., Williams, and Wodeman) of the subjects on the unsupplemented deficient diet. This was at a stage of the experiment when other methods of measuring dark adaptation showed defective vision in Golding only.

PROCEDURE

The test consists of plotting the field of vision on a modified Bjerrum screen under conditions of dark adaptation, the test objects being targets of known luminosity. Unfortunately, suitable test objects were not continuously available during the experiment owing to the difficulty of controlling and measuring the luminosity of the targets. However, some observations made with this method, though they must be regarded as provisional, are worth recording because they support the results obtained with Wald's and Craik's adaptometers, and suggest that rod scotometry may become a very useful means of discovering early stages of defective dark adaptation.

The targets used for the following measurements were at first dots of self-luminous paint, and later an electrically-lit area of opal glass. The diameter of the target was about 2 mm., the luminosity about 6×10^{-9} candle power.

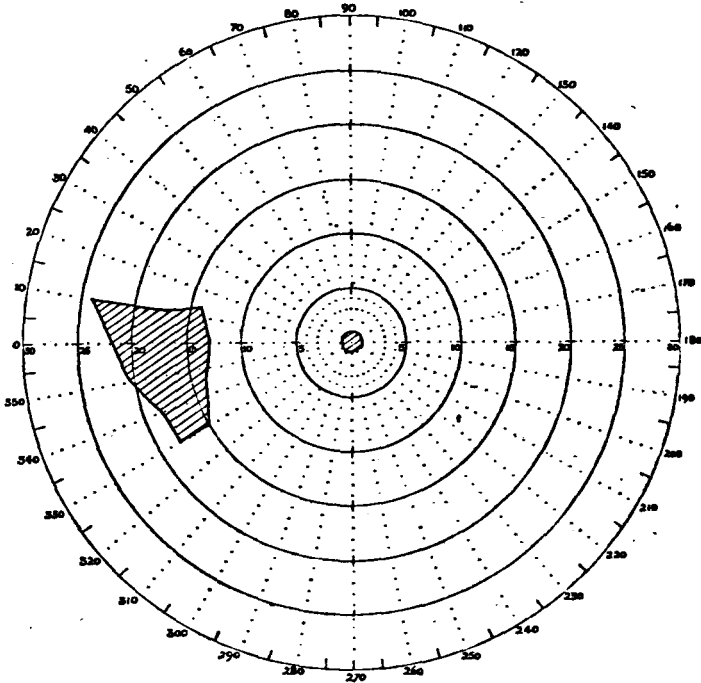


FIG. 8.—Rod scotometry : normal type of chart, showing small central scotoma and larger scotoma round the blind spot (Drake, 3.1.44).

The field of vision, as plotted on the Bjerrum screen (p. 114), was copied on to a Traquair scotometer chart. Examples of two charts, one from a non-deprived subject (Drake), the other from a deprived subject (Proctor), are shown in Figs. 8 and 9: It is obviously impracticable to reproduce all the relevant charts in this Report and, in order to simplify the presentation of the results, the total blind area on the chart was measured with a planimeter and the figure thus obtained was recorded. This proved useful for comparing results, although the figure did not take into account the shape of the blind areas and therefore did not fully convey the findings.

The units in which the area is expressed are of course arbitrary, since they depend on the size of the chart used which in this investigation was the standard Traquair scotoma chart of Weiss and Son, 287, Oxford Street, London. Owing to the limitations of the method, the figures cannot be regarded as representing a mathematically accurate expression of the field of vision. Only major differences, therefore, are likely to be of importance.

The total area of the chart, representing a zone of 30 deg. from the fovea, was 249 cm². In persons with normal night vision the total "blind" area was usually between 5 and 25cm². The "normal" blind zones consist of two, or in some cases three, different fields: the central scotoma,

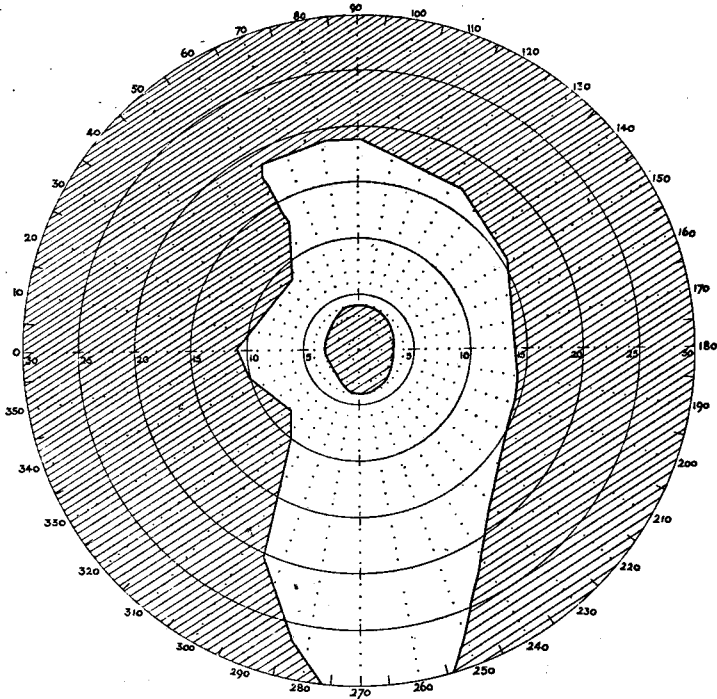


FIG. 9.—Rod scotometry : abnormal chart, showing field of vision reduced as a result of vitamin A deprivation, with central scotoma enlarged, and peripheral scotoma merging with blind spot (Proctor, 1.9.43).

the “blind spot” and, in some cases, a “peripheral contraction” usually between 25 deg. and 30 deg.

The results are recorded in Protocol C.

EFFECTS OF THE DEFICIENT DIET

Some subjects gave steady and consistent results, but others showed considerable variations on repetition of the test. In order to simplify the interpretation, the measurements taken during August and September, 1943, were averaged for each volunteer (see Table XVII). It will be seen that the mean blind areas of those whose diet was supplemented by vitamin A or carotene were between 10 and 28 cm²., but that the thirteen deprived subjects showed blind areas averaging between 42 and 249 cm². In some the fields were found to be consistently enlarged (e.g., Golding), in others the size varied. Since, at this time, the dark adaptation curve showed abnormalities in only two men (Golding and Proctor) it would appear that rod scotometry can reveal differences in night vision which other methods fail to show.*

Throughout the following year the blind area never exceeded 100 cm². in any of the non-deprived subjects, and was seldom greater than 50 cm². In contrast, every one of the deprived group gave at one time or another a field

* Sloan (1947), in a paper which appeared while this Report was being prepared, reported having examined the whole of the visual field with a Ferree-Rand perimeter, and concluded that “concentric contraction of the visual field occurs frequently in patients with ocular vitamin A deficiency”.

TABLE XVII

Rod Scotometry: Average Size of the Blind Area on the Traquair Chart during August and September, 1943 for 5 Non-deprived and 13 Deprived Volunteers

Non-deprived subjects		Subjects deprived of vitamin A for about 13 months			
Name	Blind area (cm ² .)	Name	Blind area (cm ² .)	Name	Blind area (cm ² .)
Bartley, W.	28	Bloomfield ..	89	Watson ..	46
Drake ..	13	Brown ..	120	Whitehead, A.	77
Broderick ..	10	Garling ..	103	Whitehead, D.	118
Drabble ..	11	Golding ..	249	Williams ..	134
Tridgell ..	23	Hicken ..	55	Wodeman ..	42
		Proctor ..	102	Woodhouse	58
		Thompson ..	44		

greater than 100 cm². In four (Golding, Proctor, Brown, and Whitehead) the enlargement was consistent until dosing began. In the remaining nine the size of the blind area varied. It was possible to continue measurements on five of these nine men for one year, in which their diet had a vitamin A potency of about 8,000 I.U. daily (Protocol C contains data extending from October, 1944 to September, 1945), and, with one exception, Garling, no appreciable variations occurred during this period. This suggests that the variability during the period of deprivation was not due to imperfection of the technique, but was rather an early sign of failing rod vision. This is borne out by the behaviour of the values for Watson's final rod threshold during the height of deprivation, when they showed much greater variability than at the beginning or after dosing (Protocol B).

EFFECTS OF DOSING

Striking effects of dosing were seen in Golding and Proctor (Table XVIII and Protocol C). The improvement in the field of vision approximately reflected the fall of the final rod threshold (Tables V and VI, pp. 25 and 26). Definite effects were observed also with Brown and D. Whitehead (Protocol C).

TABLE XVIII

Rod Scotometry: Effects of Dosing with Vitamin A or Carotene on the Size of the Blind Area on the Traquair Chart in 2 Depleted Subjects

Name	Time of measurement	Blind area (cm ² .)
Golding ..	Before dosing.. .. .	{ 249
	After one months' dosing with c. 1,300 I.U. vitamin A daily .. .	249
	After two months' dosing with c. 1,300 I.U. vitamin A daily .. .	152
	After six months' dosing with c. 1,300 I.U. vitamin A daily .. .	17
	After six months' dosing with c. 1,300 I.U. vitamin A daily .. .	40
Proctor ..	Before dosing.. .. .	{ 187
	After one month's dosing with c. 1,250 I.U. β-carotene daily .. .	112
	After one month's dosing with c. 2,600 I.U. β-carotene daily .. .	147
	After four months' dosing with c. 2,600 I.U. β-carotene daily .. .	113
	After four months' dosing with c. 2,600 I.U. β-carotene daily .. .	36
	After six months' dosing with c. 2,600 I.U. β-carotene daily .. .	8

who had no signs of deficiency by any other criterion. Several others (Watson, Williams, Wodeman, and Woodhouse), as already mentioned, showed less fluctuation after dosing than before.

CONCLUSION

Two subjects, Golding and Proctor, whose final rod threshold was definitely raised, showed also grossly abnormal fields of vision. The third subject, Watson, whose final rod threshold was raised, did not give consistently abnormal fields of vision. In others, notably Brown and D. Whitehead, abnormal fields of vision seemed to be the only feature associated with the vitamin A deficiency. These observations suggest that rod scotometry may detect slighter defects in night vision than are recorded by adaptometers. Further work will be required to assess the diagnostic value of this method.

Rod scotometry takes time and needs competent co-operation from the subject. There were no difficulties in obtaining this from the subjects of this experiment, but improvement of the field of vision was found to occur on repetition of the test in some of a random sample of hospital out-patients. In view of such "learning" effects, care is obviously necessary in interpreting the results obtained from unskilled subjects.

K.—Effect of Consumption of Alcohol on Capacity for Dark Adaptation and Plasma Values for Vitamin A

Clausen *et al.* (1940) reported that 60 ml. ethanol in 20 per cent. solution, given by stomach tube to dogs weighing between 17 and 24 kg., caused a rise in the blood value for vitamin A in all cases. In three dogs a biopsy of the liver was performed and "There seemed to be a positive correlation between the increase of vitamin A in the serum after the administration of the alcohol and the concentration of vitamin A originally present in the liver". The authors continue "It is hoped that this correlation may lead to the development of methods for the study of vitamin A reserves in the body".

Later, Clausen *et al.* (1941) reported that drinking alcohol to the extent of "the usual 'social' evening drinking common in this country" (U.S.A.) raised the vitamin A content of the blood of human beings. Pett (1939 ; 1940), using his own apparatus for measuring dark adaptation, found abnormally rapid recovery times after bleaching on the day after alcohol had been taken. Later investigators reported varying results. Yudkin (1941a) observed transient improvements of the final rod threshold after a dose of 20 ml. alcohol, but no rise in the vitamin A value. Mandelbaum (1941) found that a quantity of whisky equivalent to 180 ml. alcohol lowered the capacity for dark adaptation by shifting the whole adaptation curve upwards to a higher level by 0.3 log. units. Brenner and Roberts (1943) gave alcohol to one normally fed person, and to three female and two male subjects who had received a vitamin A deficient diet for 31 and 20 weeks, respectively. They found that consumption of 50 ml. had no definite effect on the vitamin A content of the blood, but conclude that "75 to 100 c.c. caused a noticeable rise 4 hours after consumption. After 12 hours the vitamin A in the blood returned to its pre-alcoholic level in most instances".

TABLE XIX

Effect of Alcohol Consumption on Level of Final Rod Threshold

Name	Date of test	Amount of alcohol taken	Period in dark room (minutes)	Rod threshold (log. μm lamberts)	
				Individual values	Average values
Proctor ..	7.1.44	Before alcohol Alcohol, 20 ml. and water, 80 ml., taken at 35 minutes	20	3.21	} 3.23
			30	3.22	
			40	3.26	
			50	3.03	
			60	3.39	
			70	3.06	} 2.92
			80	3.03	
			90	3.06	
			100	2.81	
			110	2.88	
			Proctor ..	8.1.44	No alcohol taken
30	3.06				
40	3.31				
50	2.99				
60	2.99				
70	3.09	} 2.68			
80	2.88				
90	2.81				
100	3.15				
110	2.81				
Proctor ..	10.1.44	Before alcohol Alcohol, 40 ml. and water, 160ml., taken at 40 minutes			
			30	2.96	
			40	2.96	
			50	2.85	
			60	3.03	
			70	3.22	} 3.08
			80	3.36	
			90	3.06	
			100	3.03	
			110	3.15	
			Proctor ..	13.1.44	Before alcohol Alcohol, 10 ml. and water, 40 ml., taken at 35 minutes
30	2.96				
40	3.08				
50	2.97				
60	3.02				
70	2.78	} 2.89			
80	2.86				
90	3.01				
100	2.97				
110	2.84				
Drabble..	20.1.44	Before alcohol Alcohol, 20 ml. and water, 80 ml., taken at 35 minutes			
			30	1.58	
			40	1.58	
			50	1.58	
			60	1.58	
			70	1.69	} 1.56
			80	1.59	
			90	1.52	
			100	1.54	
			110	1.57	
				1.54	
	1.61				
	1.62				

It seemed to be a matter of interest to make further observations bearing on Clausen's concept. A number of measurements of dark adaptation and of blood vitamin A were therefore made on volunteers at various stages of depletion. In the first series (Table XIX) two men, one deprived of vitamin A for 18 months (Proctor), the other a positive control supplemented with vitamin A (Drabble), were kept in the dark room for 100 minutes after being dosed with varying quantities of alcohol, and the final rod threshold was measured at 10-minute intervals. Drabble's threshold remained constant, but Proctor's showed a gradual improvement both without alcohol, and after 10 or 20 ml., but not after 40 ml. If the averages of the first three and last three readings are taken the decreases in the rod threshold for Proctor were :

Without alcohol	0.49 log. units
After 10 ml. alcohol	0.13 log. units
After 20 ml. alcohol	0.31 log. units
After 40 ml. alcohol	-0.03 log. units

These results suggest that alcohol suppresses the normal improvement which occurs over several hours in the night vision of persons with impaired dark adaptation. It was in these experiments that it became clear that the threshold after 20 minutes is not in these circumstances the "final" threshold, and further experiments, recorded on p. 47, were carried out to elaborate this observation.

In the second series of experiments the subjects, after receiving alcohol, were not kept in the dark room throughout, and the value for the rod threshold was measured by the "short" method. A much larger quantity of alcohol, 80 or 100 ml., was given, causing various signs of intoxication such as loquacity, drowsiness, and loss of consciousness which at times prevented the making of dark adaptation measurements. Three men, who had all received the deficient diet for 21 months, took part: Proctor, who showed a definitely raised rod threshold, Watson, who gave rather variable values, and Bloomfield, whose threshold was within the normal range. The values for the rod threshold and for vitamin A in the plasma are shown in Table XX. It will be seen that the thresholds of Proctor and Bloomfield remained constant, while that of Watson varied erratically, as it was in the habit of doing at that time, even if alcohol had not been drunk (Protocol B).

The values for plasma vitamin A also remained fairly constant. Since their failure to respond might have been due to depletion of the liver reserve of vitamin A, three other volunteers not on the deficient diet were given the same quantity of alcohol. Their plasma values for vitamin A, as will be seen from Table XX, showed no consistent trend during the subsequent 22 hours. In view of these negative results the problem was not pursued further.

In summing up the results of the alcohol experiments it must be said that they lend no support to the suggestion that the response of the plasma vitamin A level to administration of alcohol may be an index of the magnitude of the liver reserves of vitamin A. The capacity for dark adaptation was certainly not improved by alcohol; the only effect observed was a deterioration by some criteria, in accordance with the generally accepted

view that alcohol tends to depress mental processes (Medical Research Council : Alcohol Committee, 1938), if the dose given has any effect on them at all.

TABLE XX

Final Rod Threshold ("Short Method") and Plasma Vitamin A Values after Alcohol Consumption

(The alcohol was taken during one hour. The times stated in the Table are reckoned from the beginning of the alcohol intake. Varying amounts of water were added to the alcohol according to the different tastes of the volunteers)

Name	Date of test	Amount of liquid taken		Time after consumption of alcohol (hours)	Rod threshold (log. μm lamberts)	Plasma vitamin A (I.U. per 100 ml.)	
		Alcohol (ml.)	Water (ml.)			Cambridge laboratory	Oxford laboratory
Proctor ..	20.4.44	80	2,000	0	3.22	87	70
				3	3.21	72	75
				4	3.21	—	—
				5	3.11	86	79
				6	3.26	—	—
				7	3.22	88	99
				10½	3.30	—	—
				23	—	80	93
Watson ..	19.4.44	80	320	0	2.92	23	17
				2½	—	25	19
				5	—	28	20
				7	1.70	—	—
				8½	1.52	—	—
				9½	1.70	—	—
				19	2.70	25	26
				24	1.95	—	—
28	2.47	—	—				
Bloomfield	12.4.44	100	400	0	1.63	52	47
				2	—	52	50
				4	—	54	45
				6½	1.51	—	—
				8	—	52	44
				10	—	51	55
				11	1.53	—	—
				24	—	54	54
Earnshaw	3.7.44	80	2,000	0	—	76	176
				2	—	63	120
				4	—	57	134
				8	—	62	102
				22	—	54	107
				—	—	—	—
Milburn	3.7.44	80	2,000	0	—	89	—
				2	—	84	66
				4	—	87	52
				8	—	84	85
				22	—	87	47
Woolley	24.5.44	80	2,000	0	—	93	55
				1	—	95	66
				3½	—	96	94
				6½	—	96	57
				22	—	97	74

L.—An Examination of the Vitamin A Reserves in the Liver of a Sample of the Population of Great Britain, Made by T. Moore with the Assistance of A. C. Cooper (*see also Protocol D*)

Before the recent war the vitamin A reserves of the population of the United Kingdom were examined by analysing specimens of liver of subjects who had died by accident. In order to investigate the effect of the war-time diet in Great Britain on the body's reserves of vitamin A, specimens of liver were collected at various hospitals from similar subjects who had died in the period November, 1941 to November, 1944*. The tissues were posted promptly to Cambridge, and vitamin A was estimated by the antimony trichloride method, alkaline digestion being part of the technique used as in earlier surveys (Moore, 1937 ; Ellison and Moore, 1937). Values were not used from subjects who survived their accident more than seven days, since the vitamin A reserves tend to fall in prolonged illness. The individual values are set out in Protocol D.

The comparison of vitamin A reserves between different groups of subjects presents certain difficulties. The range of reserves found is very wide in persons who were sufficiently healthy to lead normal lives before their accident, as well as in diseased subjects. Thus, in the present survey, the extreme values found in accidental death were 23 and 2,400 I.U. per g. of liver, but some 80 per cent. of the values fell between the narrower limits of 100 to 900 I.U. per g., and there was a fairly even distribution of values within this range. No value could be singled out as representative of a normal reserve.

Use of the average value for the whole group is open to the criticism that the incidence of cases with low reserves, to which special interest attaches, may be masked by the disproportionate influence on the average exercised by the occurrence of a few cases with high reserves. To avoid this difficulty the special arrangement used in the previous work has been retained. The cases are divided into groups according to age and place of residence, or Service or civilian status, as indicated in the following pages. The individual values in each age group are arranged in ascending order of magnitude, and three equal sub-groups are formed : low (L), medium (M), and high (H), each containing one-third of the cases ; the cases with very high reserves are thus segregated. The mean of the middle group can be considered the " typical " or " central " reserve of the group.

Liver Reserves in 1941-44

In the earlier surveys low values were found in old age and childhood. Cases in the present survey were therefore grouped as before into a main group, aged 15 to 59 years, an " old age " group of 60 years or over, and a children's group under 15 years of age. A few specimens were received from

* Specimens of liver were sent by Major E. H. Bensley, R.C.A.M.C. ; Dr. E. Biddle, East Suffolk and Ipswich Hospital ; Dr. H. G. Cameron ; Dr. F. E. Camps, Chelmsford and Essex Hospital ; Dr. G. P. C. Claridge, Norfolk and Norwich Hospital ; Professor J. B. Duguid, Royal Infirmary, Cardiff ; Dr. J. F. Gaskell ; Professor J. Glaister ; Dr. C. V. Harrison, Liverpool University ; Professor H. A. Krebs ; Professor J. R. Marrack, London Hospital ; Dr. O. Morrey, White Lodge Hospital, Newmarket ; Dr. A. H. Robb-Smith, Radcliffe Infirmary, Oxford ; Professor B. Shaw and Dr. J. C. Spence, Royal Infirmary, Newcastle-on-Tyne ; Professor J. Shaw-Dunn and Mr. J. Carlisle, Western Infirmary, Glasgow ; Professor M. J. Stewart, General Infirmary, Leeds ; and Dr. Lucy Wills, Royal Free Hospital, London, to all of whom thanks are given.

the Services, and placed in a separate group on the assumption that the diet had been different from that of civilians. The results were as follows :

Type of subject	Age (years)	Number of cases	Mean vitamin A content of liver (I.U. per g.)			
			Sub-groups			Whole group
			(L)	(M)	(H)	
Civilian	Under 15	7	235	550	1,130	626
Civilian	15 to 59	71	122	324	911	455
Civilian	Over 60	29	87	273	645	337
Service	Under 33	11	484	750	1,017	751

If the weight of the adult human liver is taken as 1,500 g., the mean of 324 I.U. vitamin A per g. found for the main civilian group corresponds with a total reserve of about 500,000 I.U. Experiments with rats have shown that it is difficult to predict how long animals with known reserves of vitamin A will survive when restricted to a deficient diet, since there is a tendency for high reserves to be dissipated at a rate much greater than would be consistent with physiological demands alone (Davies and Moore, 1935, 1941). However, the long time taken by the Sorby volunteers to show signs of deficiency suggests that there is no undue waste in the human subject. By the rough expedient of simple arithmetic, therefore, a reserve of 500,000 I.U. should suffice for 200 days if the physiological requirement is 2,500 I.U. daily, or for 400 days if the physiological requirement is 1,250 I.U. daily.

The differences between the means of the (L), (M), and (H) sub-groups are wide enough to show that individual variations were large. In the main group, however, only five of the seventy-one subjects had reserves of less than 120 I.U. per g., or 180,000 I.U. for the whole liver. Two specimens with reserves of 23 and 75 I.U. per g. were from the only two subjects, out of a total of 20, which were described by Mr. John Carlisle, of Glasgow, as poorly nourished. Reports from other collaborators, however, show no correlation between vitamin A reserves and the state of nutrition.

In subjects over 60 years old the reserves were only slightly lower than in the main group. In the few who formed the group under 15 years and the Services group, the reserves were higher than in the main group.

Liver Reserves in 1941-44 Compared with Those Found in 1931-35

The "central" values for the corresponding age groups for the two periods are compared below :

Type of subject	Age (years)	1931-35		1941-44	
		Number of cases	Liver reserve (I.U. per g.)	Number of cases	"Central" liver reserve (I.U. per g.)
Civilian	Under 15	12	130	7	550
Civilian	15 to 59	40	220	71	324
Civilian	Over 60	16	100	29	273

The values for 1941-44 are higher than those for 1931-35 in all age groups but, in this connection, differences in the places from which the specimens came during the two periods must be considered.

Liver Reserves in Different Places

Owing to the scantiness of material, no attempt could be made to balance the distribution of the 1941-44 specimens according to place of origin against the distribution of those received in 1931-35. Specimens from the 15 to 59 age group originated as follows :

		<i>Glasgow</i>	<i>London</i>	<i>Leeds</i>	<i>Norwich</i>	<i>Elsewhere</i>
1931-35	..	24	4	6	0	6
1941-44	..	20	15	8	19	9

The values for vitamin A reserves, calculated for those places from which a reasonably large number of specimens was received; were as follows :

<i>Place</i>	<i>Age (years)</i>	<i>Period</i>	<i>Number of cases</i>	<i>Mean vitamin A content of liver (I.U. per g.)</i>			
				<i>Sub-groups</i>			<i>Whole group</i>
				<i>(L)</i>	<i>(M)</i>	<i>(H)</i>	
Glasgow	15 to 59	1931-35	24	91	264	648	334
Glasgow	15 to 59	1941-44	20	81	268	868	349
London	15 to 59	1941-44	15	180	454	960	531
Norwich	15 to 59	1941-44	19	120	307	1,022	474

It will be seen that the values for the three districts differ considerably but that those for Glasgow in the two periods are substantially the same.

Discussion

Between the periods 1931-35 and 1941-44 the intake of vitamin A in the United Kingdom was altered by war-time conditions. The available dairy products had become more evenly distributed throughout the population, the fortification of margarine had become compulsory, and there was a much greater use of vegetables, notably carrots. Children received additional milk, and medication with cod liver oil and vitamin A concentrates was more widespread. The total effect is not easy to assess, but it is not inconceivable that the 1941-44 vitamin A reserves of the population were higher than 10 years previously. The increase in the "central" value for the main group from 220 I.U. per g. in 1931-35 to 324 in 1941-44 might seem at first sight to support this view but the difference in the places from which the specimens came must not be forgotten. When results for Glasgow only are compared, the "central" values of 264 for 1931-35 and 268 for 1941-44 are closely similar. Glasgow, the one place adequately represented by samples in both periods, shows no evidence that a change in the vitamin A reserves took place between the two periods, at least in the main group, and it seems clear that the higher value found in 1941-44 for all localities combined was due to the inclusion of more specimens from regions, notably London, where the reserves appear to be higher than in Glasgow.

Whatever interpretation is placed on the results for the main groups, the higher values found during the war for the "old age" and "children's" groups cannot be explained by differences in locality for, even in Glasgow, the "central" value for the "old age" group rose from 158 I.U. per g. for eleven cases in 1931-35 to 300 for nine cases in 1941-44.

As far as can be judged from the small number of specimens examined there is no evidence that the reserves per g. of liver are now lower in childhood than in later life ; the difference in old age also, if it exists, is relatively small.

The high values found for the "Services" group which included five Canadian soldiers, four British soldiers, one British naval officer and one merchant seaman, appear to reflect superiority of the diet in the Services over that available for civilians. The lowest reserve in this group was 340 I.U. per g.

M.—A Study of the Normal Value for the Vitamin A and Carotenoid Content of Human Blood Plasma, Made by T. Moore and Z. A. Leitner

Most of the early attempts to estimate carotene and vitamin A in blood were made in Holland with the Lovibond tintometer (van Eekelen, 1931 ; Menken, 1934 ; van Eekelen and Emmerie, 1935) and the results were expressed in "Lovibond units", of which the exact equivalent value was at that time uncertain. In the light of later knowledge, however, the values for vitamin A appear to be somewhat low. It seems probable that the rapidity with which the colour faded in the antimony trichloride reaction prevented accurate observation with the tintometer of the small amounts of vitamin A in blood. Thus, for about two hundred healthy persons, Menken found a mean value per 100 ml. serum of only 26 "Lovibond units", which would appear to be equivalent to about 16 I.U.

Later the Dutch workers obtained values much nearer to modern ones. Wolff (1936) considered that values per 100 ml. of 90 to 120 I.U. represented a good state of vitamin A nutrition, that values of 45 to 90 I.U. were satisfactory, and that those under 45 I.U. were insufficient. Van Eekelen, Emmerie, and Wolff (1937) slightly modified these standards, calling values of more than 80 I.U. good, of 40 to 80 medium, and under 40 poor ; the highest value found in their survey was 195 I.U. For a group of native Batavian nursing staff, de Haas and Meulemans (1938) found an average value per 100 ml. serum of 108 I.U. for carotenoids and 90 I.U. for vitamin A. The mean values obtained by van Veen and Lanzing (1937) for a group of Europeans in Batavia were 200 I.U. for carotenoids and 91 I.U. for vitamin A. In German hospital patients Wendt (1935) found an average of 90 I.U. for vitamin A.

The Lovibond tintometer was replaced by the Zeiss step-photometer in the extensive experiments of Lindqvist (1938) and this instrument was used also by Willstaedt and With (1939). In both instances the results for vitamin A appear to be about twice as high as might have been expected. This is presumably to be attributed to the use as standard of the German commercial vitamin A preparation, "Vogan", now known to have been overvalued (Grab and Moll, 1939), rather than to any defect in the photometer.

The photo-electric absorptiometer was first used for the estimation of vitamin A by Dann and Evelyn (1938) and was applied to the examination of blood plasma by Kimble (1939). Numerous surveys have been made with the help of this instrument, and the values obtained for groups of healthy

white adults are assembled in Table XXI; all the results are from the U.S.A. except those of Yudkin (1941b) and of Highman (1944). In most instances the groups included both sexes; when, however, the authors have given separate averages for males and females, the mean of the two values has been taken without any weighting. To facilitate comparison, results for carotenoids, expressed by the original authors in $\mu\text{g.}$, have been divided by 0.6 to convert them to "I.U.", and results expressed as $\mu\text{g.}$ of vitamin A have been divided by 0.3. Some of the American authors, in calculating the values for vitamin A, do not appear to have made any correction for the blue colour contributed by carotenoids to the antimony trichloride reaction.

TABLE XXI

Vitamin A and Carotenoid Content of the Serum or Plasma of Groups of Healthy Adults
(Persons of both sexes are included)

Observers	Date	Number of subjects	Total carotenoids ("I.U." per 100 ml.)	Vitamin A (I.U. or U.S.P.U.* per 100 ml.)
1. Kimble	1939	64	294	109
2. Abels <i>et al.</i>	1941	124	325	160
3. Murrill <i>et al.</i>	1941	45	343	104
4. Yarbrough and Dann ..	1941	16	305	71
5. Yudkin	1941	23	200	113
6. Brenner and Roberts ..	1943	6	380	117
7. Popper and Steigmann ..	1943	27	135	153
8. Highman	1944	20	377	108
		Mean ..	295	117

* No distinction is made here between I.U. and U.S.P.U. The two terms were for a considerable time believed to be interchangeable. The U.S.P.U. is now held (Hume, 1943) to have only about 87 per cent. of the value of the I.U. but it is not always possible to be certain retrospectively as to which is really meant.

Hoch (1943), applying a novel photographic technique to the antimony trichloride reaction, obtained mean values of 125 "I.U." for carotenoids and 74 I.U. for vitamin A in a group of nine English subjects.

Chevallier and Manuel (1941), using Chevallier's spectrophotometric technique of measuring ultra-violet absorption before and after destruction of vitamin A, found an average value of 105 I.U. vitamin A per 100 ml. for thirty blood donors in 1939.

Results of Moore and Leitner

Specimens of plasma were collected in London from private patients, and from out-patients at St. Mary's Hospital. In order to obtain enough samples, blood was taken from subjects with illnesses not expected to affect the vitamin A value, as well as from healthy subjects. The method of estimation is described on p. 90.

No significant difference was found between the mean values for private and hospital patients, and the results have been combined. The mean vitamin A values for males tended to be slightly higher than for females; the values given in Table XXII are the simple average of the means for both sexes.

The mean value for vitamin A is almost the same as in the results of the workers assembled in Table XXI. Since, however, the U.S.P. unit may be slightly smaller than the International unit, the value of 121 I.U. shown as the mean in Table XXII may possibly be equivalent to up to 140 U.S.P. units. The difference between the present mean and that of 113 I.U. found by Yudkin, the only other English observer, is however very slight. The mean value of 153 "I.U." for carotenoids found in the present survey is much lower than most of the American values, but again it is not very far removed from Yudkin's mean of 200 "I.U.". The results of Highman (1944), who found mean values of 407, 270, and 218 "I.U." for carotenoids in groups of European, Indian, and Bantu males, respectively, indicate that carotenoid levels may differ widely according to race and diet. Her values for vitamin A of 111, 123, and 111 I.U. for the same groups show much less variation.

TABLE XXII

Content of Vitamin A and of Total Carotenoids in the Blood Plasma of Healthy Adults and of Adult Hospital Patients with Diseases not likely to Affect the Blood Values
(Persons of both sexes are included)

Condition	Number of subjects	Total carotenoids ("I.U." per 100 ml.)	Vitamin A (I.U. per 100 ml.)
Normal	83	167	128
Osteo-arthritis	29	163	131
Fibrositis	21	187	115
Hernia	19	132	125
Varicose veins	18	105	128
Fracture	13	101	95
Accident	8	167	101
Haemorrhoids	4	161	122
Total	195	—	—
Mean for all subjects	—	153	121

In Table XXIII are given the percentages of the values for carotenoids and vitamin A falling within certain ranges.

TABLE XXIII

Distribution of the 195 Cases in Table XXII According to the Magnitude of the Plasma Values for Vitamin A and Total Carotenoids

Plasma constituent	Percentage of total number of cases showing, per 100 ml. plasma, values in I.U. or "I.U." of:							
	0 to 40	41 to 80	81 to 120	121 to 160	161 to 200	201 to 240	241 to 280	Over 280
Vitamin A	4.1	15.8	29.6	34.2	11.2	2.6	1.5	1.0
Total carotenoids	1.0	11.2	26.6	25.5	14.3	9.2	9.2	3.1

It will be seen that 52.1 per cent. of the values for total carotenoids and 63.8 per cent. of the values for vitamin A fell within the range of 81 to 160 "I.U." or I.U. per 100 ml. The 4.1 per cent. of the vitamin A values within the range 0 to 40 I.U. may be too great through inclusion of subjects with very high values for carotenoids. The correction factor for the contribution made by carotenoids to the blue colour of the antimony trichloride reaction varies under different circumstances. Although many experiments were made before it was decided what correction was appropriate, it is possible that the factor chosen was still slightly too high, so that the corresponding vitamin A values would be proportionately too low.

Influence of Sex

Kimble (1939) found a slightly higher average value for vitamin A in the plasma of males than of females, while for carotenoids the position was reversed. Other American workers have confirmed this finding, at least in regard to vitamin A. In the present survey also, the average value for vitamin A was higher for males than for females, although for carotenoids it was almost identical. In Table XXIV the data obtained in the present survey for normal subjects only, of each sex, are compared with those of other workers.

All workers, therefore, agree in finding that the vitamin A level in males tends to be slightly higher than in females, the extent of the difference being about 20 I.U. per 100 ml. The carotenoid values were slightly higher for females than for males in three of the four surveys but in the fourth the opposite was found.

TABLE XXIV

Content of Vitamin A and of Total Carotenoids in the Blood Serum or Plasma of Groups of Male and Female Subjects

Observers	Males			Females		
	Number of subjects	Total carotenoids ("I.U." per 100 ml.)	Vitamin A (I.U. or U.S.P.U. per 100 ml.)	Number of subjects	Total carotenoids ("I.U." per 100 ml.)	Vitamin A (I.U. or U.S.P.U. per 100 ml.)
Kimble, 1939 ..	30	277	127	34	312	91
Abels <i>et al.</i> , 1941 ..	62	350	170	62	300	149
Murrill <i>et al.</i> , 1941 ..	25	333	111	20	355	97
Moore and Leitner, 1946 (present observations)	46	160	146	37	175	110
Mean	—	280	138	—	285	112

Influence of Season

In Table XXV all the results in the present survey have been grouped in two-monthly periods, from November and December, 1944 to September and October, 1945. The values are the averages of the means for males and females.

The results show a slight increase in carotenoids and vitamin A during the summer and autumn months. This might be expected from the more plentiful supply of vegetables in these seasons, but the present evidence is

TABLE XXV

Values for Content of Total Carotenoids and Vitamin A in the Blood Plasma of all the Subjects in Table XXII Grouped According to the Months in which the Observation was Made

	November, December, 1944	January, February, 1945	March, April	May, June	July, August	September, October
Total carotenoids ("I.U." per 100 ml.)	173	121	155	199	194	151
Vitamin A (I.U. per 100 ml.)	114	112	107	132	131	141

not sufficient to establish the existence of any widespread seasonal change. The incidence of the different categories of illness from which some of the subjects suffered was not uniform throughout the year, but no obvious cause of the variations in the blood value for vitamin A can be found in this fact.

Influence of Locality

In addition to the main survey in London, subsidiary investigations were made on specimens provided by Dr. D. R. McIntosh and Dr. R. P. Cook from schoolboys in Dundee, and by Wing-Commander T. F. Macrae from subjects in Brussels and Dortmund. A few specimens were collected also from a boys' home at Eastbourne. The results are given in Table XXVI, the London results being repeated for comparison.

TABLE XXVI

Content of Vitamin A and of Total Carotenoids in the Blood Plasma of Groups of Subjects from Various Places Compared with those for Subjects in London (Table XXII)

Place	Date	Number of subjects	Sex	Average age	Total carotenoids ("I.U." per 100 ml.)	Vitamin A (I.U. per 100 ml.)
Brussels ..	December, 1944	41	Mixed	14	138	134
Dortmund	November, 1945	26	F	40	111	127
Dundee ..	January, 1944	41	M	c.15	81	78
Dundee ..	December, 1945	60	M	15	102	88
Eastbourne	October, 1945	11	M	17	76	137
London ..	1944-45	195	Mixed	38	153	121

It would appear that the mean values for both carotenoids and vitamin A may vary considerably in different districts. The low results for the two male groups at Dundee indicate that the factor of sex may be outweighed by that of locality, since much higher results were obtained for a mixed group of about the same age in Brussels. The low carotenoid value for Eastbourne is probably to be attributed to the nature of the institutional diet.

TABLE XXVII

Content of Total Carotenoids and of α - and β -Carotene in the Faeces of Deficient Subjects in Relation to Variations in the Carotenoid Content of the Deficient Basal Diet

(Period 1. Permitted vegetables†; National Wheatmeal flour. Period 2. All vegetables excluded.
 Period 3. Permitted vegetables† included except for the eight subjects represented in period 2; bleached white flour)

Name	Period 1. (January to March, 1943)				Period 2. (June to July, 1943)				Period 3. (August to September, 1943)			
	Total carotenoids ("I.U." per day)		α - and β -carotene (I.U. per day)		Total carotenoids ("I.U." per day)		α - and β -carotene (I.U. per day)		Total carotenoids ("I.U." per day)		α - and β -carotene (I.U. per day)	
	Cambridge	Liverpool	Cambridge*	Liverpool	Cambridge	Liverpool	Cambridge	Liverpool	Cambridge	Liverpool	Cambridge	Liverpool
Bloomfield ..	2,540	983	763	192	940	705	80	60	485	407	72	109
Brown ..	1,014	319	415	72	—	—	—	—	870	450	139	45
Garling ..	1,575	1,986	595	—	840	490	117	80	755	900	131	160
Golding ..	1,730	1,075	261	—	503	490	63	50	575	660	172	75
Hicken ..	2,080	2,661	399	—	1,256	1,250	307	50	650	570	123	80
Proctor ..	1,940	—	250	158	—	—	—	—	2,140	900	165	160
Saunders ..	2,320	861	270	198	—	—	—	—	—	—	—	—
Stuart ..	1,010	587	132	84	—	—	—	—	—	—	—	—
Thompson ..	328	480	10	80	—	—	—	—	939	425	414	69
Watson ..	1,760	1,265	89	—	950	750	190	60	412	359	79	79
Whitehead, A. ...	785	656	151	95	870	390	39	90	541	285	267	56
Whitehead, D. ...	550	217	12	31	650	310	69	50	148	132	33	29
Williams ..	1,505	3,021	218	—	790	350	70	70	521	267	33	27
Wodeman ..	2,017	746	317	56	—	—	—	—	1,040	1,070	246	178
Woodhouse ..	2,570	2,157	305	264	—	—	—	—	610	627	446	117

* The Cambridge results are probably too high through including with the α - and β -carotene the xanthophyllic esters of which the largest part would probably be derived from the National Wheatmeal flour.

† For "permitted" vegetables, see Table XXX (p. 77).

Conclusions

The results of the present survey place the average value for total carotenoids in the London area at about 150 "I.U." per 100 ml. plasma, and for vitamin A at about 120 I.U.

The vitamin A level is influenced by sex, so that the mean for males is about 130 I.U. and for females about 110 I.U.

There are local differences in the blood level of both carotenoids and vitamin A. Values for the former seem to be much lower in Britain than in America, although questions of calibration are possibly involved. For vitamin A, however, the British and American levels are less divergent.

Season is another factor which may possibly have some influence.

N.—Is Carotene Synthesized in the Human Gut?

In view of what is known about the bacterial synthesis of B vitamins in the gut, the question may be raised whether important quantities of carotene can be synthesized by micro-organisms in the gut. It is known (Sobin and Stahly, 1942) that a number of bacteria, e.g., *Staphylococcus aureus*, are capable of synthesizing α - and β -carotene. A number of organisms were isolated from the faeces of deprived volunteers and those showing red, orange, or yellow colouring were grown in sufficient quantities to provide material for the quantitative estimation, after chromatographic separation, of the carotenoids. No measurable amounts of α - or β -carotene were found in the faecal organisms but it was confirmed that the pigments of *Staphylococcus aureus* include considerable quantities of α - and β -carotene. The cocci contained 750 "I.U." total carotenoids, and 340 I.U. α - and β -carotene, per g. dry weight.

Other observations made during the experiment strongly suggest that no appreciable synthesis occurred in the gut of the subjects. The deficient volunteers excreted no appreciable quantities of α - and β -carotene in their faeces (Table XXVII). Since a high proportion of ingested carotene is excreted unchanged (Table XXVIII) it would be expected that part of any carotene that was synthesized by micro-organisms would appear in the faeces. The absence of α - and β -carotene from the stools of the deficient subjects, therefore, argues against the occurrence of intestinal synthesis. This conclusion agrees with that reached by Wald, Carroll, and Sciarra (1941).

TABLE XXVIII

Amounts of α - and β -Carotene Excreted in the Faeces of Subjects Receiving the Basal Diet Deficient in Vitamin A but Supplemented with Carotene from Various Sources

Source of carotene	Name	Period of faeces collection	Content of α - and β -carotene						Percentage excreted		Maximum effective dose, average Cambridge and Liverpool (I.U. per day)	
			Cambridge			Liverpool			Average Cambridge and Liverpool	Group average	Value for individual	Group average
			I.U. per day		Percentage excreted	I.U. per day		Percentage excreted				
			Intake	Output		Intake	Output					
Carrots, canned, sliced	Bartley ..	9-14. 6.44	3,750	2,325	62	3,600	2,730	76	69	} 76	1,150	} 870
	Drake ..	8-12. 6.44	3,750	3,445	92	3,600	2,730	76	84			
Carrots, canned, sliced, purée	Bartley ..	17-22. 1.43	5,000	4,690	94	4,830	4,020	83	88	} 75	560	} 1,360
	Bartley, Mrs.	16-21. 1.43	5,000	3,270	65						1,730	
	Broderick ..	2 9. 1.43				4,830	4,890	100			1,335	
	Garnett ..	23.2-1. 3.43				4,830	2,160	43			1,815	
Carrots, canned, sliced, purée, and margarine	Bartley ..	4-10. 2.43	5,000	5,100	100					} 67	0	} 1,600
	Bartley, Mrs.	3-10. 2.43	5,000	2,000	40						3,000	
	Broderick ..	3-9. 2.43	5,000	3,525	70	4,830	2,590	54	62		1,855	
	Garnett ..	3-9. 2.43	5,000	2,700	54	4,830	4,030	83	68		1,550	
Carrots, canned, homogenized	Bartley ..	2-7. 2.44	4,720	1,595	34	5,000	2,510	50	} 41	} 44	2,960	} 2,715
		2-7. 2.44				5,000	2,330	47				
	Drake ..	22-26. 2.44	4,720	1,755	37	5,000	1,830	37	} 44			
		5-10. 2.44	4,720	1,585	34	5,000	2,770	55				
		5-10. 2.44				5,000	2,580	52				
		15-21. 2.44	4,720	2,655	56	5,000	2,630	53				

β-Carotene in arachis oil with hydroquinone	Bartley ..	18-23. 3.43	6,400	1,770	28	6,400	1,780	28	28	} 26	} 4,370	} 4,420				
		18-21. 4.43	6,400	1,285	20	6,400	1,480	23	21							
		12-18. 5.43	6,000	2,860	48	6,000	1,610	27	37							
	Broderick ..	19-25. 5.44	5,120	820	16	5,120	1,280	25	21							
		20-26. 3.43	6,400	1,975	31	6,400	2,170	34	32							
	Drake ..	12-18. 5.43	6,000	2,260	38	6,000	1,385	23	30							
		15-21. 4.43	6,400	1,020	16	6,400	1,260	20	18							
		7-11. 5.43	6,000	1,700	28	6,000	1,250	21	24							
		22-26. 5.43	5,120	1,055	21	5,120	930	18	19							
β-Carotene in margarine	Bartley ..	2-18.12.43	5,160	985	19	5,160	1,600	31	25	} 29	} 3,780	} 3,680				
		18-23.12.43	5,160	1,635	32	5,160	1,310	25	28							
	Drake ..	9-13.12.43	5,160	1,130	22	5,160	1,980	38	30							
		17-22.12.43	5,160	2,275	44	5,160	920	18	31							
Spinach, canned purée	Bartley ..	11-16. 6.43	4,180	2,785	67	4,400	3,380	77	72	} 59	} 1,300	} 1,730				
		24-30. 6.43	4,180	2,905	69	4,400	2,880	65	67							
	Broderick ..	11-17. 7.43	4,180	2,685	64	4,400	3,310	75	69							
		9-15. 6.43	4,180	2,110	50	4,400	2,380	54	52							
		22-26. 6.43	4,180	1,955	47	4,400	1,730	39	43							
	Drake ..	21-25. 7.43	4,180	2,195	52	4,400	1,080	25	38							
		27-31. 5.43				4,400	3,190	72								
		13-17. 6.43	4,180	2,865	69	4,400	2,680	61	65							
		12-15. 7.43	4,180	2,845	68	4,400	2,480	56	62							
	Spinach, canned, homogenized	Bartley ..	14-20. 4.44	6,670	3,485	52	5,180	2,830	55				53	} 57	} 2,760	} 2,530
			28.4-2. 5.44	6,670	3,705	56	5,180	2,630	51				53			
Drake ..		14-20. 4.44	6,670	3,835	57	5,180	3,080	59	58							
		27.4-2. 5.44	6,670	3,805	57	5,180	3,780	73	65							
Cabbage, dried, outer leaves : (1) 9 g. per day	Bartley ..	8-13. 8.43	4,140	2,575	62	2,310	880	38	50	} 59	} 1,035	} 1,375				
		7-13. 9.43	4,140	3,090	75	2,310	2,980	100	87							
	Broderick ..	15-19. 8.43	4,140	2,045	49	2,310	1,600	69	59							
		4-10. 9.43	4,140	2,220	54	2,310	1,280	55	54							
		Drake ..	8-13. 8.43	4,140	1,365	33	2,310	1,080	47				40			
	12-16. 9.43		4,140	2,185	53	2,310	1,680	73	63							
	(2) 18 g. per day	Bartley ..	14-20.10.43	8,280	4,550	55	4,620	4,120	89				72	} 73	} 2,115	} 2,080
			5-10.10.43	8,280	4,545	55	4,620	4,250	92				73			
		Drake ..														

III. DETAILS OF THE EVIDENCE

O.—Particulars and Management of the Volunteers

The details of the ages, weights, heights, and occupations of the twenty-three volunteers are set out in Table XXIX. Most of them lived at the Sorby Research Institute and all received the diet from there.

Before the beginning of this experiment, and during the first 6 months of it, work on scabies was being carried out on some of the subjects and several of them were experimentally infected with it.

All but one were pacifists. A number of them had served at the Sorby Research Institute for periods up to 18 months before the present experiment was started. They fully understood the nature of the experiment and could be relied on to give loyal co-operation.

The volunteers were originally asked to live on the restricted diet for about 6 months, as it was expected that deficiency would develop within this period. When this proved not to be so all of them agreed to continue for a further 6 months, but one had to abandon the diet after 6½ months owing to a serious riding accident, and another after 8½ months owing to the onset of pregnancy. Five stopped the experiment after a total period of between 12 and 14 months, and the rest after periods of between 18 and 25 months. They were under no compulsion and were at liberty to stop the experiment whenever they wished, but they continued for long periods, because they wished to make a contribution to knowledge which they believed might benefit mankind.

The experiment was directed by a team of workers who met every month in Sheffield to plan and modify the course of the experiment. The method of directing the experiment in this way, by committee, proved very satisfactory, and also gave the team an opportunity to meet the volunteers and discuss the course of the test with them.

To stress the importance of the experimental subjects as individual personalities, and to make the report more readable, it was decided to depart from traditional medical practice and publish their full names.

The success of nutritional experiments on volunteers depends on their whole-hearted co-operation, for it is obviously impossible to force a restricted diet upon them for long periods. Co-operation, in turn, depends on morale. In a group of people living as a community, morale largely depends on management.

The management of this team of volunteers was complicated by the fact that some of the customary methods of maintaining morale were not expedient. It would have been impracticable, for instance, to get rid of a troublesome person halfway through the experiment, since this would have meant a serious loss to the investigation. A special problem was to avoid the irritability that tends to accumulate in a small community living in very close contact for a long period. The Armed Forces deal with this problem to some extent by moving people about at frequent intervals, but this method could obviously not be applied to this team of volunteers.

A technique which on the whole worked satisfactorily was gradually evolved at the Sorby Research Institute after its inception in 1940. The main points were :

Care in selection of volunteers.—It was found to be helpful to invite a new candidate to live in the community for several days before a decision was made on either side. This gave the applicant an opportunity of meeting the other volunteers and learning something of their work and life, while

TABLE XXIX

Name, Sex, Age, Weight, Height, Occupation, and Period on Diet of Volunteers

Name	Sex	Age when diet started		Weight when diet started		Height		Occupation		Period on diet (months)
		(years)	(months)	(lb.)	(kg.)	(ft. in.)	(cm.)	Before experiment	During experiment*	
Bartley, M.	F	23	6	144	65.3	5 4	163	Office clerk	Office clerk	8½
Bartley, W.	M	26	6	138	62.6	5 8½	174	Laboratory assistant	Laboratory assistant	23
Bloomfield, H.	M	28	1	161	73.0	6 2	188	Various	S.R.I.	25
Broderick, J.	M	23	0	146	66.2	5 7	170	Draughtsman	Draughtsman	19
Brown, L.	M	28	3	151½	68.7	5 8½	174	Shop assistant	S.R.I.	13
Drabble, H.	M	23	0	144	65.3	6 0½	184	Student	S.R.I.	18
Drake, G.	M	30	9	116	52.6	5 2	157	Traveller	S.R.I.	15
Garling, H.	M	27	1	130	59.0	5 4	163	Income tax clerk	S.R.I.	22½
Garnett, C.	M	22	2	141	64.0	5 10	178	Civil defence worker	Civil defence worker	6½
Golding, A.	M	32	5	119½	54.2	5 6½	169	Insurance clerk	S.R.I.	22
Hicken, B.	M	24	9	141	64.0	5 8½	174	Audit clerk	S.R.I.	17
Proctor, N.	M	31	0	125	56.7	5 5½	166	Baker	S.R.I.	24
Russell, E.	F	29	9	139	63.0	5 5	165	Social worker	Social worker	11½
Saunders, P.	M	21	4	132	59.9	5 7	170	Commercial artist	S.R.I.	12½
Stuart, S.	M	25	2	142	64.4	5 9	175	Bank clerk	Bank clerk	11½
Thompson, W.	M	28	4	152½	69.2	6 3	191	Schoolmaster	Schoolmaster	20
Tridgell, I.	M	20	11	131	59.4	5 9	175	Clerk	S.R.I.	22½
Watson, C.	M	25	3	128	58.1	5 9	175	Architect's clerk	Student	23
Whitehead, A.	M	29	9	147	66.7	5 8½	174	Pharmacist	Pharmacist	18½
Whitehead, D.	F	29	3	118	53.5	5 4	163	Housewife	Shop assistant	13½
Williams, D.	M	22	1	146	66.2	5 8	173	Clerk	S.R.I.	20
Wodeman, R.	M	32	3	165	74.8	6 0	183	Various	S.R.I.	20½
Woodhouse, J.	M	23	10	144	65.3	5 11½	182	Clerk	Clerk	21½

* S.R.I. indicates volunteer in the full time service of the Sorby Research Institute.

III.—DETAILS OF THE EVIDENCE

at the same time the experienced members of the team were able to form an opinion as to whether he was likely to fit in with this somewhat unusual community.

Participation of the volunteers in the management.—The volunteers were given the greatest possible share in the responsibility of running both the community and the experiment. They were encouraged to take an intelligent and active part in the work, and to regard themselves not as mere passive guinea pigs but as partners, equal in importance to the team of investigators. Many routine duties, especially domestic duties, such as cooking and cleaning, were discharged by the volunteers and everyone was expected to play his part. The most important part of the routine work in any nutritional experiment is the cooking. The ingredients to be used, as well as standard recipes and general instructions about the diet, were laid down by the investigators but the actual preparation of the meals was made the responsibility of the volunteers themselves. One of them, a professional baker and confectioner, was in charge of the general supervision of the kitchen, while the bulk of the routine cooking was in the hands of a rota of volunteers. Kitchen duties changed weekly and all the volunteers who were not employed on work outside the Institute were on the rota. This method of changing the cook introduced some variety of style and, what was more important, greatly reduced the grumbling against him. In a long drawn out nutritional experiment with a monotonous diet, the cook is liable to be the first target for grievances. By making the volunteers themselves responsible, in turn, for the preparation of the food, the meals were virtually eliminated as a source of grumbling. The prospect of taking their own turn at cooking, and the existence of a high degree of esprit de corps, helped the volunteers to put up cheerfully with the shortcomings of the food.

Other routine work in which the volunteers took a responsible share was laboratory work, record-keeping, and clerical work.

Regular occupation.—The routine work at the Sorby Research Institute was not sufficient to occupy all the twenty-three volunteers. Those who were in a position to carry on their ordinary occupations were therefore encouraged to do so. This applied to nine of them. Others, who had special experience in the diagnosis and treatment of scabies, took part in a scabies survey conducted by K. Mellanby (1944) in schools and mental hospitals. The remainder filled in some of their spare time in work on a vegetable garden, though the dietary restrictions prevented them from eating much of the produce.

Holidays.—When it became obvious that the experiment had to be continued for a very long period, it was decided to allow leave of absence from the Institute. The restricted diet was maintained during the holidays, and the volunteers were given detailed dietary instructions. Certain foods, such as bread (except during the period when the special white flour was in use), meat, tea, sugar, and vegetables such as potatoes, white turnips, or white beans, they could buy in the shops; other items, such as dried skimmed milk, jam, and white margarine, they took with them from the Institute. The refreshing break of holiday time, even though it gave no relaxation from the monotony of the diet, helped considerably to make the prolonged restrictions endurable.

By these means the morale of the volunteers was well maintained. There was a time in the autumn of 1943, after a full year of experiment, when there were complaints of a variety of subjective symptoms, particularly of fatigue, and failure of powers of concentration and memory. This occurred at the time when objective signs of deficiency were beginning to be apparent in one or two subjects, though not in the majority. It was at this point that Dr. Russell Davis came into the team to investigate the relationship of these complaints to the deficient diet.

It eventually became clear that these troubles were common to the deprived and non-deprived subjects alike, and that they arose from strain, originating partly from conflict as to the relative usefulness to the community of continuing with the experiment or doing something else. A considerable part must also have been played by the monotony of the diet and the irksomeness of the necessarily restricted conditions of life.

It is important to be aware that such symptoms of strain and doubt may arise in the course of a prolonged human experiment, especially in the best type of volunteer, so that they should not be confused with the effects of dietary deprivation.

P.—Diet

COMPOSITION OF THE BASAL DIET

The diet was planned to be as low as possible in vitamin A and its precursors, but to be complete in all other ways. Since it had to be consumed for a long period, every effort was made to include in the list of permitted foods (Table XXX) as many items as possible from which choice could be made, and to supply sufficient amounts of those foods that make a diet acceptable. Rationing regulations and war-time difficulties of supply

TABLE XXX

Vitamin A Deficient Basal Diet :

Foods of Low Vitamin A and Carotene Content from which Selection was Permitted

Flour	Turnips, white
Bread	Beetroot, red
Biscuits, plain	Radishes
Wheat products, any other	Horseradish
Breakfast cereals	Celery, white part
Oatmeal	Onions
Barley	Spring onions, white part
Rice, white	Leeks, white part
Custard powder	Shallots
Cornflour	Artichokes, Jerusalem
Tapioca	Cucumber, peeled
Semolina	Cauliflower, flower only
Spaghetti, without tomato sauce	Raspberries
Macaroni, not made with egg	Redcurrants
Walnuts, not green	Strawberries
Chestnuts	Apples, white fleshed, peeled
Peanuts, groundnuts	Apples, dried
Hazel nuts	Pears, peeled
Brazil nuts	
Beans, haricot, dry or canned	Meat, lean: beef, mutton, veal, rabbit, no internal organs
Beans, butter, dry or canned	Cooked meats, pork products only
Potatoes	<i>No sausages</i>
Potato crisps	
Parsnips	Fish, white : cod, whiting

TABLE XXX—*cont.*

Skimmed milk powder	Pepper
Egg white	Mustard, small amounts
Food yeast (<i>Torulopsis utilis</i>)	Ginger
Tea	Vinegar
Coffee	Margarine, non-vitaminized
Nescafé	Lard
Cocoa, non-vitaminized from reliable source	Oil, groundnut, arachis
Beer	Sugar
Ginger beer	Honey
Lemon drinks	Golden syrup
Cider and apple juice	Treacle
Bovril	Jam, strawberry, special supply
Marmite	Peanut butter
Oxo	Chocolate, special supply
Torox	Gelatine
Lemon flavouring	Boiled sweets, clear, not toffee
Vanilla flavouring	Chewing gum
Salt	Pickles, onion and cauliflower in vinegar only, no piccallilli

Note.—The following foods were added to the list during the course of experiment :
 Fat, mutton and beef, no yellow fat to be eaten
 Corned beef
 Bramble jelly, special supply
 Mushrooms.

limited the amounts of some foods but the Ministry of Food arranged for amounts, in excess of the ration, of meat, bacon, margarine, sugar, skimmed-milk powder, jam, chocolate, and some foods on points. The amounts of food entering the kitchen during a week in March and another in August, 1943, are given in Table XXXI. Full details of food consumption by

TABLE XXXI

Quantities of Each Food Consumed on Basal Diet in Two Sample Weeks (Gross amounts entering kitchen)

Foodstuff	For 18 people (One week, March, 1943)		For 8 people (One week, August, 1943)	
	(lb.)	(kg.)	(lb.)	(kg.)
Rolled oats	7	3.2	3	1.4
Bacon	9	4.1	4	1.8
Bread	100	45.4	50	22.7
Tea	2	0.90	1	0.45
Sugar	14	6.4	8	3.6
Syrup	8	3.6	4	1.8
Biscuits	4	1.8	2	0.90
Potatoes	81	38.1	42	19.1
Parsnips	14	6.4	—	—
Beetroot	14	6.4	6	2.7
Cauliflower, flower only	12	5.4	—	—
Meat	15	6.8	10	4.5
Pork meat	2½	1.1	1	0.45
Skimmed milk powder ..	28	12.7	28	12.7
Rice	4	1.8	2	0.90
Macaroni	2	0.90	1	0.45
Margarine	12	5.4	7	3.2
Lard	2½	1.1	1½	0.56
Jam	14	6.4	7	3.2
Marmite	½	0.22	¼	0.11
Baking powder	½	0.22	—	—
Custard powder	1	0.45	1	0.45
Chocolate	6½	3.0	2	0.90
Beans, tinned	12	5.4	6	2.7
Shredded wheat and corn flakes	3	1.4	1½	0.67

TABLE XXXII

Vitamin A Potency and Total Carotenoid Content of Certain Foodstuffs Included in the Deficient Diet

Foodstuff	Report		Total carotenoids ("I.U." per 100 g.)	α- and β-carotene (I.U. per 100 g.)	Remarks
	Date	Laboratory			
Bread, National Wheatmeal	11. 5.43	Cambridge	170	—	—
	11. 5.43	Cambridge	160	—	—
	11. 5.43	Cambridge	115	—	—
Hovis	11. 5.43	Cambridge	200	—	—
	11. 5.43	Cambridge	180	—	—
	11. 5.43	Cambridge	165	—	—
Biscuits, Ryvita	23. 6.43	Cambridge	79	0	—
Weetabix	23. 6.43	Cambridge	63	0	—
Shredded Wheat.. ..	23. 6.43	Cambridge	65	0	—
Flour, white, imported Canadian Government Regulation	5. 7.43	Cambridge	68	8.6	Bleached with agene (nitrogen trichloride)
			60	6.3	Bleached with novadelox (benzoyl peroxide)
	12. 8.43	Liverpool	98	—	Bleached with agene, pigment almost completely xanthophylllic
			149	—	Unbleached
Flour, National Wheatmeal	11. 5.43	Cambridge	180	—	—
Jam, strawberry	27. 6.44	Cambridge	15	—	—
Jelly, bramble	27. 6.44	Cambridge	14	3	—
Milk, dried, skimmed ..	25. 1.44	Liverpool	7.4	—	Vitamin A not detected
Potatoes	28.10.43	Liverpool	128	14	—
	22. 3.44	Liverpool	82	<10	Uncooked
	22. 3.44	Liverpool	75	<10	Cooked
	27. 3.44	Cambridge	200	32	King Edward, cooked ("I.U." per 100 g. wet weight)
	27. 3.44	Cambridge	85	20	Potatoes from Sheffield, cooked ("I.U." per 100 g. wet weight)
	12. 4.44	Reading	70	10	—
Dripping, beef	21. 6.44	Liverpool	95	—	Vitamin A not more than 130 I.U. per 100 g.
	23. 6.44	Cambridge	100	—	Vitamin A not detected

individuals during a representative week in January, 1944, together with the food values, are given in Table XXXV, p. 82.

Special supplies of certain foods were arranged by the Ministry of Food and through the courtesy of the commercial firms concerned :

Margarine without colouring matter or supplementary vitamins was supplied by Marcom, Ltd., 1, Watergate, London, E.C.4, by arrangement with Messrs. Lever Bros. and Unilever.

Chocolate and cocoa without supplementary vitamins were supplied by Messrs. Rowntree, York.

Jams. Strawberry jam and bramble jelly were supplied by Messrs. Chivers and Sons, Cambridge.

Skimmed-milk powder in large batches was supplied through the Ministry of Food, Milk Powder Pool.

The supplies of many of the items of food used were specially tested for vitamin A potency before being admitted to use. The details are given in Table XXXII.

The bread eaten during the first year of the experiment was baked from National Wheatmeal (85 per cent. extraction), which was the only flour available to the general public at that time. From 10th August, 1943, onwards this was entirely replaced by a white flour, Canadian Imported Government Regulation Flour, which was specially bleached by treatment with 7 g. agene (nitrogen trichloride) per 280-lb. sack at the mills of Messrs. Cranfield Bros., Ipswich.

The object of using the bleached flour was to facilitate the estimation of carotene in the faeces by reducing the content of total carotenoids. The actual effect achieved by substituting the bleached flour was to reduce the value for total carotenoids from 149 to about 70 "I.U." per 100 g. of flour.

When National Wheatmeal was not used, an adequate supply of the B vitamins was ensured by including in the bread 1 per cent. of food yeast (Medical Research Council, 1945b), supplied from the National Chemical Laboratory, Teddington, by courtesy of Dr. A. C. Thaysen.

Diet without vegetables.—In the hope of accelerating depletion, all vegetables including potatoes, and all fruits with the exception of 1 lb. of strawberry jam per head per week, were withheld from a group of the volunteers from 22nd May, 1943, to 15th September, 1943. Only the foods listed in Table XXXIII were allowed to this group. In Table XXXIV are shown the amounts of food entering the kitchen for one representative week of this period.

TABLE XXXIII

Foods Permitted on "Vegetable-Restricted" Diet

Breakfast cereals	Pork products	Flavours and essences
Rice	Mutton	Margarine
White flour, white bread	Beef, no fat	Syrup
Tapioca	White fish	Strawberry jam
Semolina	Bacon	Tea
Sago	Skimmed milk powder	Lard
Cornflour		Sugar
Spaghetti		
Macaroni		

TABLE XXXIV

*Food Consumption on "Vegetable-Restricted" Diet in a Sample Week
(Gross amounts entering kitchen)*

<i>Foodstuff</i>	<i>For 9 people (1 week, August, 1943)</i>	
	<i>(lb.)</i>	<i>(kg.)</i>
Oats	3	1·4
Bacon	5	2·3
Bread	50	22·7
Tea	1	0·45
Sugar	8	3·6
Syrup	4	1·8
Biscuits	2	0·90
Meat	13	5·9
Pork meat	1½	0·68
Skimmed milk powder	14	6·4
Rice	7	3·2
Macaroni	3	1·4
Margarine	7	3·2
Lard	1½	0·55
Jam	8	3·6
Custard powder	1	0·45
Wheat flakes	3	1·4

Supplements.—The basal diet was supplemented throughout with 50 mg. ascorbic acid, as "Redoxon Roche", per head daily. During the winter months a weekly dose of 3,000 I.U. vitamin D₂, as "Radiostol" of the British Drug Houses, was given to each person. For a short time, from early December, 1942, to early January, 1943, when some volunteers showed a lowering of haemoglobin values, possibly as a result of frequent blood withdrawals, a dose of four 3-grain tablets of exsiccated ferrous sulphate per week was given, but it was found unnecessary to continue this.

ADEQUACY OF THE DIET

Analysis of the food values was made on a representative sample of the diet collected on the seven days from the 13th to 19th January, 1944. On each of the 7 days a different volunteer was asked exactly to duplicate his selection of food for the day and, after recording the amount, to set it aside for analysis. The quantities of food eaten on the days of collection are set out in Table XXXV, together with the food values for the edible portion calculated from the Medical Research Council's (1945a) Tables of Food Composition. The food collected was pooled and kept in the refrigerator, and at the end of the week was taken to Cambridge in an insulated container where it was delivered to Sergeant J. A. Childs of the R.A.F. Nutrition Team, under Wing Commander T. F. Macrae, to be dried by the method used by Macrae, Henry, and Kon (1943) for Royal Air Force diets. It was thoroughly mixed, frozen, and ground. The larger portion was dried in an oven at a temperature not greater than 70° C., and the rest was vacuum-ice-dried. The whole was stored at -20° C.

The amount of dry food consumed proved on the average to be 650 g. per head daily. Analyses were made on the oven-dried material for calories, total nitrogen, fat, and carbohydrate at Sheffield; for calcium and phosphorus at Reading; and on the vacuum-ice-dried material for iron

TABLE XXXV

Quantities of Food Consumed by Individual Volunteers on the Seven Days, 13th to 19th January, 1944, with their Food Values Calculated from the Tables for the Edible Portion (Medical Research Council, 1945a) (for cooking losses the percentage deducted from the total value for the diet was 15 for vitamin B₁ and 50 for vitamin C. The value for riboflavin does not include that for the items marked *)

Foodstuff	13th Jan., Bloomfield	14th Jan., Wodeman	15th Jan., Garling	16th Jan., Proctor	17th Jan., Watson	18th Jan., Thompson	19th Jan., Woodhouse	Average per man per day
Milk, skimmed (ml.)	500	254	350	400	635	200	500	
Porridge* (g.)	480	800	285	398	155	350	390	
Potatoes (g.)	482	360	250	370	335	70	262	
Bread, white (g.)	535	465	490	290	330	510	464	
Margarine (g.)	100	37	39	50	20	70	36	
Beetroot (g.)	60	42	69	150	40	35	—	
Meat (g.)	46	—	38	31	40	135	70	
Beans (g.)	107	43	30	—	32	—	464	
Bacon (g.)	—	23	8	18	10	25	16	
Apple pie* (g.)	109	—	—	—	—	150	—	
Custard (g.)	55	62	—	—	—	70	72	
Peanut butter* (g.)	80	—	—	20	—	30	—	
Spam (g.)	25	56	26	30	28	—	—	
Scone (g.)	—	118	—	—	—	—	—	
Jam* (g.)	—	25	30	20	16	60	—	
Rice pudding (g.)	—	—	252	240	170	—	—	
Beans, rice, onion mixture (g.)	—	—	100	—	80	—	—	
Corn flakes (g.)	—	—	12	—	—	—	—	
Gravy (g.)	—	—	—	34	—	—	—	
Syrup tart (g.)	—	—	—	—	—	50	—	
Syrup (g.)	—	—	—	—	—	55	90	
Steamed pudding (g.)	—	—	—	—	—	—	106	
Onions (g.)	—	—	—	—	—	—	40	
<i>Dietary constituents</i>	<i>Calculated food values</i>							
Calories	3,660	2,870	2,800	2,560	2,100	3,310	2,990	2,900
Protein (g.)	139	117	102	92	87	108	133	111
Carbohydrate (g.)	436	446	459	358	340	427	463	418
Fat (g.)	152	69	61	84	44	130	68	87
Calcium (g.)	1.5	1.4	1.1	1.3	1.3	0.9	1.4	1.3
Iron (mg.)	18.4	15.9	9.1	12.5	12.8	14.0	18.0	14.4
Vitamin B ₁ (mg.)	1.9	2.1	1.5	1.9	1.6	2.1	1.8	1.8
Vitamin C, including 50 mg. supplement (mg.)	86	81	77	86	80	67	75	79
Riboflavin (mg.)	3.0	3.0	2.4	2.3	2.1	2.3	3.1	2.6

by Sergeant Childs, and for riboflavin and nicotinic acid by Sergeant S. Parkin. These values, per portion of 650 g. dry food, are set out in Table XXXVI, where they are compared with the average values for the week, obtained by calculation as described above and set out in Table XXXV, and with the requirements formulated by the League of Nations Technical Commission on Nutrition (1938) and in the U.S.A. by the National Research Council (1941). The particulars of the analysis were as follows :

Calories.—Bomb calorimeter estimations by Mr. W. A. Batley, Department of Fuel Technology, University of Sheffield : 1,000 g. food gave 4,852 Calories. On the assumption that the physiological heat values of fat and carbohydrate are 97 per cent., and of protein 75 per cent., of the calorimetric value, and with no deduction for indigestible roughage, the daily ration of 650 g. contained 2,930 Calories.

Protein.—Total nitrogen (Kjeldahl) : 0.507 g. diet gave 14.8 mg. nitrogen. Thus, the daily ration contained 19.0 g. nitrogen. With a factor of 6.0 for the calculation of protein, in view of the considerable proportion of wheat products in the diet (*see* Medical Research Council, 1945a), and with no deduction for non-protein nitrogen, the daily protein intake was 114 g.

Carbohydrate.—Method of McCance and Lawrence (1929) : 1.00 g. diet contained 0.70 g. glucose after acid hydrolysis. Thus, the daily ration contained 407 g. starch.

Fat.—Soxhlet extraction : 11.50 g. diet contained 1.54 g. ether-soluble matter. Thus, the daily ration contained 87.0 g. fat.

Calcium.—Method of McCrudden (1911) : 100 g. diet contained 0.2066 g. calcium. Daily intake 1.34 g.

Phosphorus.—Strychnine molybdate method of Embden-Felter as described by Peters and van Slyke (1932) : 100 g. diet contained 0.2988 g. phosphorus. Daily intake 1.94 g.

Iron.—Method of McCance (1933) : 10 g. of freeze-dried diet contained 0.478 mg.

TABLE XXXVI

Average Daily Food Values, Calculated and Found by Analysis, of Diet Consumed During a Single Week in January, 1944
(Average weight of 7 Volunteers taking part in sampling, 67.6 kg.)

	Calculated food value (see Table XXXV)	Food value found by analysis of dried sample	Requirements of moderately active man weighing 70 kg.	
			Standards of National Research Council (1941)	Standards of League of Nations (1938)
Calories	2,900	2,930	3,000	3,000
Protein (g.)	111	114	70	70
Carbohydrate (as starch) (g.)	418	407	—	—
Fat (g.)	87	87	—	80 to 125
Calcium (g.)	1.3	1.3	0.8	0.75
Iron (mg.)	14.4	31.1	12	10
Vitamin B ₁ (mg.)	1.8	—	1.8	0.9
Riboflavin (mg.)	2.6	2.8	2.7	—
Nicotinic acid (mg.)	—	18.3	18	—
Ascorbic acid (mg.)	79	—	75	30

Riboflavin.—Microbiological method of Snell and Strong (1939), modified.

Nicotinic acid.—Microbiological method of Snell and Wright (1941), modified.

The staple items providing most of the food value were bread, potatoes, porridge, margarine, and skimmed milk. Bread and milk alone supplied about 40 per cent. of the calories and protein. Of the protein, between one-quarter and one-third was of animal origin. The consumption of the staple items was fairly constant throughout the experiment, except for the period when some of the subjects were deprived of potatoes. The data collected for the sample week may therefore be regarded as being typical.

It can be seen from Table XXXVI that, except for vitamin A, the above food values compare very favourably with the standards of requirements of the National Research Council (1941). The adequacy of the diet was confirmed by the clinical observations during the course of the experiment, since perfect health was maintained when the diet was supplemented by vitamin A or its precursors, and in the deprived group there were no signs of malnutrition that could not be directly attributed to vitamin A deficiency. The deficient diet maintained bodyweight.

The amount of iron found by direct analysis was more than double that obtained by calculation from the Food Tables. The kitchen equipment included chipped enamel and iron pots, and knives not of stainless steel, from which iron is liable to pass into the food, as has been shown by Widdowson and McCance (1943).

VITAMIN A POTENCY OF THE BASAL DIET

The vitamin A potency of the diet was tested chemically and biologically.

Chemical Test

The chemical test was made on the oven-dried material at the laboratories in Cambridge and Liverpool. The methods used were those described for foods (p. 99).

The average daily intake of total carotenoids, and of α - and β -carotene, was found to be as follows :

Total carotenoids ("I.U.")		α - and β -carotene (I.U.)	
Cambridge	Liverpool	Cambridge	Liverpool
325	466	65	62

The intake of biologically active carotenoids was thus very small.

Biological Test

The biological test was not carried out on the dried sample.

The diet consumed by the volunteers was tested on rats for its vitamin A potency by Mr. W. A. Broom, of Messrs. Boots Pure Drug Co., Ltd., Nottingham.

The food was collected twice weekly, on Monday and Thursday, for dispatch on Tuesday and Friday. On the two days of collection, food was served for one extra man and this portion was put on one side; it was roughly representative of the average amount eaten per head but the quantity was not weighed. At the end of the day the total amount of food

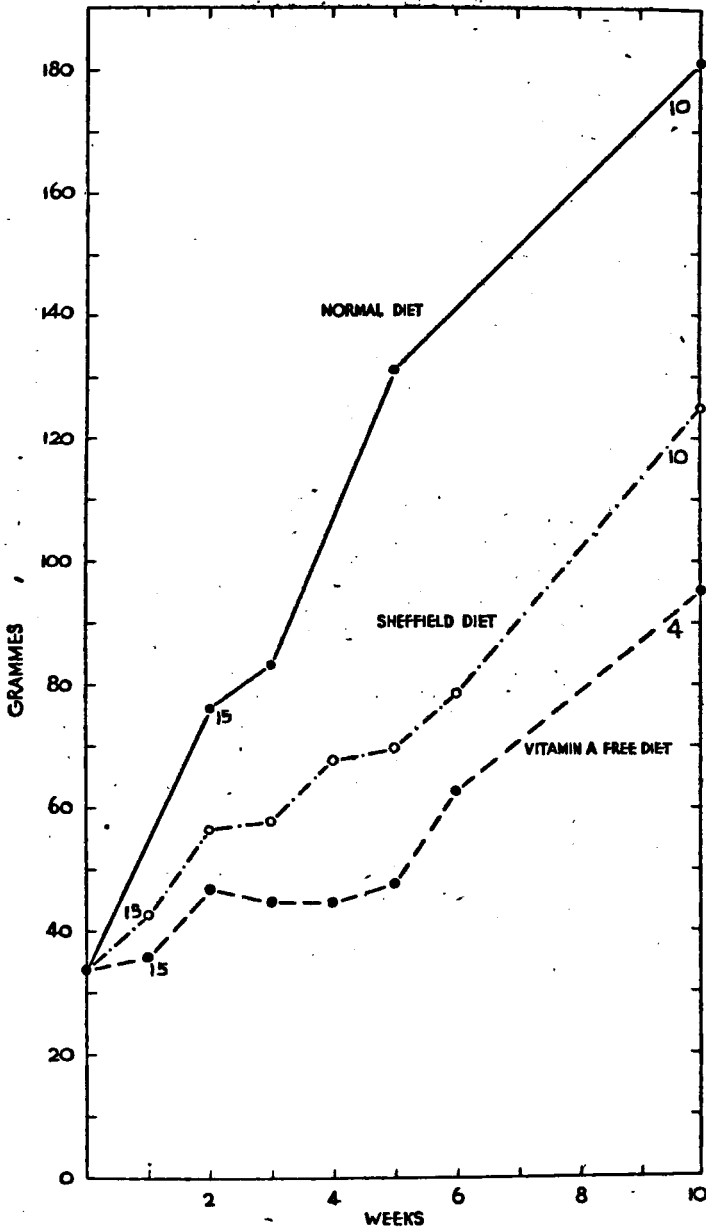


FIG. 10.—Average rate of weight increase of rats receiving a normal diet, the human (Sheffield) diet deficient in vitamin A, and a rat diet deficient in vitamin A. The numerals on the chart indicate the number of rats from which the average was derived.

excluding the liquid milk was put through a mincer. The liquid milk was then added and the whole well mixed. No other fluids were added. The food was sent to Nottingham by passenger train, a distance of 40 miles.

Three groups of rats were formed, littermates being distributed among them according to the established method.

The first group of young rats, 4 to 5 weeks old, received to appetite a basal rat diet deficient in vitamin A consisting of :

Casein (Glaxo, ashless, extracted) ..	20	} parts, by weight.
Rice starch	60	
Salt mixture	5	
Dried yeast	6	
Arachis oil	10	
Vitamin D ₂	10 I.U. per rat weekly.	

A similar group of rats received a normal diet, and the third group the human experimental diet from the Sorby Institute, at Sheffield.

The experiment lasted 10 weeks, by the end of which time most of those receiving the basal rat diet deficient in vitamin A were dead, some with xerophthalmia. When they died, the livers of some of them and of littermates in the other two groups killed on the same day were sent to Cambridge for estimation of the vitamin A reserves by the method regularly used there (Davies, 1933). The livers of five rats in each group were thus analysed during the experiment and, at the end, all the survivors were killed and their livers analysed.

The rats receiving the Sorby Institute diet did not die, but their growth was much less than that of the rats receiving the normal diet, though better than that of the rats wholly deprived of vitamin A (Fig. 10), and they did not show xerophthalmia though their coats were poor.

The reserves of vitamin A found in the liver are shown in Table XXXVII. They were *nil* for all the nine rats receiving the vitamin A deficient basal rat diet, on which analyses were made, whether they died during the experiment or were killed at the end of it. The reserves were *nil* also in all the fifteen rats receiving the Sorby Institute diet, except in one animal killed during the experiment, in which the total reserve was 18 I.U. In the rats receiving a normal diet the total reserve ranged from 75 to 600 I.U. for those killed during the experiment, and from 225 to 1,800 I.U. for those killed at the end.

The results of the biological test are thus in agreement with those of the chemical test, and indicate the presence in the Sorby Institute diet of a small residual amount of vitamin A potency, so that young rats fed on it survived the experimental period of 10 weeks but suffered total depletion of their liver reserves of vitamin A.

SUPPLEMENTS OF VITAMIN A AND CAROTENE

Choice of Supplements

For the curative experiments, β -carotene in oil and vitamin A in oil were the only materials that it was possible to test, but for the prophylactic experiments it was planned to use different materials in successive periods. It was desired to test certain natural foodstuffs, and particularly to make a comparison between green and yellow vegetables. The bulk that could conveniently be consumed as a daily dose was to some extent a factor limiting

TABLE XXXVII

Vitamin A Reserves in the Liver of Littermate Rats of Average Initial Weight 34 g., Maintained for Periods up to 10 Weeks on a Normal Diet, on an Experimental Rat Diet Deficient in Vitamin A, and on the Sheffield Experimental Human Diet Deficient in Vitamin A

Diet	Litter marking of rats	Sex	Weight, final (g.)	Date when animal died or was killed	Total vitamin A content of liver (I.U.)
Normal	3R1L	M	182	17.5.44	600
	3L1R	M	125	18.5.44	75
	2L1R	M	158	18.5.44	225
	2L	F	114	18.5.44	225
	2R1L	M	152	22.5.44	225
	Plain	F	142	9.6.44	1,800
	1R	F	144	9.6.44	1,200
	1L	F	142	9.6.44	1,200
	2R	M	200	9.6.44	600
	1R1L	M	200	9.6.44	750
	2R2L	M	240	9.6.44	1,200
	3R	M	193	9.6.44	750
	3L	F	125	9.6.44	225
	3L2R	M	245	9.6.44	450
	3L2R	F	177	9.6.44	750
	Vitamin A deficient for rats	3R1L	M	40	17.5.44
3L1R		M	34	18.5.44	0
2L1R		M	58	18.5.44	0
2L		F	37	18.5.44	0
2R1L		M	53	22.5.44	0
1R		F	74	9.6.44	0
1L		F	65	9.6.44	0
2R2L		M	127	9.6.44	0
3R	M	115	9.6.44	0	
Vitamin A deficient for humans (as used in present study at the Sorby Institute)..	3R1L	M	54	17.5.44	0
	3L1R	M	68	18.5.44	0
	2L1R	M	84	18.5.44	0
	2L	F	66	18.5.44	18
	2R1L	M	85	22.5.44	0
	Plain	F	120	9.6.44	0
	1R	F	125	9.6.44	0
	1L	F	115	9.6.44	0
	2R	M	115	9.6.44	0
	1R1L	M	165	9.6.44	0
	2R2L	M	94	9.6.44	0
	3R	M	144	9.6.44	0
	3L	F	80	9.6.44	0
	3R2L	M	160	9.6.44	0
	3L2R	F	131	9.6.44	0

choice, so that only foods with a fairly high concentration of carotene were suitable.

The test periods were not short, so that it was imperative that each test material should consist of a single uniform batch to obviate the need for repeated analyses. Fresh vegetables, therefore, were not used but single consignments were obtained of two brands of canned carrots and two of canned spinach. In order to test cabbage, a supply of the dehydrated vegetable was obtained.

Analyses were usually made at Cambridge and Liverpool, sometimes also at Reading. The methods used are described on p. 99. All the values obtained are assembled in Table XXXVIII.

Values for Vitamin A Potency of Supplements

For assessing the vitamin A potency of the supplements in relation to the state of vitamin A nutrition, figures based on the results of analyses by several laboratories were used. Agreement was good except for dried cabbage, which was hard to sample. The values arrived at in this way and used for the above purpose are set out in Table XXXVIII.

A different procedure was used for assessing the value of the supplements as a basis for calculating the percentage of carotene or of total carotenoids excreted in the faeces. The value adopted for the supplement was in this case the one obtained by the same laboratory that had made the analysis of faeces. In averaging the results of more than one laboratory the figures averaged were in this case those for the percentage excretion.

Nature of Supplements and Mode of Administration

Vitamin A.—The concentrate of vitamin A was a mixture of distilled natural esters supplied by the British Drug Houses. The potency was determined spectrophotometrically at intervals at Liverpool. The results obtained were as follows :

<i>Bottle number</i>	<i>Date of report</i>	<i>Vitamin A (I.U. per g.)</i>
1	2.11.43	1,450
1	26. 1.44	1,340
2	26. 1.44	1,220
2	3. 2.44	1,275
2	27. 3.44	1,160
3	27. 3.44	1,180
3	15. 5.44	1,100
4	15. 5.44	1,450

In the earlier part of the experiment the amount to be administered was measured daily in a specially graduated pipette, run into a watch glass and licked off ; later, it was weighed directly into the watch glass.

Carotene margarine was specially prepared by Messrs. Lever Bros. The appropriate daily dose was weighed.

β -Carotene in oil.—The crystalline β -carotene used at first was obtained from Messrs. Lever Bros. and later under Lease-Lend from the U.S.A., where it was manufactured by the S.M.A. Corporation, Chagrin Falls. The latter product contained 10 per cent. α -carotene.

The solution in arachis oil which was used for dosing was prepared by the Department of Biological Standards of the National Institute for Medical Research by the same method as is used for the International β -Carotene Standard. The solution was made as strong as possible and contained about 330 I.U. per g. together with 0.1 per cent. hydroquinone. The appropriate amount of solution was measured daily in a specially graduated test tube.

Dehydrated cabbage.—The dried cabbage was prepared by a manufacturing process but, with the help of the Low Temperature Research Station at Cambridge, a special batch was prepared in which the largest proportion possible of green outer leaves was selected. The daily dose was weighed,

TABLE XXXVIII

Content of α - and β -Carotene and of Total Carotenoids in Materials Used Prophylactically and Curatively as Supplements to the Diet Deficient in Vitamin A

Nature of supplement	Date of report	Total carotenoids ("I.U." per g.)			α - and β -carotene (I.U. per g.)			Values used for calculating amounts of supplements (I.U. per g.)*
		Cambridge	Liver-pool	Reading	Cambridge	Liver-pool	Reading	
Carrots, canned, sliced	7. 8.42	—	—	—	133	—	—	133 (1942 and 1943) 98·6 (1944)
	11.10.43	—	—	—	134	—	—	
	20-23.6.44	144	132	—	99	96	—	
		133	—	—	97	—	—	
152	—	—	—	104	—	—		
Carrots, canned, sliced, purée	11.10.43	180	—	—	117	—	—	133
Carrots, canned, homogenized	23.12.43	—	160	—	—	125	—	127·5
	23.10.44	160	—	—	118	—	—	
		150	—	—	—	135	—	
					140			
					138			
Cabbage, dried, outer leaves	19.8.43	—	812	—	—	261	—	358
		—	853	—	—	230	—	
	24.8.43	—	1,330	—	—	483	—	
	13.9.43	—	990	—	—	280	—	
	11.10.43	—	840	—	459†	210	—	
28.10.43	—	1,020	—	—	192	—		
			654			134		
Spinach, canned, purée	17.5.43	—	138	—	87	73	—	71·7
	9.6.43	—	—	—	70	—	—	
Spinach, canned, homogenized	17-23.12.43	—	113	107	35	31	32	41·3
		—	—	—	—	—	30	
	18-21.1.44	—	—	97	41	—	33	
					44			
					42			
β -Carotene in margarine (Lever Bros.)	22.11-8.12.43	197	—	—	173	172	—	172
β -Carotene in arachis oil with hydroquinone (Lever Bros.) (S.M.A. Corporation)	12.3.43	—	—	—	—	361	—	Value frequently re-adjusted
	29.4.43	—	—	—	—	345	—	
	7.6.43	—	—	—	—	356	—	
	2.11.43	—	—	—	—	352	—	
	22.11.43	—	—	—	—	320	—	
	4.1.44	—	—	—	—	307	—	
	26.1.44	—	—	—	—	348	—	
	21.3.44	—	—	—	—	356	—	
	28.4.44	—	—	—	—	330	—	
	10.5.44	—	—	—	339	—	—	

* The figures given in this column were based on results which were subsequently modified by the Cambridge Laboratory's introduction of a small correction factor, after most of the Report had been written. Since the actual change involved is very small, it was not thought necessary to recalculate the values throughout the whole Report in the light of this modification. The values given in Protocol B are the Cambridge Laboratory's corrected values.

† Mean of six estimations, 442, 455, 456, 467, 468, 470.

put in water and cooked for 20 to 30 minutes. Any water not taken up by the cabbage was drunk and the vegetable was eaten with dinner.

Canned carrots, sliced, and purée.—The canned carrots came from a single batch supplied by Messrs. Chivers and Sons. The bulk supply was stored at about 10° to 15° C. The carotene content after 14 months' storage (from 7th August, 1942, to 11th October, 1943) seemed unchanged, but 9 months later (June, 1944) the carotene content was found to be about 75 per cent. of the original value; this phenomenon of a fall beginning after an induction period was also found for carotene in canned tomato juice by Guerrant, Vanich, and Dutcher (1945). The daily dose was weighed after the water had been drained off and was then eaten. When made into purée the drained carrot was passed through a "Latapie" mincer and the dose weighed.

Homogenized carrots were provided by Messrs. Nestlé's Milk Products, Ltd. The material belonged to the one batch AVWMRL.

Homogenized spinach was provided by Messrs. Nestlé's Milk Products, Ltd. The material belonged to the one batch AAEURL.

Spinach purée, "Lin Can" brand made by Messrs. Lincolnshire Cannery, Ltd., was all of one consignment obtained from a wholesaler.

The daily dose of homogenized carrots, homogenized spinach, or spinach purée was the appropriate weighed amount of the total contents of the tin.

Q.—Methods Used for the Estimation of Vitamin A, Total Carotenoids and Carotene

BLOOD PLASMA

Sampling

Blood was drawn from the cubital vein, usually between 7.30 and 9 a.m., and mixed with sodium oxalate and sodium fluoride. The plasma was separated from the cells on the centrifuge and divided into portions for dispatch by express post to Cambridge, Liverpool, and Oxford. The samples usually reached their destination 24 hours after collection. Cambridge required about 6 ml., Liverpool 15 to 25 ml., and Oxford 2.5 ml.; Liverpool, in view of the large amount needed, was usually supplied at four-weekly intervals, while Cambridge and Oxford received samples fortnightly.

Sterility was not maintained after the withdrawal from the vein and no preservatives were added. During the first 8 months an equal volume of ethanol was added to the Oxford sample before dispatch, this being the first step of the analytical procedure at Oxford. It was later established, however, that samples thus treated often yielded low values for vitamin A and for carotene, because the alcohol was added rapidly with the result that vitamin A and carotene were not properly extracted from the thick coagulum formed. The addition of ethanol at the Sorby Institute was, therefore, abandoned and all data obtained with this procedure were discarded. On the other hand, it was found that the vitamin A and carotene content remained constant for several days in non-sterile human plasma, to which alcohol had not been added.

Procedure at Cambridge

At one time the method used in this laboratory included digestion with alkali but the advisability of this procedure was questioned by Robertson (1941), who compared various methods with and without the use of alkali. He found that higher results were often obtained by the addition of ethanol to the plasma, followed by simple extraction with ether and light petroleum, than by the same treatment preceded by heating the plasma with potassium hydroxide. The extent of the difference decreased with the thoroughness of the digestion, and finally disappeared after prolonged heating. Since the same results were achieved more quickly by extracting the plasma without digestion, the use of alkali was discarded as merely running an unnecessary risk.

The procedure used throughout the present experiments differed only slightly from Yudkin's (1941b) modification of Kimble's (1939) method. In a corked centrifuge tube 6 ml. plasma were shaken by hand with 6 ml. ethanol; 12 ml. light petroleum were then added, and the mixture was shaken for 10 minutes in a mechanical shaker. After being centrifuged 10 ml. of the light petroleum layer were evaporated to dryness on a hot waterbath at diminished pressure, and were made up in 5 ml. of a mixture of four parts of light petroleum and one part ethanol. The purpose of adding the ethanol was to prevent turbidity. Carotenoids were then estimated in a photoelectric absorptiometer with a blue filter. The solution used in the absorptiometer cell was restored to the flask and was again evaporated to dryness and made up in 1 ml. chloroform, which was carefully twirled round the sides of the flask. A cell containing 2 ml. antimony trichloride reagent, to which one drop of acetic anhydride per ml. had been added to prevent turbidity, was placed in position in the absorptiometer with a red filter, and 0.3 ml. of the plasma extract was introduced rapidly with quick stirring. When the stirring rod was withdrawn the "hesitation point" in the galvanometer reading was noted. A second reading was then made, and the average taken.

The absorptiometer was made with a single photo-cell and was illuminated by a lamp with a variable resistance. The blue filter consisted of one layer of Wratten No. 47 between pieces of plain glass. The red filter consisted of five layers of Wratten No. 25 between a piece of "Calorex" glass and a piece of plain glass. A solution of the International Standard carotene was used to make a calibration curve for carotenoids. A specimen of vitamin A acetate, having the value $E_{1\text{ cm.}}^{1\%} = 325 \text{ m}\mu = 1,480$ was used to calibrate for vitamin A, the factor for converting the values for $E_{1\text{ cm.}}^{1\%}$ into I.U. per g. being taken as 1,600. Difficulty was experienced in finding the correct amount which should be deducted for the contribution made by carotenoids to the blue colour in the antimony trichloride test, since the correction varied according to the medium in which the carotene was present. After numerous experiments with human and bovine plasma a deduction from the vitamin A value of one-quarter of the carotenoid value, as expressed in I.U. of β -carotene on a colour basis, seemed the most reasonable estimate. In order to ensure continuity in the calibration of the absorptiometer through

all the running repairs which were necessary from time to time, sets of blue and yellow Lovibond glasses were used as subsidiary standards.

Procedure at Liverpool

Two methods were used during the investigation; the first, in which the plasma was saponified, was discontinued when the second method was found to be effective.

Method A.—To every 10 ml. plasma were added 3 ml. 60 per cent. aqueous potassium hydroxide and 8 ml. ethanol, addition in that sequence preventing precipitation of protein. Saponification was carried out by heating on a waterbath for 2 minutes after the mixture had begun to boil; constant shaking was required to prevent excessive frothing. After being cooled the mixture was added to an equal volume of water in a separating funnel. The residue in the flask was washed into the funnel with again the same volume of water, and the funnel was rinsed with 5 to 10 ml. ethanol.

The contents of the separating funnel were extracted three times with ether freshly redistilled over reduced iron. The ether extracts were combined and washed successively, once with distilled water, once with 0.05 *N* potassium hydroxide,* and then with distilled water until the ether layer was free from alkali as shown by a test with phenolphthalein. The ether was distilled off on a warm waterbath and the last traces of moisture were removed from the unsaponifiable fraction by heating it twice with 1 to 2 ml. absolute ethanol in a current of nitrogen.

Method B.—This was essentially similar to that described by Yudkin (1941b). The plasma was run slowly and with constant shaking into an equal volume of absolute ethanol. By this means the plasma proteins were precipitated, and the vitamin A, together with the lipids, was removed by two extractions with an equal volume of light petroleum of b.p. 40° to 60° C. The total light petroleum extract was evaporated to dryness and the residue was blown dry with alcohol under nitrogen.

Both the unsaponifiable fraction and the lipid extract were examined in the same way. The dry residue was dissolved in a suitable volume of light petroleum of b.p. 40° to 60° C., and the "carotene" absorption was measured in a Hilger Nutting visual spectrophotometer at *c.* 450 $m\mu$.

Optimum accuracy in the region of 450 $m\mu$ is obtained when the instrument records an extinction ($E = \log \frac{I_0}{I}$) *c.* 1.5. This optimum condition of accuracy is rarely attained in practice, usually because the sample of blood plasma is too small or because the concentration of carotenoid pigment is too low. The cells usually used in this laboratory for estimation of vitamin A and carotene were 2 cm. deep with an internal diameter of *c.* 1.5 to 2.0 cm. and needed 5 ml. of the test solution to fill them. They were fitted with side tubes by which the solution under examination could be recovered quantitatively for further use. The objection to diluting the plasma extract to 5 ml., and thus weakening the colour, was overcome by constructing a special holder by which a microcell, which gave a 2 cm. light path and required only 2 ml. of solution, could be placed in the light beam of the spectrophotometer.

* It was later found that washing three times with tepid water at 30° C. is sufficient to remove all traces of soaps and alkali, and the washing with 0.05 *N* potassium hydroxide was omitted after this.

Example :

Extract from 8 ml. blood plasma in 2 ml. light petroleum (400 per cent. by volume) ; E at 447 m μ with a 2 cm. cell = 1.41.

$$E \frac{1\%}{1 \text{ cm.}} 447 \text{ m}\mu^* = \frac{1.41}{400 \times 2} = 0.001762$$

$$E \frac{1\%}{1 \text{ cm.}} 450 \text{ m}\mu^* \text{ pure } \beta\text{-carotene in light petroleum} = 2,500.$$

$$\begin{aligned} \text{Percentage of carotene in plasma} &= \frac{0.001762 \times 100}{2,500} \\ &= 0.000705 \end{aligned}$$

i.e., 70.5 μ g carotene per 100 ml. plasma
or 117.5 I.U. per 100 ml. plasma.

The solution used for the carotene estimation was recovered from the cell (*see below*) and the solvent was removed under nitrogen ; 2 ml. of the Carr Price reagent (saturated solution of antimony trichloride in chloroform) were added directly to the extract or to an aliquot part of it in chloroform, according to the amount of plasma used and its probable potency. The vitamin A content was determined by measuring the intensity at 617 m μ of the blue colour produced.

Example :

Extract from 19 ml. of blood plasma in 2 ml. antimony trichloride reagent (950 per cent. by volume) ; E at 617 m μ with 2 cm. cell = 2.10

$$E \frac{1\%}{1 \text{ cm.}} 617 \text{ m}\mu = \frac{2.1}{950 \times 2} = 0.001105$$

$$E \frac{1\%}{1 \text{ cm.}} 617 \text{ m}\mu = 1.0 \text{ (pure vitamin A)} \equiv 510 \text{ I.U. per g. (ml.)}$$

$$\begin{aligned} E \frac{1\%}{1 \text{ cm.}} 617 \text{ m}\mu &= 0.001105 \equiv 0.563 \text{ I.U. per ml.} \\ &= 56.3 \text{ I.U. per 100 ml.} \end{aligned}$$

Corrections applied to vitamin A values when measured in the presence of carotenoids.—Both carotene and xanthophyll give blue-green colours with antimony trichloride (λ_{max} . carotene 585 m μ , xanthophyll 621 m μ), which in some small degree interfere with the assessment of the blue colour developed by the vitamin A present. The intensities of the colours produced with the pigments are so much weaker than with vitamin A ($E \frac{1\%}{1 \text{ cm.}} 585 \text{ m}\mu$ for carotene = 420 ; $E \frac{1\%}{1 \text{ cm.}} 617 \text{ m}\mu$ for vitamin A = 5,600) that, with a method in which the maximum of absorption for antimony trichloride is isolated and measured at 617 m μ , as it is in the spectrophotometric method, the interfering effect of carotenoid absorption can reasonably be ignored. This is not the case with colorimetric determinations of vitamin A in which the best filters available do not transmit anything approaching monochromatic light.

* The small difference in λ_{max} . for the plasma and for pure β -carotene is due to the presence of xanthophylls in the former.

Accuracy of the method. Vitamin A.—In examining blood plasma for vitamin A two factors militate against maximum accuracy :

(a) Only one test is generally possible for each sample.

(b) As a result it is not possible in a second test to adjust the concentration of the sample so that optimum extinction values can be obtained ($E = c \cdot 2.0$). This may result in the instrument not being in the best adjustment for the extinction obtained, e.g., good accuracy at $E = c \cdot 1.0$ can be achieved by judicious use of the slit of the spectroscope. When both these factors are taken into consideration it is not possible to claim an accuracy greater than ± 10 per cent. for the spectrophotometric readings in a single estimation of blood vitamin A.

It should be pointed out that a photo-electric spectrophotometer combines the advantages of using monochromatic light, of speed in measuring a transient colour, and of accuracy in measuring weak colours. These advantages permit trustworthy results to be obtained and duplicated with quite small samples of plasma. At the time this work was carried out it was necessary to make the best of the apparatus available ; this meant that some determinations were less accurate than could have been desired, but the picture as a whole is accurate enough.

Carotenoids.—Accuracy in estimating the carotenoids is not affected to such a large extent by the factors discussed above. The colour is stable and a number of readings can be taken on the same solution. When the carotenoid content is low the error in reading E values is increased, but an overall accuracy of ± 5 per cent. for the results of these determinations can safely be claimed.

Stability of vitamin A and carotenoids in blood plasma.—Blood plasma was obtained fresh from a "blood bank" and part of it was examined immediately. The remainder was divided into two portions in stoppered tubes which were kept for two days in the ice chest or in the laboratory at room temperature. The results (Table XXXIX) indicate that over this short storage period the vitamin A and carotene in the plasma were quite stable

TABLE XXXIX

Stability of Vitamin A and Carotene in Various Portions of a Single Sample of Blood Plasma

<i>Treatment of serum</i>	<i>Vitamin A (I.U. per 100 ml.)</i>	<i>Total carotenoids (“ I.U.” per 100 ml.)</i>
Fresh	(a) 101	225
	(b) 125	—
	Mean 113	225
Kept 24 hours in ice chest ..	(a) 92	218
	(b) —	238
	Mean 92	228
Kept 24 hours at room temperature	(a) 122	214
	(b) 130	—
	Mean 125	214
Kept 48 hours at room temperature	(a) 132	220
	(b) 99	208
	(c) 115	227
	Mean 115	218

irrespective of the temperature of storage. Thus, it was confirmed that the transport of blood plasma through the post from the Sorby Institute to the various laboratories would be unlikely to have any adverse effect, if the tests were made promptly afterwards.

Factors used in expressing vitamin A and carotene content.—In estimating vitamin A and carotene in all experiments carried out in this laboratory, the following factors were used to evaluate the results in international units :

Vitamin A.—The spectrophotometric results were converted into I.U. on the basis of

$$E \frac{1\%}{1 \text{ cm.}} 617 \text{ m}\mu = 1.0 \equiv 510 \text{ I.U. per g.,}$$

i.e., a conversion factor of 510.

α - and β -carotene.—In these experiments no account was taken of the presence of α -carotene for the following reasons :

(1) In most samples β -carotene was by far the predominating pigment.

(2) The spectroscopic data for α -carotene closely resemble those for β -carotene. Consequently the α - and β -carotene content of samples has been evaluated by comparing their values for $E \frac{1\%}{1 \text{ cm.}}$ at 447 m μ with that of 2,500

for pure β -carotene in light petroleum. The weight of β -carotene thus obtained was converted into I.U. on the basis 1 I.U. \equiv 0.6 μ g. β -carotene.

Total carotenoids and "non-carotene" fractions.—For comparative purposes the amounts of these two fractions in any sample have been evaluated against pure β -carotene. The I.U. values obtained in this way are without biological significance and throughout the Report are indicated by "I.U."

Procedure at Oxford

The method was modified from that of Clausen and McCoord (1938), Kimble (1939), and Yudkin (1941b). With a pipette, 2 ml. of serum or plasma were introduced into a 15 ml. heavy test tube ; 2 ml. of 95 per cent. ethanol were added from a 50 ml. burette drop by drop ; the test tube was shaken throughout after each addition, this shaking being most important. To the solution were added 4 ml. of pure light petroleum of b.p. 40° to 60° C. The tube was corked and shaken by hand for 10 minutes, after which it was centrifuged for 2 minutes at 2,000 r.p.m.

From the light petroleum layer 2 ml. were pipetted into the colorimeter tube, 0.25 ml. of light petroleum was added, and the whole was mixed. The colour due to the carotenoids was measured in an Evelyn photo-electric colorimeter, adapted for $\frac{1}{2}$ -inch tubes, with a 440-m μ filter. The solution was evaporated to dryness in the colorimeter tube on a water bath at c. 40° C. and then at 70° C. for a few seconds ; 0.2 ml. of chloroform and one drop of acetic anhydride were added and mixed. The tube was placed in the photo-electric colorimeter with light intensity already adjusted and a 620-m μ filter, 2 ml. of the Carr Price antimony trichloride reagent were added from an automatic pipette, and the galvanometer was read when it became temporarily steady, after about 6 seconds, at the minimum value. The tube was removed at once and the result was not accepted if the solution was turbid. For estimation of both carotene and vitamin A, the galvanometer was set to give a blank reading of 100.

The galvanometer readings were converted to I.U. of carotene and vitamin A per 100 ml. plasma or serum by reference to a calibration chart, made with a standard solution of β -carotene and a solution of a vitamin A ester. The carotene for the purpose was crystalline β -carotene obtained from the British Drug Houses and dissolved in arachis oil. The vitamin A preparation was a fish liver oil of known potency, $E \frac{1\%}{1 \text{ cm.}} 325 \text{ m}\mu = 32.3$, obtained from Glaxo Laboratories Ltd., and the conversion factor used was 1,600. A correction curve obtained with β -carotene was used for the blue colour due to carotenoids in the Carr Price reaction.

Chromatographic Separation of Carotenoids in Blood Plasma

Procedure at Liverpool.—The lipid extract of the blood plasma was obtained as described above. It was dissolved in specially purified light petroleum of b.p. 40° to 60°C. , and was run through a very small column ($4 \times 1 \text{ cm.}$), of defatted bone meal (Mann, 1944). On elution with further small quantities of light petroleum, α - and β -carotenes were removed from the column, and were estimated by reducing the light petroleum eluate to a suitable volume and measuring its absorption at $450 \text{ m}\mu$. The residue which remained on the column was mainly xanthophyllin in character though small amounts of neo- β -carotene (Mann, 1944 ; Zechmeister, 1944,) and oxidation products might be present also. This "non-carotene" fraction was eluted from the bone column with a small volume of acetone. The acetone was removed under nitrogen and the residue in solution in the light petroleum was analysed in the usual way.

The acetone extract contained also the vitamin A fraction of the plasma extract and could be used for estimating vitamin A, if it were so desired.

Example :

Extract from 34 ml. plasma in 10 ml. light petroleum (340 per cent. by volume) ; E at $442 \text{ m}\mu$ with a 2 cm. cell = 1.54.

$$E \frac{1\%}{1 \text{ cm.}} 442 \text{ m}\mu = 0.002265 \equiv 90.6 \text{ }\mu\text{g. per 100 ml.}$$

$$\equiv 151 \text{ "I.U." per 100 ml.}$$

Light petroleum eluate (α - and β -carotene) in 5 ml. light petroleum (680 per cent. by volume) ; E at $448 \text{ m}\mu$ with a 2 cm. cell = 0.98.

$$E \frac{1\%}{1 \text{ cm.}} 448 \text{ m}\mu = 0.0007206 \equiv 28.8 \text{ }\mu\text{g. per 100 ml.}$$

$$\equiv 48.0 \text{ I.U. per 100 ml.}$$

Acetone eluate (non-carotene fraction) in 5 ml. light petroleum (680 per cent. by volume) ; E at $442 \text{ m}\mu$ with a 2 cm. cell = 1.76.

$$E \frac{1\%}{1 \text{ cm.}} 442 \text{ m}\mu = 0.001294 \equiv 51.76 \text{ }\mu\text{g. per 100 ml.}$$

$$\equiv 86.3 \text{ "I.U." per 100 ml.}$$

Procedure at Reading.—One volume (usually 10 ml.) plasma and one volume absolute ethanol were mixed gradually with shaking ; $2\frac{1}{2}$ volumes light petroleum were then added and the whole was shaken for 5 minutes. A second extraction was carried out with another $2\frac{1}{2}$ volumes light petroleum. The products of the two extractions were combined and the light petroleum removed by evaporation and finally *in vacuo*. Five ml. absolute ethanol and

1.1 ml. 60 per cent. potassium hydroxide were added to the residue, which was then heated on a steam bath for 15 minutes. The mixture was taken up in 20 ml. distilled water and after being cooled the material was extracted three times with 20, 13, and 13 ml. of di-ethyl ether. The extracts were combined and the ether removed by evaporation, which was completed *in vacuo*. The residue was dissolved in 3 ml. hexane, or, if vitamin A was not to be estimated, in 2 ml. The vitamin A was measured in duplicate by adding 2 ml. of antimony trichloride reagent to 0.5 ml. of hexane solution and reading the intensity of the blue colour at wavelength 618 $m\mu$ in the photo-electric spectrophotometer of Thompson (1942).

Two ml. of solution were used for estimating the total carotenoids with the same instrument at wavelength 451 $m\mu$. The hexane solution was then transferred quantitatively back to the flask and most of the hexane removed *in vacuo*. The small volume of solution remaining was poured on to a column of Savory and Moore's standard alumina approximately 3 mm. in diameter and 30 mm. long. The β -carotene was eluted with 1 or 2 per cent. acetone in hexane; lycopene, which was present in small amounts, with 10 per cent. acetone in hexane; an unidentified pigment with 20 per cent. acetone in hexane; and lutein with 8 per cent. ethanol in hexane. Each fraction was made up to 2 ml. with hexane, and readings were made in the photo-electric spectrophotometer in the same way as for total carotenoids.

FAECES

Sampling

For the analysis of the carotene content, faeces were collected over a 7-day period, carmine being used as a marker. The food under investigation was given for at least 3 days before carmine was taken. The dose of carmine was 0.6 g., taken in several gelatine capsules at the beginning and end of a 7-day period, and all faeces between the disappearance of the first pink colouration and the appearance of the second pink colouration were collected. Thus, the total collection corresponded to 7 days' food intake, though the actual period between the first and the last collection was often less or more than a week. Each portion of faeces was voided into the scale pan of a special balance, weighed and immediately transferred to a mortar containing anhydrous sodium sulphate, where it was ground up to a dry homogeneous powder, more sodium sulphate being added as required. The powder was stored in the dark until the end of the collection period. The weekly total of dry powder was thoroughly mixed in a mechanical mortar or a ball mill and weighed. A sample was dispatched for analysis.

Procedure at Cambridge

The faeces mixed with sodium sulphate, as received from the Sorby Institute, were extracted in a Soxhlet extractor with ether. The portions taken varied from 1 to 20 g. at various periods of the experiment, but were seldom less than 5 g. and were usually 10 or 20 g. Total carotenoids were estimated in the ether extract by means of a Zeiss step photometer with a blue filter, S.47. The extract was then evaporated, dissolved in light petroleum, and passed through an adsorption column. For this purpose di-calcium phosphate was used at first (Moore, L. A., 1940) but, since this material was rather difficult to obtain in adequate amounts in the correct state of activation, anhydrous sodium carbonate was later preferred (Kernohan, 1939). The

column was washed with light petroleum and the carotene fraction passed through without further treatment. Carotene was estimated in the filtrate.

Results obtained with columns of anhydrous sodium carbonate and correctly activated di-calcium phosphate were identical. The procedure with sodium carbonate does not allow the separation of lycopene from carotene, and is, therefore, inferior to that with alumina for general work (Booth, 1945). In the absence of lycopene and other unusual pigments, however, sodium carbonate has the advantage of extreme simplicity in manipulation and of ease of supply.

Procedure at Liverpool

A weighed quantity of 20 to 40 g. of the dried faeces was placed in a conical flask and extracted four times with 100 ml. ether, freshly redistilled over reduced iron. The extraction was carried out by placing the flask on a tepid waterbath and vigorously stirring the mixture with a glass rod for three minutes. The mixture was then allowed to stand until the solid had settled, when the ether was decanted off. The final traces of ether were removed by filtration at the pump with a Büchner funnel. The solid residue was transferred to the original flask and the process repeated twice. The combined ether extracts were evaporated to dryness and the residue examined in one of two ways.

Estimation of total carotenoids and of α - and β -carotene.—The residue was saponified by adding about 1 ml. 60 per cent. aqueous potassium hydroxide and 10 ml. absolute ethanol, and heating the mixture on a waterbath for 10 minutes. The unsaponifiable fraction was prepared as previously described (p. 92). By the process of saponification chlorophylls and other material which might interfere with the chromatographic separation were destroyed.

The residue was dissolved in light petroleum, of b.p. 40° to 60° C., and the total carotenoids were estimated by measuring the absorption of the solution at a wavelength of about 450 $m\mu$ in the Hilger Nutting spectrophotometer. An aliquot part of this solution was then run through a column of defatted bone meal, measuring 15 \times 3 cm., and the pigments were separated into two fractions: α - and β -carotene, and the "non-carotene" fraction.

Estimation of the α - and β -carotene fraction only.—With this method the residue was not saponified but was made up directly in light petroleum and run through the bone meal column. Elution with light petroleum removed α - and β -carotene only, and their concentration in the solution could be measured in the usual way.

Example :

Total carotenoids

Faeces + Na_2SO_4 (1 week) = 5,194 g., *i.e.*, 742 g. per day ; 20 g. mixture extracted and saponified ; "non-sap." in 20 ml. light petroleum 100 per cent. diluted 4/10 (40 per cent. by volume) ; E at 443 $m\mu$ with a 2 cm. cell = 0.92.

$$\begin{aligned} E \frac{1\%}{1 \text{ cm.}} 443 m\mu &= 0.0115 \equiv 4.61 \mu\text{g. per g. dry faeces} \\ &\equiv \frac{(4.61 \times 742)}{0.6} \text{ "I.U." per day} \\ &= 5,700 \text{ "I.U." per day.} \end{aligned}$$

α- and β-carotene

Petrol eluate from aliquot of above (≈ 16 g.) after chromatography, in 25 ml. (64 per cent. by volume) ; E at 448 m μ with a 2 cm. cell = 0.89.

$$E \frac{1\%}{1 \text{ cm.}} \text{ 448 m}\mu \text{ (}\alpha\text{- and } \beta\text{-carotene)} = 0.0070 \approx 2.8 \mu\text{g. per g.}$$

$$\approx 3,450 \text{ I.U. per day.}$$

Stability of carotenoids in dried faeces.—Two samples of freshly dried faeces were examined immediately for carotenoids. They were then re-examined after storage in a glass bottle at room temperature for 3 and 2 weeks, respectively. The results (Table XL) indicate that the carotenoids in the preparations were completely stable.

TABLE XL
Stability of Carotenoids in Dried Faeces

	Total carotenoids ("I.U." per g.)	α - and β -carotene (I.U. per g.)	Non-carotene fraction ("I.U." per g.)
<i>Sample A :</i>			
Fresh	5.3	3.7	1.4
Stored three weeks	5.6	3.7	1.3
<i>Sample B :</i>			
Fresh	7.7	4.6	3.5
Stored two weeks	7.9	4.3	3.1

FOODSTUFFS

Procedure at Cambridge

For flour, bread, dried diet, and similar materials, the method of estimation was the same as for faeces. For carrots and green vegetables a weighed sample of the material was ground with quartz powder with a mixture of three parts of light petroleum and two of acetone until all the colour had been extracted. Dehydrated cabbage was moistened with water beforehand to facilitate the extraction. The combined extracts were evaporated under reduced pressure, and made up in light petroleum. Total carotenoids were estimated directly in this solution, and carotene after chromatography, as described for faeces.

Procedure at Liverpool

Two methods were used which gave results in good agreement.

Method A.—A weighed quantity of 2 g. of the sample was heated on a waterbath with 5 ml. 60 per cent. aqueous potassium hydroxide until disintegration was complete ; 15 ml. ethanol were then added and the mixture was heated for a further 10 minutes. The mixture was then treated and extracted with ether as described under Method A for the estimation of vitamin A and carotene in blood plasma (p. 92). The dried ethereal extract was dissolved in light petroleum and submitted to chromatographic analysis through a column of bone meal as described on p. 96.

Method B.—A weighed quantity of 2 g. of the sample was ground in a mortar with silver sand and anhydrous sodium sulphate (except for dehydrated materials where this step was unnecessary). The pulverized mass was then extracted and its carotenoid content was evaluated in the same way as for

dried faeces (p. 98). If total carotenoids were to be estimated, the ether extract had to be saponified to break down the chlorophylls before chromatography. If the values for α - and β -carotene only were required, this preliminary saponification was not necessary.

The experimental diet after dehydration was examined in the same way.

Estimation of vitamin A in concentrates of distilled natural esters.—The method was that generally used for measuring the potency of vitamin A concentrates. A spectrogram of the solution of the esters in cyclohexane was taken and the extinction coefficient at 325 $m\mu$ was measured. The instrument used was a Hilger E3 quartz spectrograph, run in conjunction with a long-focus rotating-disc sector photometer, and with an arc struck between iron and nickel electrodes as source of light.

Example :

For a 2 per cent. solution of esters in a 6 mm. cell, $E_{325 m\mu} = 1.06$

$E_{\frac{1\%}{1 \text{ cm.}}} 325 m\mu = 0.883$

$E_{\frac{1\%}{1 \text{ cm.}}} 325 m\mu$ for pure vitamin A = 1.0 \equiv 1,600 I.U. per g.

Potency of esters = $0.883 \times 1,600 = 1,413$ I.U. per g.

Procedure at Reading

The material was exhaustively extracted with acetone and the pigments were transferred to di-ethyl ether by addition of water. The ether solution was washed with water, dried over sodium sulphate, and evaporated to dryness *in vacuo*. The residue was saponified, extracted twice with ether, and dried over anhydrous sodium sulphate. Ether was removed on the waterbath and the non-saponifiable residue was taken to dryness *in vacuo*, and finally dissolved in *n*-hexane (Gillam, Henry, and Kon, 1937). The absorption of the total carotenoids was then read at 451 $m\mu$ in the photo-electric spectrophotometer of Thompson (1942).

Chromatographic separation was carried out on an aluminium oxide column (Savory and Moore's standardized alumina). The α - and β -carotene fraction was eluted with 1 per cent. acetone in *n*-hexane and read as above.

R.—Measurement of the Capacity for Dark Adaptation

MEANING AND CHARACTERISTICS OF THE DARK ADAPTATION CURVE

The points in the physiology of dark adaptation of which use is made in assessing the degree of vitamin A deficiency are not familiar to all readers interested primarily in nutrition and it may not be out of place, therefore, to give a brief account of the relevant matters ; papers by Hecht (1937), Hecht and Mandelbaum (1939), and by Mandelbaum (1941) may be consulted for further information.

Measuring the capacity for dark adaptation involves finding the minimum, or threshold, brightness which makes a given surface visible. The value for the threshold brightness is high after exposure of the retina to bright light and gradually decreases in darkness. A graph in which the threshold brightness is plotted against time spent in the dark gives the "dark adaptation curve" ; according to the method of Hecht (1937), the time is plotted on the horizontal axis on a linear scale, while the light intensity is

plotted on a vertical axis on a logarithmic scale. The change of brightness perceived, as sojourn in darkness is prolonged, may extend over 10^5 or 10^6 units and, with an ordinary scale, the graph would have to be enormous to cover the whole range in detail, and would cramp some portions of the data and exaggerate others.

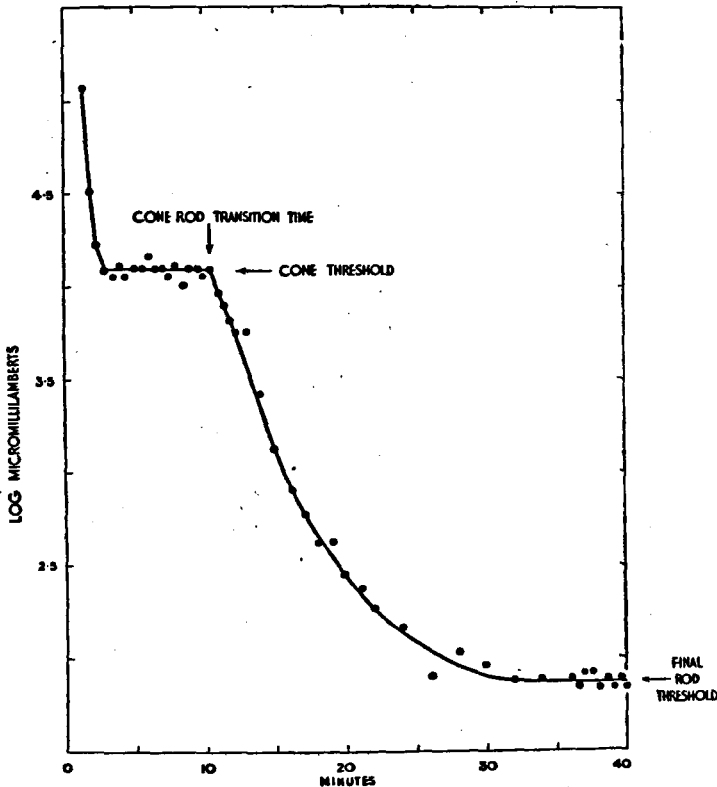


FIG. 11.—Normal curve of dark adaptation showing cone threshold, cone-rod transition time and final rod threshold (Drabble, 19.5.44).

A normal dark adaptation curve is shown in Fig. 11. It will be seen that dark adaptation proceeds in two stages. The first is rapid and is completed within a few minutes; the second begins after 5 to 10 minutes and continues for from 20 to 30 minutes. The first stage records the progressive adaptation of the cones, the second that of the rods (*see* Hecht, 1937). The exact shape of the curve depends on the intensity and duration of exposure experienced by the retina before dark adaptation begins, on the size, colour and duration of the test light used, and on the part of the retina tested. Good adaptometers are designed to control all these factors.

The chief characteristics of the curve are :

The "cone threshold": *i.e.*, the brightness value of the plateau reached towards the end of the first stage.

The "cone-rod transition time": *i.e.*, the time when vision changes from being predominantly with the cones to being predominantly with the rods, as shown by a sudden drop of the threshold values.

The "final rod threshold": *i.e.*, the brightness value of the final plateau reached at the end of the second stage.

When it is not practicable to reproduce the full curves it is customary to record the characteristics instead.

ADAPTOMETER OF WALD

Apparatus

The adaptometer was made available by the Oxford Nutrition Survey. It has been described in detail by Wald (1941), and by Dow and Steven (1941). It consisted of a box containing batteries, a control panel, and a test unit. The test unit was built from a section of brass tubing about 4 inches long and 2 inches in diameter. Mounted in the base was the test lamp, which was on a potentiometer circuit and was calibrated photometrically. The test lamp illuminated the test field of opal glass at the other end of the tube in flashes of 1/50 second duration, through a camera shutter mounted in the central portion. The lamp for the fixation spot was on a separate circuit and was mounted in a small housing on top of the test unit. It shone through a pinhole aperture, covered by a deep red filter (Wratten No. 70). Attached in front of the test unit was a funnel-shaped mask, to hold the head of the subject in the correct position, 11 inches from the test field. The relative positions of the subject's eyes, the test field, and the fixation point were such that the test field subtended an angle on the retina of $2^{\circ} 27'$, at 6° above the fixation point. The whole test unit was mounted on an adjustable camera tripod.

The test unit was connected with the box containing the batteries and control panel by a length of light insulated wire. On the control panel were switches for the test and fixation lamp circuits, a resistance attached to a scale for varying the brightness of the test field, and a second resistance and voltmeter for setting the line voltage of the test circuit.

The bleaching light used for light adaptation consisted of a 500-watt bulb, in front of which was an opal glass diffuser 1 inch from the bulb. Behind the bulb was a metal reflector. The average brightness of the opal diffuser over the bleaching area was about 880 millilamberts.

Since the brightness of the test light was controlled by a resistance, the spectral composition of the test light changed somewhat with the luminosity. The proportion of the longer wavelengths increased as the luminosity fell.

The brightness of the test field was calibrated with the Craik low brightness photometer, the range of which was 0.1 to 0.0000001 equivalent foot candles. The procedure was as follows :

The metal cone was removed from the adaptometer and the camera shutter was fixed in the open position. The voltage was set at 5 volts with the dial reading at 100 scale divisions. The scale was then turned to 0, the adaptometer being supported in a convenient position, and the lights extinguished. The photometer field brightness and the brightness of the adaptometer opal were matched, and the photometer reading was recorded. At least six consecutive readings were taken, the line voltage being checked between every three readings. The dial setting was then moved successively to 10, 20, 30, 40 scale divisions and so on, and the procedure repeated. No calibration was attempted above 70 scale divisions but it was assumed that the calibration curve below 0 and above 70 followed the same straight line as between these two figures.

From the figures obtained a calibration chart was made from -50 to $+100$. The calibration was checked at regular intervals.

During the summer of 1944, the original Wald apparatus was showing signs of wear beyond repair; the camera shutter was becoming erratic in action and the resistances were worn in places. It became necessary, therefore, to build a new apparatus which included the two following improvements introduced by W. Bartley:

(1) A neutral wedge was used to dim the light, instead of the original resistance. This prevented the shift to the red end of the spectrum, which occurred on dimming the light in the original apparatus.

(2) A self-calibrating unit was incorporated in the new machine, consisting of a photo-cell connected directly to a micro-ammeter. The new unit, with the bulb in position, was calibrated against the Craik low brightness photometer. The line setting of the micro-ammeter then remained as standard for any further use of the apparatus. No further calibration was necessary.

The presentation time of the flash, the magnitude of the test field, and the angle between it and the red fixation light were the same as in the original apparatus. The results obtained on the new apparatus were in very close agreement with those obtained on the old machine. The new apparatus was not ready for use until 16th September, 1944; very few of the readings were, therefore, made with it.

Description of the apparatus as modified by Bartley (see Fig. 12).—A 2.5-volt torch bulb (E), standardized by a resistance, photo-cell (D) and micro-ammeter (A), provided a constant source of light. A neutral wedge dimmed the light without altering its quality. A camera shutter (N) allowed a timed flash of light of a given intensity to be shown to the subject. A red light (R) provided a fixation point.

The apparatus consisted of a metal box 3 inches square in cross-section and 6 inches long, on the back of which was mounted a micro-ammeter (A), connected (B) with a photo-cell (D) of approximate area 3 square inches. In front of the photo-cell, mounted on the base of the box, was the 2.5-volt torch bulb (E), providing the source of illumination. The bulb was wired (S) to terminals on the outside of the box and thence to the battery box. Directly in front of the bulb was a fixed metal partition (H) with a hole half an inch in diameter immediately opposite the bulb filament. Mounted on top of the partition was a small mirror (G), which threw light forwards and downwards through a red filter (F) on to the scale attached to the top of the neutral wedge carrier (J). Immediately apposed to the hole in the fixed partition was a neutral Ilford wedge (K), gradient one log. unit per inch, mounted on a metal wedge carriage and moved by means of a rack and pinion device (LM). On the top of the wedge carriage was an ivorine scale giving the log. of the value for the brightness in micromillilamberts. Slotting into the box immediately in front of the wedge was a metal slide carrying a camera shutter (N), by means of which a flash could be presented to the subject. In front of the camera shutter was a piece of opal glass (T) slotted into the box, and immediately in front of this a metal slide (V) with a hole half an inch in diameter opposite the camera shutter aperture and bearing on the top a small tubular housing for the red fixation light (R). The hole in the slide was the area visible to the subject. The source of the red fixation light was a torch

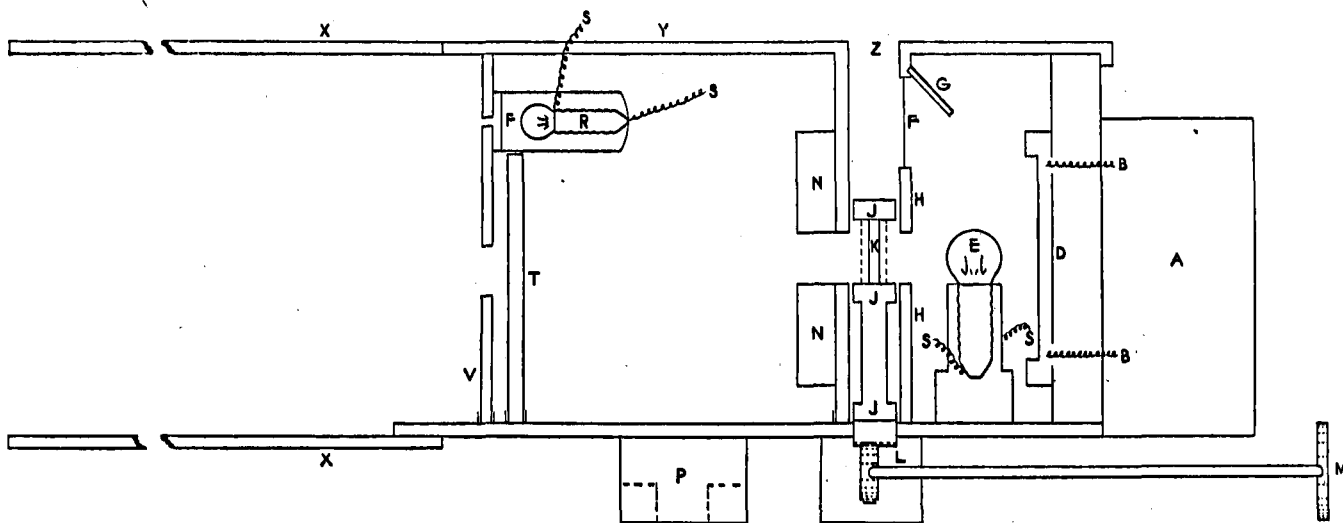


FIG. 12.—Plan of Bartley's adaptation of the adaptometer of Wald.

- | | | | |
|-----|--|----|---------------------------------------|
| A | Micro-ammeter. | M | Knob for moving pinion. |
| BB | Connections between A and the photo-cell. | NN | Camera shutter. |
| D | Photo-cell. | P | Attachment to retort stand. |
| E | Test bulb. | R | Red light unit. |
| FF | Red filters. | SS | Connections from bulb to battery box. |
| G | Mirror mounted on top of partition. | T | Opal glass. |
| HH | Fixed metal partition with $\frac{1}{4}$ -in. hole. | V | Metal partition with hole. |
| JJJ | Wedge carriage bearing scale. | XX | Metal hood. |
| K | Neutral wedge. | Y | Lid to test unit. |
| L | Rack and pinion mechanism for moving wedge carriage. | Z | Aperture for viewing scale. |

bulb connected to terminals on the outside of the box and thence to the battery box. In front of this bulb was a slot containing a 0.6 neutral filter and a red filter (F). The neutral filter could be removed if an extra-bright red light was required. The red light was viewed by the subject through a small hole in the metal slide. Attached to the front of the box was a metal hood (X); the length of the hood, and the distance between the red fixation light and the test field, were so arranged that an area of the retina $6^{\circ} 20'$ from the fovea was under test. The box had a lid (Y) with a small square aperture (Z) for viewing the scale reading. The battery box contained two batteries, each connected through a variable resistance and a switch to a plug socket on the outside of the box, which in turn was connected by wires to the terminals on the apparatus.

The apparatus was calibrated by means of the Craik low brightness photometer at any convenient position of the wedge. The micro-ammeter reading corresponding to this calibration was noted and this became the future setting for the apparatus.

Procedure

Determination of complete dark adaptation curve.—First, the retina was bleached. The subject was asked to gaze at the opal screen of the bleaching apparatus from a distance of 6 inches for exactly 3 minutes measured with a stop watch. At the end of this period the bleaching light was extinguished and the testing room was kept in complete blackness till the test was finished. The subject was seated comfortably with his face against the mask and his eyes were focused on the red fixation point. The operator then illuminated the test field by releasing the camera shutter. Starting with the brightness of the test field at a sub-threshold level, he increased it by a small regular amount of about 0.05 to 0.06 log. unit between each exposure until the subject reported seeing it. The time, the end of the bleaching period being taken as zero, and the brightness of the flash were recorded in the light of a dim red torch. This procedure was repeated at half-minute or minute intervals for 40 minutes. During the last 10 minutes normal subjects gave consistent readings indicating that the final rod threshold was reached. The red fixation light was turned off between tests to avoid fatigue.

Short method: determination of final rod threshold only.—Bleaching was omitted. The subject was kept in complete darkness for 20 minutes and testing then proceeded as described above until consistent readings were obtained. In normal subjects this required as a rule no more than 10 minutes. The results obtained under these conditions agreed closely with the final rod threshold values as measured 35 to 40 minutes after bleaching.

Rod Threshold

Normal range of the final rod threshold.—The literature contains only a few data on the "normal" dark adaptation curve as measured with the portable adaptometer of Wald. A number of measurements on "normal" persons was therefore made.

Table XLI shows the final rod threshold of a number of persons who had received a comparatively large dose of vitamin A, 24,000 I.U. or more daily, for about a week before the measurement was made. It can be assumed that in these people the supply of vitamin A was not a factor limiting dark adaptation. The Table includes measurements on volunteers, made at the end of the trial after they had received a large dose of vitamin A, usually

1,000,000 I.U., to replenish their stores. It will be seen that the "normal" threshold values varied between 1.37 and 2.05 log. μm lamberts, except in one person, Tridgell, whose threshold varied between 2.10 and 2.30 log. μm lamberts. These figures agree with the range given by Steven and Wald (1941), and Dow and Steven (1941).

TABLE XLI

Normal Range of Final Rod Threshold in Subjects "Saturated" with Vitamin A: Wald Adaptometer

Name	Date	Final rod threshold (log. μm lamberts)	Name	Date	Final rod threshold (log. μm lamberts)
<i>A. Volunteers serving in the experiment</i>					
Bartley, M. ..	11. 9.44	1.61	Proctor	9. 9.44	1.93
	12. 9.44	1.64		11. 9.44	1.83
				21. 9.44	2.05
Bartley, W. ..	12. 1.45	1.45	Russell	18. 9.44	1.75
	5. 2.45	1.45		18. 9.44	1.75
	9. 3.45	1.48		26. 9.44	1.75
Bloomfield ..	18. 9.44	1.60	Saunders	13. 8.43	1.48
	18. 9.44	1.65		22. 8.44	1.87
	19. 9.44	1.65			
Broderick	24. 2.44	1.88	Stuart	19. 9.44	2.03
				19. 9.44	2.00
Brown	3. 9.43	1.37		26. 9.44	1.87
	23.10.43	1.59			
Drabble			Thompson	25. 5.44	1.72
	20. 5.44	1.72		27. 7.44	1.77
	21. 8.44	1.64		20. 9.44	1.85
	21. 8.44	1.47			
Drake	26. 8.44	1.93	Tridgell	23.10.44	2.20
	7. 9.44	1.84		24.10.44	2.10
	18. 9.44	1.80		9.11.44	2.30
Garling			Watson	15. 8.44	1.61
	30. 8.44	1.84		25. 8.44	1.61
	11. 9.44	1.70		7. 9.44	1.51
	16. 9.44	1.94			
Garnett	28. 9.44	1.99	Whitehead, A. ..	24. 9.44	1.95
	9.10.44	1.90	Whitehead, D. ..	24. 9.44	1.65
Golding	1. 9.44	1.73	Williams	22. 7.44	1.47
	17. 9.44	1.73		14. 8.44	1.37
	20. 9.44	1.85		22. 9.44	1.60
Hicken	26. 4.44	1.53	Wodeman	28. 7.44	1.80
	18. 9.44	1.68		16. 8.44	1.77
				8. 9.44	1.75
			Woodhouse	29. 8.44	1.73
			18. 9.44	1.94	
			20. 9.44	1.95	
<i>B. Other persons</i>					
Ashton	22. 5.44	1.52	Holiday	22. 5.44	1.55
Daniell	24. 2.44	1.59	MacDonald	22. 5.44	1.59

Variations of the final rod threshold within brief periods.—In order to ascertain the consistency of measurements, the final rod threshold was determined at frequent intervals within a short space of time on a number of subjects. The results are shown in Table XLII. It will be seen that occasionally (Proctor) variations up to 0.5 log. unit might occur within a few hours, and even greater variations, up to 0.7 log. unit, within three days. The scatter varied from person to person.

TABLE XLII

Variations of the Final Rod Threshold Recorded at Frequent Intervals Within a Short Space of Time : Wäld Adaptometer

Name	Date	Time	Final rod threshold (log. μ m lamberts)	Range
Bloomfield	2.11.42	9 a.m.	1.63	1.48 to 1.88
		4 p.m.	1.48	
	3.11.42	9 a.m.	1.48	
		4 p.m.	1.88	
	4.11.42	9 a.m.	1.68	
		4 p.m.	1.53	
	5.11.42	9 a.m.	1.73	
		4 p.m.	1.80	
	6.11.42	9 a.m.	1.88	
		4 p.m.	1.83	
	7.11.42	9 a.m.	1.68	
		4 p.m.	1.83	
	8.11.42	9 a.m.	1.60	
		4 p.m.	1.68	
Brown	25. 8.43	1 p.m.	1.37	1.37 to 1.44
		8 p.m.	1.41	
	26. 8.43	11 a.m.	1.37	
		8.30 p.m.	1.44	
	27. 8.43	12 noon	1.37	
7 p.m.	1.44			
Garling	3. 9.43	3 p.m.	2.02	1.62 to 2.02
		8 p.m.	1.62	
Proctor	2.11.42	9 a.m.	2.13	1.63 to 2.35
		4 p.m.	1.63	
	3.11.42	9 a.m.	1.83	
		4 p.m.	2.03	
	4.11.42	9 a.m.	2.33	
		4 p.m.	2.05	
	6.11.42	9 a.m.	2.35	
		4 p.m.	2.25	
	7.11.42	9 a.m.	2.18	
		4 p.m.	2.03	
8.11.42	9 a.m.	2.18		
	4 p.m.	1.98		
Watson	2.11.42	9 a.m.	1.88	1.73 to 2.03
		4 p.m.	1.88	
	3.11.42	9 a.m.	1.75	
		4 p.m.	1.83	
	4.11.42	9 a.m.	2.03	
		4 p.m.	1.98	
	6.11.42	9 a.m.	2.03	
		4 p.m.	1.83	
	7.11.42	9 a.m.	1.88	
		4 p.m.	1.75	
	8.11.42	9 a.m.	1.83	
		4 p.m.	1.73	

TABLE XLII—*contd.*

Name	Date	Time	Final rod threshold (log. μ m lamberts)	Range
Barrett	2.11.42	9 a.m.	2.02	1.80 to 2.27
		4 p.m.	1.87	
	3.11.42	9 a.m.	1.80	
		4 p.m.	2.05	
	4.11.42	9 a.m.	2.20	
		4 p.m.	2.20	
	5.11.42	9 a.m.	1.90	
		4 p.m.	2.03	
	6.11.42	9 a.m.	2.03	
		4 p.m.	2.27	
7.11.42	9 a.m.	2.05		
	4 p.m.	2.17		
Burn	2.11.42	9 a.m.	1.40	1.40 to 1.68
		4 p.m.	1.52	
	3.11.42	9 a.m.	1.47	
		4 p.m.	1.52	
	4.11.42	9 a.m.	1.48	
		4 p.m.	1.68	
	5.11.42	9 a.m.	1.48	
		4 p.m.	1.67	
Mannock	2.11.42	9 a.m.	1.53	1.47 to 1.82
		4 p.m.	1.47	
	3.11.42	9 a.m.	1.47	
		4 p.m.	1.62	
	4.11.42	9 a.m.	1.62	
		4 p.m.	1.55	
	5.11.42	9 a.m.	1.62	
		4 p.m.	1.72	
	6.11.42	9 a.m.	1.68	
		4 p.m.	1.82	
	7.11.42	9 a.m.	1.68	
		4 p.m.	1.63	
	8.11.42	9 a.m.	1.53	
		4 p.m.	1.55	

Cone Threshold

The available apparatus did not permit a very accurate measurement of the cone threshold. As Mandelbaum (1941) points out, a red filter should be used to eliminate rod vision when it is desired to determine the cone threshold. The dark adaptation curve as measured by the Wald apparatus frequently failed to show a consistent "plateau". Instead, fluctuating values were recorded; this often made it impossible to fix a definite threshold value and, in view of their doubtful character, these measurements are not presented.

Cone-Rod Transition Time

The "cone-rod transition time" was taken to be the interval in minutes between the extinction of the bleaching light and the end of the cone threshold plateau, the latter being the moment when readings started to show a consistent fall.

"Normal" values of the cone-rod transition time are given in Table XLIII. They refer to persons who had been "saturated" with vitamin A before

TABLE XLIII

Normal Range of Cone-Rod Transition Time in Subjects "Saturated" with Vitamin A

Measured with the Wald Adaptometer under Standardized Conditions (see p. 105)

Name	Date	Cone-rod transition time (minutes)	Name	Date	Cone-rod transition time (minutes)
<i>A. Volunteers serving in the experiment</i>					
Bartley, M.	11. 9.44	7	Russell	18. 9.44	11
	12. 9.44	8		18. 9.44	9
Bartley, W.	12. 1.45	7	Saunders	26. 9.44	7
	5. 2.45	8		22. 8.44	10
	9. 3.45	7		19. 9.44	7
Bloomfield	18. 9.44	5	Stuart	19. 9.44	10
	18. 9.44	5		26. 9.44	7
	19. 9.44	7		25. 5.44	8
Broderick	24. 2.44	5½	Thompson	27. 7.44	9
				20. 9.44	8
				23.10.44	6
Brown	—	—	Tridgell	24.10.44	7
				9.11.44	9
Drabble	20. 5.44	10½	Watson	15. 8.44	7
	21. 8.44	9		25. 8.44	8
Drake	26. 8.44	9	Whitehead, A.	7. 9.44	6
	7. 9.44	12		24. 9.44	7
	18. 9.44	6		28. 9.44	5
Garling	30. 8.44	7	Whitehead, D.	24. 9.44	5
	16. 9.44	6		14. 8.44	7
Garnett	28. 9.44	9	Williams	22. 9.44	8
	9.10.44	10		23. 9.44	7
				28. 7.44	7
Golding	1. 9.44	10	Wodeman	16. 8.44	5
	17. 9.44	8		8. 9.44	8
	20. 9.44	7		29. 8.44	9
Hicken	26. 4.44	7	Woodhouse	18. 9.44	10
	18. 9.44	6		20. 9.44	9
Proctor	1. 9.44	8			
	9. 9.44	8			
	21. 9.44	7			
<i>B. Other persons</i>					
Beverley	29. 2.44	6	Middleton	18. 4.44	8
Blagden	29. 2.44	5½	Smith	29. 2.44	8½
Daniell	24. 2.44	10	Squire	29. 2.44	7½
Goodinson	29. 2.44	5½	Thirwell	29. 2.44	10½
Holland	22. 2.44	10	Trotter	10. 3.44	7

the measurement. The Table includes the results obtained on the volunteers after the conclusion of the deficient diet. The range of the values in the thirty-three persons examined was from 5 to 12 minutes.

ADAPTOMETER OF CRAIK (see PLATE I)

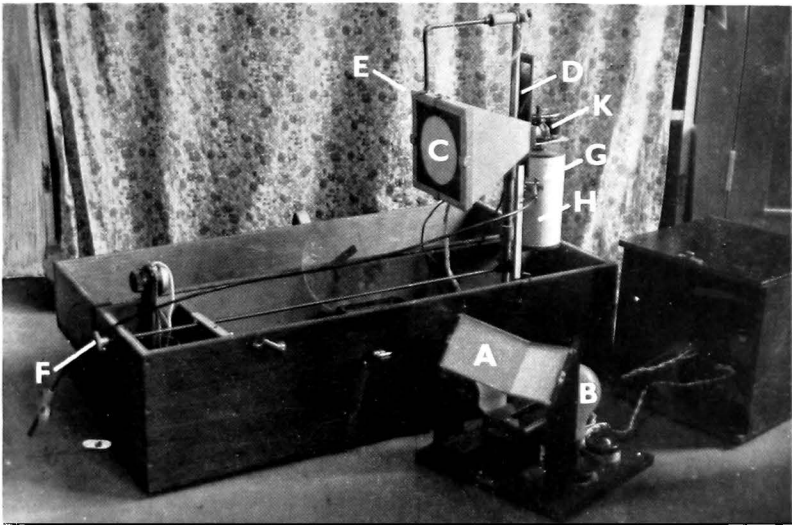
Apparatus and Procedure

The subject was first bright-adapted for 5 minutes by looking at a surface of 750 equivalent foot candles' brightness covering the whole visual field except for slight gaps at the base of the nose, the head being placed close to two sheets of flashed opal glass (A) inclined to one another at 120° and illuminated by two 100-watt lamps (B) placed behind them. The subject was then instructed to turn to the adaptometer, consisting of a 4-inch diameter disc of flashed opal glass (C) in the front of a box ; it was evenly illuminated from behind by a pea-lamp shining through a flashed opal diffuser and a 0 to 6 density neutral gelatine wedge (D). The opal disc was at a distance of 2 feet 6 inches from the subject's eyes ; vision was binocular, with natural pupils. A lamp shining through a red filter and down a pointed glass rod provided a fixation point (E) 6° above the centre of the field ; the brightness of the fixation point could be adjusted with a variable resistance. The adaptometer lamp was run at a constant voltage measured on an accurate meter. The subject moved the neutral wedge by a knob (F) on a shaft 2 feet 6 inches long ; he was instructed to keep the patch of light just visible by operating the knob while looking in the general direction of the fixation spot but not staring so rigidly at it as to cause local fatigue of the retina. He was also warned that in the later stages of dark adaptation the disc of light would look blurred at the threshold and that he must not try to brighten it to produce a sharp edge. A pencil lead (G) connected to the neutral wedge frame recorded the subject's threshold settings continuously on paper on a drum (H) turned once an hour by an electric clock motor (K). The record was subsequently measured with a special ruler having appropriate time and brightness scales. The brightness range of the instrument was from 0.000001 to 0.01 equivalent foot candles, at a colour temperature of 2,360° K. Though there are numerous methods of measuring dark adaptation, by perception of flashes or simple figures with or without rigid fixation of the eye, the above provided a simple means of obtaining a continuous curve and relieved the experimenter of the necessity of operating shuttles or figure-slides and of recording readings in darkness ; the method was completely automatic after the bright-adaptation period was complete, and the resulting curve was in the form in which such results are usually plotted, with the logarithm of threshold brightness against linear time, since a neutral wedge itself gives a logarithmic scale. A black V which could be rotated on a transparent disc could be placed in front of the test field to determine the subject's acuity at low illuminations on completion of the threshold test.

The results are expressed as the logarithm of the brightness in equivalent foot candles.

The values given in this Report are based on a calibration of the instrument in the Cambridge Psychological Laboratory which was checked with a low brightness photometer before and after each set of tests. At the end of the

PLATE I.



Adaptometer of Craik and bleaching unit.

- | | | | |
|---|--|---|---------------------------------|
| A | Flashed opal glass. | E | Red fixation light. |
| B | Pearl bulb, 100 watt. | F | Knob, moving wedge. |
| C | Test field of flashed opal glass. | G | Pencil lead, recording on drum. |
| D | Neutral wedge, Ilford, density 0 to 6. | H | Drum. |
| | | K | Electric clock motor. |

whole experiment the apparatus was sent to the National Physical Laboratory to be calibrated against an absolute standard. The results were as follows :

<i>Readings marked on Craik adaptometer (e.f.c.)</i>	<i>Readings of National Physical Laboratory (e.f.c.)</i>
0·01	0·0053
0·003	0·0013
0·001	0·00036
0·0005	0·00016

It will be seen that the calibration did not extend beyond a brightness of 0·0005 equivalent foot candles but that most of the rod threshold readings lay between 0·00001 and 0·000001 equivalent foot candles. As this range is not covered by the calibration of the National Physical Laboratory no correction has been made. The calibration suggests that the rod threshold values given in the Report are too high by about 0·6 or 0·7 log. unit. The readings made throughout the experiment are, however, strictly comparable with each other.

Measurements of the complete dark adaptation curve were made on most of the subjects on 15 occasions between 4th September, 1942, and 19th June, 1944. The data obtained for the final rod threshold and the cone-rod transition time are given in Tables XLIV and XLV.

Low Brightness Photometer

This instrument was used for the calibration of the adaptometer of Craik and also, in the later stages of the experiment, for the calibration of the adaptometer of Wald and the scotometry targets. It was originally designed by the late K. J. W. Craik for work in connection with the Royal Air Force*, such as measurements of the brightness of the night sky, of instrument panels, and of searchlight beams. For these purposes it had to be portable and robust, and an accuracy of ± 5 per cent. could be regarded as sufficient. A neutral wedge is used for dimming the reference light and the scale is therefore logarithmic, giving equal percentage accuracy over a large range of brightness extending from 0·1 to 0·000001 equivalent foot candles; filters (e.g., $\frac{1}{100}$ and $\frac{1}{10000}$ transmission) can be used to extend the range. The entire optical and electrical systems are mounted in a box $12\frac{1}{2} \times 6 \times 4$ inches. Fig. 13, p. 114, shows a plan of the instrument (omitting the lid).

The observer sees an aperture (6) in a mirror (7) sharply focused by a lens (4). Through the aperture he sees the background whose brightness is being measured; the surrounding part of the mirror reflects an opal screen (5) illuminated by a bulb (9), the light from which passes through a neutral wedge (12). This can be moved by rubber friction rollers and its position is read on a scale when the central and outer fields have been matched in brightness. The light from the bulb is standardized by a photo-cell (10) connected directly to a micro-ammeter (11); the current through the bulb is varied by a resistance (17) until the standard reading is obtained on

* Acknowledgment is due to Air Marshall Sir Harold Whittingham, K.C.B., K.B.E., Director-General of Medical Services, Royal Air Force, for permission to publish the description of the photometer.

TABLE XLIV

Values for all Observations (in log. equivalent foot candles) of the Final Rod Threshold Measured by Craik's Adaptometer (beginning of dosing indicated by horizontal line)

Date	Barley, M.*	Barley, W.*	Bloomfield	Broderick*	Brown	Drabble*	Drake*	Garling	Garnett*	Golding	Hicken	Proctor	Russell	Saunders	Stuart	Thompson	Tridgell*	Watson	Whitehead, A.	Whitehead, D.	Williams	Wodeman	Woodhouse
4.9.42	-5.9	-5.55	-6.05	-5.7	-6.05			-6.05	-5.5	-5.65	-5.7	-6.35	-5.5	-5.85	-5.8	-5.6	-5.7	-6.1	-6.05	-5.4	-5.45	-5.95	-5.5
21-23.12.42	-5.6	-5.3	-6.1	-5.85	-6.3			-5.8	-5.4	-6.0	-5.8	-5.5	-5.2	-5.85	-5.35	-5.75	-5.8	-6.0	-5.65	-4.85	-5.25	-6.3	-5.5
18-20.2.43	-5.6	-5.6	-6.0	-6.05	-6.1	-6.0		-5.8		-5.7	-6.0	-5.8	-5.3	-5.7	-5.45	-5.8	-6.5	-5.8	-4.9	-5.25	-6.05	-5.5	-5.5
9.4.43	-5.95	-5.35	-5.9	-5.8	-6.15	-6.3		-5.95		-5.7	-5.8	-5.35	-6.15	-5.7	-5.4	-5.65	-5.7	-5.95	-4.9	-5.3	-6.4	-5.4	-5.4
31.5.43			-6.25	-5.8	-5.95	-6.2	-6.1	-5.95		-5.85	-5.95	-6.0	-5.45	-6.1	-5.5	-6.1	-5.85	-6.3	-6.1	-4.9	-5.3	-6.3	-5.45
17.6.43		-5.65		-5.65	-6.4	-5.9	-5.7	-5.7		-5.6	-5.6	-5.8	-5.4	-5.8	-5.4	-6.1	-5.9	-6.3	-5.95	-4.9	-5.4	-6.25	-5.6
19-20.7.43		-6.0	-5.9	-5.75	-5.8	-6.15	-6.0	-5.55		-5.65	-5.95	-5.85	-5.4	-6.0	-5.8	-5.95	-5.7	-6.15	-5.7	-4.5	-5.3	-6.3	-5.4
16-18.8.43		-5.95	-6.0	-5.75	-6.1	-6.5	-5.95	-5.5		-5.3	-6.1	-5.9		-6.0	-5.8	-5.95	-5.8	-5.95	-5.8	-4.75	-5.5	-6.4	-5.55
24-27.8.43					-6.18†																		
28-31.8.43					-6.19‡																		
12-14.9.43		-6.0	-6.1			-6.3	-5.95	-5.95		-5.2	-6.1					-6.05	-5.6	-6.05	-5.7	-4.85	-5.3	-6.4	-5.4
2.10.43										-5.2													
4-30.10.43										-5.32§													
1-2.11.43		-5.7	-5.8			-6.4	-5.6	-5.7		-5.65		-5.5				-5.85	-5.6	-5.8			-5.1	-6.3	-5.3
19-20.12.43		-6.25	-5.6	-5.8		-6.3	-5.5	-5.4		-5.5		-5.35				-5.7	-5.4	-6.05	-5.7		-5.1	-6.0	-5.5
11.1.44												-5.4											
6-7.2.44		-6.3	-5.8	-5.7		-6.3	-5.75	-5.4		-5.4		-5.4				-6.0	-5.7	-6.0	-5.2		-5.3	-6.05	-5.55
19-20.3.44		-6.35	-5.9			-6.5	-5.9	-5.45		-5.7		-5.65				-5.55	-6.1	-5.4				-6.05	-5.4
8-9.5.44		-5.9	-5.7			-6.2	-5.7	-5.15		-5.8		-5.5					-5.65	-5.7					-5.35
18-19.6.44		-5.95	-5.7				-5.6					-5.7						-6.25					

* Received supplement of carotene or vitamin A for all or most of the time. † Average of 7 results before dosing. ‡ Average of 20 results after dosing. § Average of 10 results after dosing.

TABLE XLV

Values (in Minutes) for all Observations of the Cone-Rod Transition Time Measured by Craik's Adaptometer (beginning of dosing indicated by horizontal line)

Date	Bartley, M.*	Bartley, W.*	Bloomfield	Broderick*	Brown	Drabble*	Drake*	Garling	Garnett*	Golding	Hicken	Proctor	Russell	Saunders	Stuart	Thompson	Tridgell*	Watson	Whitehead, A.	Whitehead, D.	Williams	Wodeman	Woodhouse
4.9.42	5½	8	6	5	6			5½	6½	7	10	5	5		7½	5	7	5	7	6½	7	4½	7
21-23.12.43	4	6½	7	5	5	7		5½	7½	7	5	7	5½		7½	5	8	5½	7½	7½	8½	6	5
18-20.2.43		7	8	6	7½	5		4	5	4	5½	7	5		5	7	9	5	4½	6	6	4	4
9.4.43	5	5½	7		7	7	8½	7		5	5½	6	5		6	6	7	3½	6	5½	9	7	8½
31.5.43			6½	6	6	6½	7	6		7	5½	7	7½		8	6	7	5	7	7	6	6	10
17.6.43		8			9	7	8½	5		7	8	7	7½		9	6	8	5	8	7	6	6	9½
19-20.7.43		4½	6½	5	7	7	9	7		9½	6	6	7		8	8	4½	4	7	6	5	5	8
16-18.8.43		6	7	6	7½	8½	8½	5½		14½	6	7	7½		8		7	5	7	7	9	6	8
12 14.9.43		8	7			6	7	5½		21½	7	10				5	6	4½	7	10½	6	6	8
1-2.11.43		8	7½			7	7	6½		7½		10				8½	6½	5			8	5	10
19-20.12.43		7	6½	7½			8½	7½		5		13½				10	6	6	6½		5½	6	8
11.1.44												17											
6-7.2.44		9½	7			7	9	7		6		10½				7½	7½	8	7½		7	6	8½
19-20.3.44		7½	8			5½	10	7		6		10				8	6½	16½				7½	11
8-9.5.44		6	10			7	6	7½		5½		7½					7½	20					8½
18-19.6.44		7½	9½				9					8					8	6					

* Received supplement of carotene or vitamin A for all or most of the time.

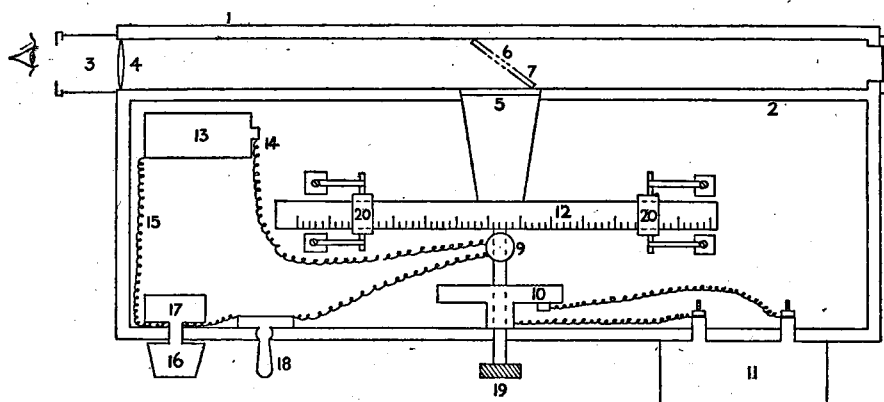


FIG. 13.—Plan of portable low brightness photometer of Craik.

- | | | | |
|----|------------------------------------|----|--|
| 1 | Wooden case. | 12 | Neutral wedge, Ilford, density 0 to 6. |
| 2 | Partition in wooden case. | 13 | Single dry cell. |
| 3 | Eye tube. | 14 | } Clip and contact for dry cell. |
| 4 | Lens, 6-in. focus. | 15 | |
| 5 | Flashed opal diffuser. | 16 | Knob of resistance. |
| 6 | Aperture in mirror. | 17 | Circular resistance, 10 ohms. |
| 7 | Silvered surface of mirror. | 18 | Switch. |
| 9 | Spotlight bulb, 2.5 volts. | 19 | Knob on shaft operating friction roller. |
| 10 | Selenium oxide photo-cell (Eel). | 20 | Rubber rollers. |
| 11 | Micro-ammeter, 0 to 50 micro-amps. | | |

the micro-ammeter; this ensures that the light from the bulb is kept constant. The lid of the instrument has a circular hole with a cross-wire by means of which the scale on the wedge can be read.

Calibration of the low brightness photometer.—A standard lamp of candle power 25.2 was set up at a distance of $\sqrt{\frac{25.2}{0.1}}$ ft. from an aluminium plate smoked with magnesium oxide, thus producing a brightness of the magnesium oxide screen of 0.1 equivalent foot candles. This screen was viewed with the photometer wedge set at 0.1 equivalent foot candles and, by adjusting the variable resistance of the photometer, the brightnesses of the photometer field and the screen field were matched. The micro-ammeter was read and the reading thus obtained was kept constant with the help of the resistance throughout the subsequent use of the photometer.

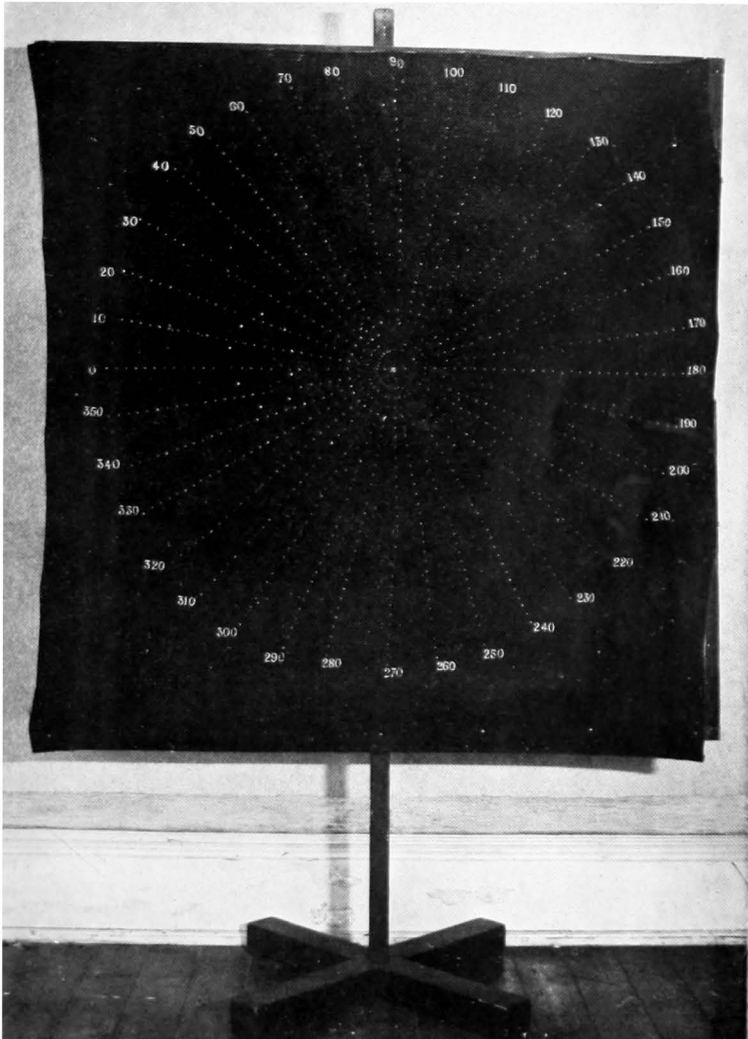
TECHNIQUE OF ROD SCOTOMETRY

*Procedure**

The test was made in a completely darkened room. The investigator could use a torch dimmed by a red filter. The subject was seated opposite a modified Bjerrum screen (*see* Plate II) on which a 30° field was marked with white and red dots. Fixation of the eye under test was maintained by projecting a pencil of red light from the centre of the screen through a tube suitably baffled. This provided a source of light 1 mm. in diameter which immediately became invisible if the eye under test wandered; cone fixation

* The description of the method follows partly Livingston's (1944) account, and partly verbal information received from him.

PLATE II.



Bjerrum screen modified for use in rod scotometry.

was thus maintained without disturbance of rod adaptation. This is a point of much importance in view of the natural tendency of the eyes to undergo automatic movements in the dark. A metal collar was fitted round the base of the tube through which the red light passed. Behind this, in a groove, was placed a band of self-luminous paint, which indicated the position of the centre of the screen to the examiner standing on one side of the apparatus, but remained invisible to the subject under test. This assisted in locating the centre of the screen.

The first test objects were supplied by Air Commodore P. C. Livingston ; they were prepared from a radium sulphide paint at a Royal Air Force depot. The size of the self-luminous dot was about 2.5 mm. in diameter. The luminosity of the test objects was measured at the National Physical Laboratory. Targets between 3×10^{-9} and 13×10^{-9} candle power were found to be suitable for differentiating between normal and defective rod vision. The luminosity of the principal targets used was about 6.2×10^{-9} and 13.5×10^{-9} candle power.

The subject was adapted for 30 minutes by sitting in the dark, after which he was seated in front of the screen with the eye to be tested in a direct line with the red fixation light. An eye shade was put over the eye not under test. The subject was requested to search for the red light, to fix it continuously with his eye, and to state when he could or could not see the luminous target moved by the examiner about the screen. The target was placed first about 10 cm. to the left of the fixation spot if the left eye was being tested, care being taken to keep the target facing the subject. When the subject had identified the target, it was moved directly to the left, the red light being kept steadily fixed all the time. The subject was asked to indicate the moment when he ceased to be able to see the target, and a pin with a coloured head was put into the screen to mark the point where the target disappeared from the field of vision. The target was then moved further to the left until the subject could see it once more, and another pin was inserted in the screen at this point. From midway between these two points the target was next moved upwards until the subject again saw it. This process was repeated with the target starting midway between the first two pins and moving downwards and then outwards along the four diagonals. This gave eight points in all, outlining the blind spot. The target was then placed about 10 cm. above the red fixation light, on the 90° line, and was moved outwards until the subject could no longer see it and a pin was inserted at this point. This was repeated, the target being moved along the 0°, 45°, 130°, 180°, 225°, 270°, and 315° lines. In this way eight points were obtained mapping the outer limits of the field of vision. The target was then put about 10 cm. from the fixation light in the direction of the 90° line, and moved in towards the centre ; where the target disappeared a pin was inserted. This procedure was repeated at the 0°, 270°, and 180° lines. The resulting area within the pins marked the central scotoma. The subject was rested from time to time during the test. Various points were checked by repetition in order to estimate the ability of the subject to reproduce results. Unless readings were consistent they were discarded.

Experience with over a hundred subjects showed that some people failed to give satisfactory results. Some were unable to see targets of luminosity

6×10^{-9} c.p. although their daylight vision and the appearance of the fundus and media were normal. Others were unable to give consistent readings, as might be expected in a test requiring intelligent co-operation from the subject. Some persons were found to show a relatively large blind area when tested for the first time and to improve on repetition of the test. "Learning" effects of this sort were not seen among the Sorby volunteers, presumably because they were sufficiently trained by previous experience for this type of test. The volunteers all gave consistent results, except on certain occasions when genuine difficulties were encountered in locating the test object. On these occasions the subject was unable to give prompt answers. He sometimes had glimpses of the target when it was kept still, but could not see it all the time. This state of affairs is recorded as "fluctuating sensitivity".

Targets

Self-luminous targets.—The equipment provided by Air Commodore Livingston included two targets whose nominal luminosities were 6.5×10^{-9} and 13.5×10^{-9} candle power. Both targets were used in most cases. They had been calibrated by the National Physical Laboratory at an unspecified time previously.

After about $2\frac{1}{2}$ months it became obvious that the targets had lost some of their original luminosity. On 20th November, 1943, the target with the nominal luminosity of 6.5×10^{-9} was returned for recalibration but unfortunately was lost in transit. The target with the nominal luminosity of 13.5×10^{-9} had by that time lost about half its original luminosity and was used during January, 1944, as the standard target.

Electrical targets.—Deterioration of the self-luminous targets was a very serious defect of the method and the difficulty of calibration was another drawback (see Stiles, 1944). It was therefore decided to construct a different type of target in which the source of light was an electric bulb whose luminosity was reduced by means of a neutral silver wedge.* On principle, such a target would have two advantages: the source of light would be more easily reproducible than self-luminous dots, and the calibration could be carried out at a brightness a thousand or ten thousand times greater than that of the target; at this level photometry is relatively simple, so that it is possible to recalibrate the target at frequent intervals. The outcome of various trials was the instrument depicted in Fig. 14.

The target was an area of opal glass illuminated by an underrun ophthalmoscope bulb (B) whose luminosity was reduced by means of an Ilford neutral silver wedge (W). The ophthalmoscope bulb was housed in a small metal box (M). In front of the bulb was the neutral wedge, a green gelatine filter, Ilford 401 (F), and an opal glass (O). The gradient of the wedge was approximately 1 log. unit per 2 inches, exact information on the gradient being supplied by the manufacturers. The wedge was movable by hand and its position was read on a steel scale (S), divided into fractions of $1/64$ th of an inch, as manufactured for depth gauges. The metal scale was provided with a stop against which the end of the wedge rested. A mark on the metal box opposite the centre of the aperture indicated the length of the wedge in use. The cap (C) on the front of the box consisted of a

* A light source of this type has been used by Sloan (1939) for testing the light minimum in photopic and scotopic vision, and the rate of dark adaptation.

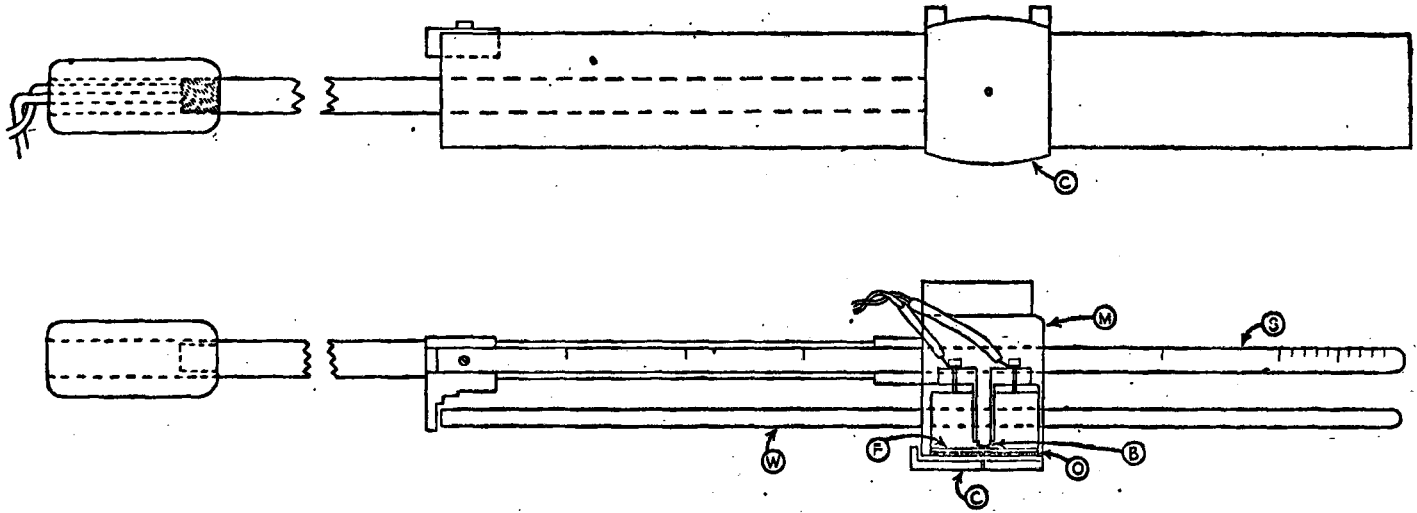


FIG. 14.—Plan of electrical scotometry target.

B Ophthalmoscope bulb.
 C Cap.
 F Filter.
 M Metal box.

O Opal glass.
 S Steel scale.
 W Neutral wedge.

detachable piece of brass with a centre hole. A set of caps with holes of different size was available ; the size of the hole used for routine work was 2 mm.

The bulb was connected in series with an accumulator of 2 volts, a milli-ammeter of 0 to 250 milli-amp. and a variable resistance of 10 ohms.

Calibration of the electrical target.—The calibration of the brightness of the opal glass was carried out with the low brightness photometer of Craik. A circular area of about 12 mm. diameter, sufficiently large for making the measurements, could be exposed by removing the cap. With the cap removed, the wedge was set at a convenient position, usually at 1 or 2 inches, so that the brightness of the opal glass was between 0·1 and 0·01 equivalent foot candles. The milli-ammeter of the target was adjusted to a figure somewhere between 0·13 and 0·19 amp., this range being chosen in order to underrun the ophthalmoscope bulb. The target was then placed on its back in a convenient position and the opal glass was viewed through the photometer. The photometer was adjusted until the brightness of the photometer field matched that of the opal glass. The photometer reading was recorded and the procedure was repeated at least six times. The results, which should be consistent within 0·02 log. unit, were averaged. The calibration was best performed in the dark, although it was possible with experience to obtain consistent results with a moderate intensity of lighting. The result obtained represented the brightness of the opal glass. The intensity of illumination, in candle power, emitted through the hole of 2 mm. diameter in the cap (C) is calculated with the help of the following formula :

$$\text{Candle power of area of 2 mm. diameter} = \frac{\text{photometer reading in e.f.c.} \times \pi}{144 \times 25 \cdot 4 \times \pi}$$

The position of the wedge required to give the desired candle power (usually $6 \cdot 5 \times 10^{-9}$) is calculated as follows :

Let

L_1, L_2 , etc. = different distances from thin end of wedge ;

A_1, A_2 , etc. = the logarithms of the brightness of the opal at L_1, L_2 , etc. ;

d = density gradient of the wedge ;

then $A_1 = A_2 - (L_1 - L_2)d$.

If L_1 is the position of the wedge during the calibration, A_1 the brightness of the 2 mm. diameter area, A_2 the brightness desired, L_2 the unknown position of the wedge to give the brightness A_2 , then

$$L_2 = L_1 + \frac{A_1 - A_2}{d}$$

Example : Current recorded by ammeter 0·140 ; wedge set at 1 inch ; optical density of the wedge 0·4936 ; photometer readings (log. e.f.c.) : $\bar{2} \cdot 96, \bar{2} \cdot 98, \bar{2} \cdot 96, \bar{2} \cdot 96, \bar{2} \cdot 98, \bar{2} \cdot 95$; average photometer reading $\bar{2} \cdot 965$; calculated brightness of opal glass 0·09226 e.f.c. ; candle power of 2 mm. hole $993 \cdot 1 \times 10^{-9}$. Luminosity required : $6 \cdot 5 \times 10^{-9}$ c.p.

Length of wedge to give $6 \cdot 5 \times 10^{-9}$ c.p.

$$= 1 + \frac{\log. (993 \cdot 1 \times 10^{-9}) - \log. (6 \cdot 5 \times 10^{-9})}{0 \cdot 4936}$$

$$= 5 \cdot 424 \text{ (or } 5\frac{2}{3} \text{) inches.}$$

Calibration of the electrical target by the National Physical Laboratory.—In order to check the above procedure of calibration a target was sent to the National Physical Laboratory to be calibrated by the method of Stiles (1944), which determines directly the candle power of the target, with the wedge and cap in the position in which they were used for scotometry. It therefore checked the photometric measurement made at the Sorby Research Institute and the accuracy of the series of values allotted to the luminosities obtained by progressive reduction with the wedge and by cutting down the area of the opal glass.

With the target was sent information concerning the position of the wedge, and the current for the ophthalmoscope bulb, which had been used in the Sorby Institute test. By the Sheffield method of calibration the intensity of illumination was found to be 6.5×10^{-9} c.p. when the reading on the steel rule was 5 inches and the strength of the current was 180 milli-amp. The relevant section of the report received from the National Physical Laboratory is as follows :

“The scotopic candle power of the electrical scotometry target measured on a line through the centre of, and perpendicular to, the aperture was found to be 6.3×10^{-9} . It is estimated that, under the conditions used, this value is correct to within 12 per cent.”

The agreement between the results obtained by the Sorby Institute method and by the National Physical Laboratory was therefore satisfactory.

Scotometry Measurements on Vitamin A Deficient Volunteers

Owing to the circumstance that the method was being developed during the course of the experiment and that the luminosity of the targets was not consistent, the measurements do not represent a homogeneous series. Nevertheless at least two types of comparable data are available, both relying on measurements with the same target within short periods during which the luminosity of the target may be assumed to have been virtually constant.

(1) Results obtained from different individuals at the same time, allowing the comparison of controls and deficient subjects.

(2) Results obtained from the same individual before and after treatment with vitamin A or carotene. If, on treatment, rod vision improved, this must be regarded as a real improvement. The gradual deterioration of the target would have the opposite effect, since it would simulate a deterioration of rod vision, and any improvement observed would thus have been smaller than the actual improvement.

All results obtained with targets of approximately 6.5×10^{-9} candle power, and expressed as square cm. blind area on the Traquair chart, are recorded in Protocol C. Five different targets were used.

Target 1.—Self-luminous target, supplied by Air Commodore Livingston, of a nominal value of 6.5×10^{-9} candle power. This target had been calibrated by the National Physical Laboratory at an unspecified date previously. It was used from 3rd August until 20th November, 1943. At the end of this period its luminosity had dropped to about half the original value.

Target 2.—This was another self-luminous target received at the same time as target 1. Its luminosity at the time of calibration was 13.5×10^{-9} candle power. It had fallen to about half its value when the recorded measurements were made. It was used between 1st January and 2nd February, 1944.

Target 3.—This was the first electrical target constructed at the Sorby Research Institute. It was slightly different from that described on p. 116, in that a 3·5-volt spotlight bulb was used as the source of light and a 6-inch optical wedge, density gradient 1 per inch, was employed to cut down the luminosity. It was calibrated as described and adjusted to $6·5 \times 10^{-9}$ candle power. It was used between 1st January, 1944 and 30th March, 1944.

Target 4.—This was an electrical target, as described, except that a 6-inch wedge was used with a density gradient of 1 per inch. It was used between 31st March and 2nd May, 1944.

Target 5.—The same as target 4 except that an 8-inch wedge of 0·5 density gradient per inch was substituted. It was used from 3rd May, 1944 onwards.

Since the last three targets had very nearly the same candle power, results obtained with these targets are comparable.

S.—Particulars of Medical Examinations

ENLARGEMENT AND HYPERKERATOSIS OF THE HAIR FOLLICLES

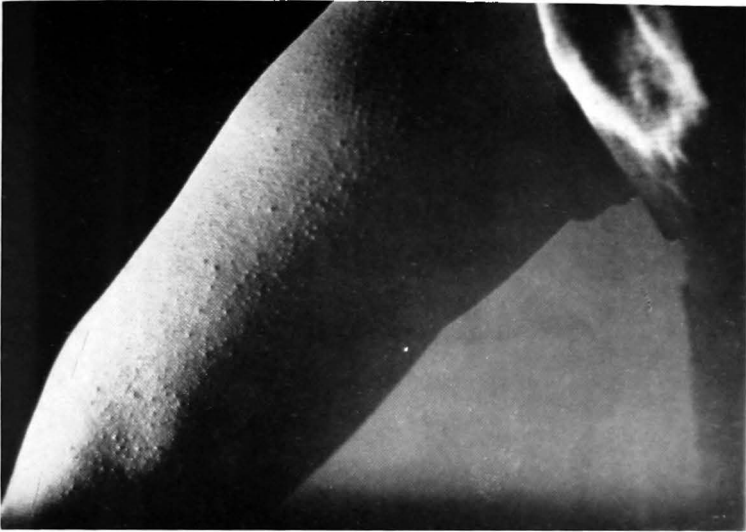
Two stages in the changes of the hair follicles were recorded—simple enlargement, and hyperkeratosis in addition to enlargement. The latter was recorded when a tiny spine of horny material could be seen and felt protruding over the orifice of the follicle. The changes were mostly found on the extensor aspects of the upper arm and on the buttocks. Occasionally eruptions appeared on the trunk especially over the scapulae, and on the calves.

The observations on the volunteers are recorded in Table XLVI. It will be seen that two of the seven receiving supplements and five of the sixteen deprived subjects had neither of the signs throughout. The others showed varying degrees at various times. Only in Golding did the eruptions increase in number and size as the depletion proceeded and then decrease on dosing. In the others the variations were unrelated to the intake of vitamin A.

Minute structure and histology.—Pieces of skin were taken from three deprived subjects, Golding, Proctor, and Bloomfield. The pieces were of elliptical shape, about 2 cm. long and 4 mm. wide at the widest part, and extending down to the fat. They were taken from the extensor aspect of the right arm, with the exception of one piece from Golding, which was taken from the left upper quadrant of the abdomen.

Golding.—The first examination was made in September, 1943, after he had been on the deficient diet for 14 months, when the deficiency was at its height. The photograph showing the naked eye appearance of the arm is shown in Plate III. With the binocular dissecting microscope the elevations appeared to consist of semi-translucent horn containing one or sometimes two coiled hairs. The coils looked like a watch hair-spring lying on the skin and in some places one or more coils appeared to have burst through, one side of the plug standing perpendicular to the skin surface. The plugs blocking the mouths of the hair follicles were soft and could fairly easily be detached. As a result the hair sprang outwards, bearing the plug attached to its distal end. Once the plug was detached the follicle appeared normal. There was no abnormality of the hair shaft.

PLATE III.



Photographs of the outer aspect of the upper arm of Golding (*see* p. 120).

Above : At height of depletion (September, 1943), showing marked follicular hyperkeratosis.

Below : After six months' treatment with 1,300 I.U. vitamin A daily (March, 1944). The eruptions are smaller and less numerous but still apparent.



TABLE XLVI

Incidence of Enlargement and Hyperkeratosis of the Hair Follicles among the Volunteers in Relation to the Diet

Name	Nature of supplement to basal diet (duration in months)	Site and degree of enlargement or hyperkeratosis of hair follicles
Bartley, M. ..	Carotene (8½)	None throughout.
Bartley, W. ..	Carotene (22) ; none (1) ..	Slight enlargement on buttocks at 17 months ; on arms at 22 months ; still present after 3 months' unrestricted diet.
Broderick ..	Carotene (13½) ; none (5½) ..	Slight enlargement on arms at beginning only.
Garnett ..	Carotene (6½)	Slight enlargement on buttocks at 4 months.
Drabble ..	None (2½) ; vitamin A (15½)	Slight enlargement on arms at beginning ; slight hyperkeratosis on arms at 14 months.
Drake ..	Vitamin A (1) ; carotene (14)	None throughout.
Tridgell ..	None (5½) ; vitamin A (17) ..	Slight enlargement on arms at beginning, at 7, and at 18 months ; still on back after 3 months' unrestricted diet.
Bloomfield ..	None (24½) ; carotene (½) ..	Slight enlargement on arms at beginning ; moderate about 16 months ; hyperkeratosis on arms at 21 months ; enlargement and hyperkeratosis on buttocks at 23 months.
Brown ..	None (13)	None throughout.
Garling ..	None (21½) ; vitamin A (1) ..	None throughout.
Golding ..	None (14½) ; vitamin A (7½)	Slight enlargement and hyperkeratosis on arms at beginning ; at 4 months spreading to legs and scapular region ; less after dosing with vitamin A but persisting in a mild degree.
Hicken ..	None (17)	Slight enlargement on arms at beginning ; hyperkeratosis on arms at 5 months ; slight enlargement and hyperkeratosis on buttocks at 5 months.
Proctor ..	None (18½) ; carotene (5½) ..	Moderate enlargement on arms at 18 months ; none after 3 months' unrestricted diet.
Russell ..	None (10½) ; carotene (1) ..	None throughout.
Saunders ..	None (12½)	None throughout.
Stuart ..	None (10½) ; carotene (1) ..	None throughout.
Thompson ..	None (18) ; vitamin A (2) ..	Slight enlargement on arms at beginning ; involving trunk at 6 months.
Watson ..	None (22) ; carotene (1) ..	Slight enlargement on buttocks at beginning ; slight enlargement and hyperkeratosis on arms at 18 to 20 months ; none after 3 months on unrestricted diet.
Whitehead, A.	None (14½) ; carotene (4) ..	Moderate enlargement and hyperkeratosis on arms at beginning ; the same after 3 months' dosing with carotene.
Whitehead, D.	None (13½)	Slight enlargement on arms at beginning.
Williams ..	None (18) ; vitamin A (2) ..	Slight enlargement on back at beginning ; slight enlargement and hyperkeratosis on buttocks at 5 months ; none after 3 months' unrestricted diet.

TABLE XLVI—*contd.*

Name	Nature of supplement to basal diet (duration in months)	Site and degree of enlargement or hyperkeratosis of hair follicles
Wodeman ..	None (18½) ; vitamin A (2) ..	Slight enlargement on buttocks at 5 months ; moderate enlargement and hyperkeratosis on buttocks at 18 months ; none 2 months after dosing with vitamin A ; slight enlargement on arms noted for first time 2 months after dosing with vitamin A ; none after 3 months' unrestricted diet.
Woodhouse ..	None (20) ; carotene (1½) ..	Slight enlargement on arms and buttocks at 5 months ; slight enlargement on arms and chest after 3 months' unrestricted diet.

Histologically there was no increase of superficial keratin, though the lamellae were closer and appeared to stain more deeply with eosin. The horny plugs in the follicles did not appear as elevations, which suggests that the papillae found in life may owe their protrusion to the action of the well-formed *erector pilae* muscles in this area. The plugs were formed by intra-follicular keratinization with consequent dilatation of the follicle. There were no sebaceous glands visible and their probable site was marked by small clusters of lymphoid cells round the follicle. Elsewhere, the arterioles had a sheath of round cells, but not greater than in normal skin. There was no evidence of inflammation. The excision did not go deep enough to include the sweat glands except at one point where they were found to be normal. The basal layer of the epithelium appeared rather active ; the cells appeared swollen and showed many mitoses.

A piece of skin from the abdomen was excised at the same time. Macroscopically this was normal. Histological examinations showed no keratinization of the follicles ; the sebaceous glands were intact.

A further excision from the arm was made in March, 1944, after about six months' treatment with 1,300 I.U. vitamin A daily. Histologically this skin contained abundant sweat glands and sebaceous glands. There appeared to be a slight increase in the superficial keratin, and the mouths of the hair follicles contained considerable amounts of dark staining keratin. The macroscopic appearance at that time is shown on Plate III. It will be seen that the eruptions, though smaller, were still present.

A third excision was made in July, 1944. In the interval treatment had continued with a dose of about 2,600 I.U. vitamin A daily. Histologically the epidermis was normal. The hair follicles had widely dilated mouths filled with laminated keratin in which a hair was coiled. The keratosis did not appear to extend far down the follicle. In serial sections one abnormal follicle shaft was seen. It was tortuous and the enclosed hair was even more coiled, so that at one point the hair had broken out of the epithelial tube and lay between the tube and the surrounding fibrous tissue. Sebaceous glands were absent but there were occasional areas of dense connective tissue and round-cell infiltration adjacent to the hair shaft. The blood vessels of the corium were rather dilated and made conspicuous by a mantle of round cells. The sweat glands were normal.

Two series of sections were cut from this material and five hair follicles were seen. None of these had sebaceous glands. Macroscopically the eruption had nearly cleared up at that time.

A fourth excision was made in November, 1944, after further treatment with vitamin A. The histological picture differed from the last only in that the vessels in the corium were not so dilated and had little or no cellular mantle. Serial sections of one hair showed the same dilatation of the mouth of the follicle and the same absence of sebaceous glands.

In summing up the observations on Golding it must be said that the histological findings did not closely follow the macroscopical changes. Macroscopically the eruptions increased in number and size during the period of depletion and receded, though they did not disappear completely, on treatment with vitamin A. Microscopically the sebaceous glands seemed to be absent from the hair follicles at the end of the period of deprivation; they were present after 5½ months' dosing, but disappeared again later in spite of continued treatment with vitamin A. At all stages plugging of the hair follicles and coiling of the hairs were noted.

Proctor.—A biopsy was made in November, 1943, after 16 months' deprivation. There was no macroscopical enlargement or hyperkeratosis at that time, and histologically the appearance of the skin was normal. There were abundant sebaceous glands and no keratinization of the hair follicles.

Bloomfield.—A biopsy was made in August, 1944, after he had been deprived of vitamin A for 24 months. Macroscopically he showed slight follicular enlargement and hyperkeratosis at this time. Histologically there was no notable abnormality of the keratin. The hair shafts were straight. There were no sebaceous glands round the two hairs seen in twenty serial sections, but the roots were somewhat irregular and there was some increase of connective tissue round them. The sweat glands looked normal. The arterioles and precapillaries were made very prominent by cellular thickening of their walls. Since no sebaceous glands were seen, a further series of sections was cut from the same block; these sections showed two hairs with well developed sebaceous glands.

Summary.—Macroscopic and microscopic examinations of the skin showed that enlargement and hyperkeratosis of the hair follicles occurred among the subjects. The chief microscopic feature was plugging of the mouths of the hair follicles with horny debris, coiling of the hair and atrophy of the sebaceous glands.

The condition occurred in the supplemented as well as in the deprived group. The extent and size of the eruptions seemed to fluctuate independently of the state of vitamin A nutrition. The observations, therefore, do not support the view that, in the conditions of the present experiment, lack of vitamin A was a causal factor in the development of follicular hyperkeratosis.

When the question of whether vitamin A deficiency is the specific cause of follicular hyperkeratosis was discussed at a meeting of the Royal Society of Medicine, Stannus (1945) arrived on general grounds at much the same conclusions as the present investigators.

SUMMARY OF THE REPORT BY W. J. WELLWOOD FERGUSON ON THE SLIT-LAMP EXAMINATION OF THE EYE (*see also Protocol E*)

All volunteers were subjected to slit-lamp examinations at frequent intervals during the experiment. Three types of opacity of the conjunctiva were noticed at the beginning of the experiment: (1) general milkiness of the

conjunctiva ; (2) floccular opacities ; (3) larger areas of opacity of the type known as pinguiculae. The areas and type of opacity in each case were carefully charted. Throughout the period of observation no perceptible changes were recorded in any of the individuals. None of the cases exhibited at any time wrinkling of the conjunctiva of the type mentioned by Kruse (1941) amongst the signs of vitamin A deficiency.

The examination of a large number of persons not connected with the experiment confirms the view that the conjunctival signs ascribed by Kruse to vitamin A deficiency are due to various non-dietary factors, such as exposure, increasing age, or individual idiosyncrasy (Ferguson, 1944). In particular, it is noteworthy that the conjunctiva in children and young persons is normally thicker and less transparent than in those past middle age. In elderly persons the superficial layers of the conjunctiva tend to become thinner and more transparent, although as a rule studded with a variable number of areas of hyaline degeneration.

These observations are in accordance with those of Berliner (1942; 1943) and of Anderson and Milam (1945).

SUMMARY OF THE REPORT BY J. L. BURN ON AUDIOMETRY MEASUREMENTS

(see also *Protocol F*)

The capacity for hearing of fourteen of the volunteers was tested by means of the pure tone audiometer. A continuous series of results, consisting of five to seven measurements spread over two years, was obtained on Bartley, Drabble, Golding, Proctor, Watson, Whitehead, A., and Woodhouse.

The audiometer which was used enabled notes of a known frequency to be presented to the subject at an intensity ranging between -15 and 120 decibels.

The subject listened to the note through an earphone, or by an attachment clipped directly on to the head when hearing by bone conduction was being measured. Notes of subthreshold intensity were presented and gradually increased in volume until the subject became aware of them ; this he indicated by pressing a switch which operated a torch bulb visible to the tester. The frequencies 256, 512, 1,024, 4,096, and 8,192 were used in testing hearing by air conduction, and three frequencies 256, 1,024, and 4,096 for testing hearing by bone conduction. The threshold values in decibels obtained at the different frequencies were averaged separately for both methods of hearing.

The data were submitted to J. O. Irwin for statistical analysis. His findings supported the following conclusions :

(1) The two subjects, Bartley and Drabble, receiving supplements of vitamin A or carotene, showed no significant change of hearing throughout the experiment.

(2) Of the five subjects deprived of vitamin A, Golding, Proctor, and Whitehead, A., showed significant worsening of hearing and significant improvement on dosing. It should be recalled that the first two were definitely deficient by other criteria.

(3) The remaining two deprived subjects, Watson and Woodhouse, showed fluctuations but no consistent trend.

SUMMARY OF THE REPORT BY D. RUSSELL DAVIS ON THE PSYCHOLOGICAL STATE OF THE VOLUNTEERS

Some of the subjects reported in the Autumn of 1943 that they became tired more easily than before in both mental and physical activities. It was decided that the complaints must be investigated to try to discover whether

they were due to deprivation of vitamin A, and correlated with any demonstrable deterioration of performance in mental and physical tasks. The investigation was begun in January, 1944, and continued until October, 1944.

Investigation of Subjective Symptoms

All subjects, whether they had been deprived of the vitamin or not, were questioned about all matters which might be relevant. Two of the five non-deprived, and nine of the twelve deprived, subjects made definite complaints of tiredness and lack of energy, although only of a slight degree. *Prima facie* this difference of incidence, though not significant statistically, was strongly suggestive of an effect due to the deprivation, but since the subjects were fully informed of their nutritional state the possibility of suggestion could not be ruled out.

The nature and course of development of the symptoms of several subjects were not those which would be expected from a vitamin deficiency. Difficulty in concentration, absent-mindedness, and even insomnia were prominent in several cases and, if they had any significance at all, these symptoms, with frequency of urination complained of by one subject, were more readily explained as a result of anxiety than of a vitamin deficiency. Moreover, supplementing the diet did not influence the symptoms except in Golding. Proctor, for example, whose symptoms of lassitude and sleepiness during the day time were more like those of a deficiency than the symptoms of most of the subjects, did not feel any better after being dosed, his complaints being most definite 6 weeks after restoration of the diet to normal.

Only in Golding did the nature and course of the symptoms suggest a real effect of deprivation. He said that he became aware of a "general feeling of lassitude and tiredness" in the autumn of 1942, and that the feeling became gradually worse until October, 1943, when his diet was supplemented. At the first interview in January, 1944, he felt very much better, but his feeling of tiredness was accompanied by a soreness round the eyes, and this did not improve, being still present in July, 1944. He had also developed migraine-like headaches (p. 23); his account of them and of his feelings of fatigue did not suggest a psychological origin.

Tests of Mental and Physical Deterioration

The mental and physical powers of the subjects had not been measured by special tests before they were deprived of vitamin A, and reliance had to be placed entirely on comparisons made before and after the restoration of the diet to normal. By the time the psychological investigation was begun, it had been decided for other reasons to supplement the diet of the majority within a relatively short period of time. This was another handicap because there was not sufficient time to devise and standardize really suitable tests, or to test the subjects often enough to reduce the effects of individual variation to a degree permitting the demonstration of relatively small changes in ability. Also the number of subjects was small and the uncontrolled variables could not be properly randomized. Several special tests were applied, however, including the "pennies" test which was a modification of the agility test used by Frankau (1943), an "adding" test, a test of the strength of the handgrip with a dynamometer, and tests of memory and span of apprehension. Only in Garling was the performance

appreciably worse before than after the restoration of the diet to normal. The difference was small and was restricted to the " pennies " test ; thus, on two occasions (28th April, 1944, and 28th May, 1944) he was more distressed and had a higher pulse rate after a slower performance than on any earlier or later occasion. These results indicated a small loss in his physical powers, but a definite conclusion was not possible, since there were no other positive findings. The results of the special tests proved that there was no serious impairment of powers in any of the subjects, including Garling; although a small deterioration was not excluded in the majority. Even a small deterioration was proved to be very unlikely in the case of Proctor, whose performance in the pennies test was better before his diet was supplemented than at any time subsequently.

There were other grounds, as well as the results of the special tests, for believing physical and mental powers to be well maintained. None of the four subjects deprived of the vitamin, who were employed regularly outside the Sorby Institute, encountered any special difficulty in doing his work. For example, Thompson, a schoolmaster, carried on, at normal pressure, the full day's work to which he was accustomed, and Woodhouse, a clerk in a local-government office, was able to bear the extra burden of work put on him by staff shortage. The case of Watson, an undergraduate, was more difficult to assess, but neither he nor Broderick showed any signs of being handicapped in their work. Proctor cycled many times home to York, 50 miles from Sheffield, and the trip became a kind of informal test of his physical condition. He made his fastest journey immediately before his diet was supplemented in January, 1944, when he happened to be accompanied by a racing cyclist. This performance, by itself, made it very unlikely that there was any significant impairment of his abilities, and confirmed the conclusion drawn from the pennies test.

Thus, no evidence was obtained that deprivation of vitamin A had impaired the powers of the subjects, and several other factors were found which might more readily explain the complaints. Many ordinary people complain of lack of concentration, tiredness, poor memory, poor sleep, and so on, if they are given the opportunity to do so, and such a tendency would be strengthened by the influence of suggestion from the circumstances of the experiment. The majority of the subjects were of stable personality, but the group did contain several individuals more than ordinarily predisposed to nervous symptoms of one kind or another.

There were still other factors which tended to make the symptoms more prominent in the autumn and winter, 1943-44. The volunteers were mostly individuals particularly keen to contribute to social welfare, but they found themselves living for a long period without sufficient occupation or satisfying achievement at a critical period of the war, and their position as pacifists was socially precarious. There were also difficulties within the community through conflicts of personalities and duties. Thompson, for example, two days before his diet was supplemented, decided to give up his job as a schoolmaster and join a group of welfare workers ; for several critical weeks beforehand he had been dissatisfied with his mode of life and had complained of tiredness, absent-mindedness, and inability to concentrate, but these symptoms disappeared as soon as the decision was made, and his recovery was complete before his diet was supplemented.

T.—Statistical Report on the Values for the Contents of Vitamin A and Total Carotenoids in the Blood Plasma

by J. O. Irwin

The basic data of this study are the values for vitamin A and for total carotenoids in those samples of blood plasma on which all three laboratories, Cambridge, Liverpool, and Oxford, carried out analyses. The figures for each subject were included until that subject was specially dosed towards the end of the experiment. The subjects were divided into those receiving the basal diet without supplement, those receiving it with the carotene supplement, and those receiving it with the vitamin A supplement. At the beginning, in May, 1943, there were sixteen subjects in the first group, three in the second, and two in the third. By 17th April, 1944, these were reduced to three, two, and two, respectively. The calculations were not continued with the data available beyond this date, since the number of subjects remaining was considered too small.

Comparison of the Three Diets

For each laboratory's set of determinations, at each date, in each diet group, mean values were calculated. These are shown in Tables XLVII and XLVIII.

The content of total carotenoids in the blood is much greater for the group receiving carotene than for the other two groups, between which there is no significant difference. The vitamin A content of the blood is greatest in the group receiving the carotene supplement and least in the group having the basal diet without supplement, and the differences are significant taken as a whole, though not always on individual occasions.

An unweighted mean of the 15 mean counts in Tables XLVII and XLVIII gives the following figures :

Mean Values for Total Carotenoids ("I.U." per 100 ml. plasma)

	Cambridge (C)	Liverpool (L)	Oxford (O)	Laboratories in descending order
No supplement	25.45	29.22	31.39	O L C
Carotene supplement ..	140.58	149.59	165.33	O L C
Vitamin A supplement ..	24.57	29.67	28.77	(L O) C

Mean Values for Vitamin A (I.U. per 100 ml. plasma)

	Cambridge (C)	Liverpool (L)	Oxford (O)	Laboratories in descending order
No supplement	65.86	56.97	65.85	(C O) L
Carotene supplement ..	112.08	88.74	95.30	C O L
Vitamin A supplement ..	79.12	74.97	82.90	O C L

When thus averaged, Cambridge got the lowest values for total carotenoids and Liverpool the lowest for vitamin A.

Comparison of Variation between the Experimental Subjects on the Same Date and Receiving the Same Diet, as Estimated by the Three Laboratories

Variation from person to person on the same date in groups on the same diet is here compared. From the sum of squares of deviations from the

TABLE XLVII

Content of Total Carotenoids in the Blood Expressed as "I.U." per 100 ml. Plasma

(The tabulated values are the means of the values obtained by each laboratory on any one day for all the subjects tested, on basal diet with and without supplements, only those results being used for which all the three laboratories carried out analyses on the same sample)

Date	Cambridge			Liverpool			Oxford		
	No supplement	Carotene supplement	Vitamin A supplement	No supplement	Carotene supplement	Vitamin A supplement	No supplement	Carotene supplement	Vitamin A supplement
3. 5.43	47.00	104.00	50.00	39.18	104.00	44.00	22.09	172.00	19.00
31. 5.43	25.25	145.50	18.00	45.83	162.00	61.50	33.25	237.50	33.00
28. 6.43	19.15	109.50	20.00	32.31	159.00	35.50	16.38	250.00	16.50
19. 7.43	19.31	134.33	17.00	34.85	163.67	31.00	13.54	250.00	13.00
16. 8.43	25.20	81.33	26.00	30.50	120.67	30.50	54.90	217.00	64.00
13. 9.43	25.60	84.50	26.50	24.70	85.50	22.50	35.30	83.00	31.00
11.10.43	22.88	107.00	24.00	25.50	90.00	27.00	29.75	71.00	24.00
8.11.43	28.44	172.50	39.00	26.78	121.50	22.50	27.56	148.00	34.50
6.12.43	23.89	113.50	22.50	25.44	143.50	21.50	25.89	114.00	27.50
3. 1.44	16.88	157.00	15.50	27.50	109.00	26.00	29.38	104.00	26.00
31. 1.44	26.75	141.00	19.00	20.00	188.50	21.50	39.50	155.50	30.00
28. 2.44	39.00	170.00	35.00	23.00	154.50	27.50	47.00	146.50	32.00
27. 3.44	22.67	157.50	16.50	27.33	183.00	23.50	39.00	140.00	33.50
17. 4.44	20.67	213.00	19.50	22.33	218.50	18.50	27.33	190.50	18.50
16. 5.44	19.00	218.00	20.00	33.00	240.50	32.00	30.00	201.00	29.00
<i>Mean for all dates</i> ..	25.45	140.58	24.57	29.22	149.59	29.67	31.39	165.33	28.77

TABLE XLVIII

Content of Vitamin A in the Blood Expressed as I.U. per 100 ml. Plasma

(The tabulated values are the means of the values obtained by each laboratory on any one day for all the subjects tested, on basal diet with and without supplements, only those results being used for which all the three laboratories carried out analyses on the same sample)

Date	Cambridge			Liverpool			Oxford		
	No supplement	Carotene supplement	Vitamin A supplement	No supplement	Carotene supplement	Vitamin A supplement	No supplement	Carotene supplement	Vitamin A supplement
3. 5.43	62·10	—	29·00	69·60	—	67·00	87·20	—	78·00
31. 5.43	74·00	96·50	69·00	79·85	106·50	79·50	65·85	39·50	67·00
28. 6.43	70·73	100·50	76·33	74·82	91·50	80·00	88·36	94·50	90·00
19. 7.43	70·67	114·50	126·00	77·75	125·50	102·00	70·50	79·50	90·00
16. 8.43	63·43	78·67	49·00	69·29	97·33	100·50	75·14	86·67	118·50
13. 9.43	65·00	95·00	57·50	62·43	69·50	72·00	76·71	89·00	100·50
11.10.43	83·88	133·00	82·50	53·00	57·00	61·50	66·75	118·00	32·00
8.11.43	62·78	105·00	75·50	53·00	50·50	54·00	73·44	135·00	83·50
6.12.43	58·00	95·50	101·50	44·88	84·00	71·00	66·00	100·50	91·00
3. 1.44	50·38	110·00	67·50	58·75	92·50	76·50	68·00	100·50	90·00
31. 1.44	71·50	122·00	103·00	46·75	106·50	58·00	74·00	104·50	113·50
28. 2.44	65·50	157·50	108·50	41·75	96·50	73·00	56·50	122·00	95·50
27. 3.44	67·00	117·00	85·00	45·00	58·50	60·00	49·33	101·00	85·50
17. 4.44	66·33	131·50	85·50	34·00	84·50	62·50	31·67	86·00	59·50
16. 5.44	56·67	112·50	71·00	43·67	122·00	107·00	38·33	77·50	49·00
<i>Mean for all dates</i>	65·86	112·08	79·12	56·97	88·74	74·97	65·85	95·30	82·90

mean within the three diet groups, pooled estimates of the standard deviations were made and are as follows :

<i>Person to Person Variation in Content of Total Carotenoids</i> (expressed as "I.U." per 100 ml. blood plasma)			
	Cambridge	Liverpool	Oxford
<i>Carotene supplement* :</i>			
Standard deviation	21.40	15.21	27.99
Coefficient of variation (per cent.)	15.22	10.17	16.93
<i>Other two groups :</i>			
Standard deviation	8.14	9.09	12.77
Coefficient of variation (per cent.)	32.55	30.88	42.45
<i>Person to Person Variation in Content of Vitamin A</i> (Expressed as I.U. per 100 ml. blood plasma)			
	Cambridge	Liverpool	Oxford
<i>All three groups pooled :</i>			
Standard deviation	25.33	17.50	23.38
Coefficient of variation (per cent.)	29.56	23.79	28.74

The carotene values obtained at Oxford are absolutely and relatively more variable than the others, otherwise there is no significant difference. The vitamin A values obtained at Liverpool are absolutely and relatively less variable than the others.

Examination of the Significance of Personal and Laboratory Differences in Determinations Made on the Same Date

For each date when determinations were made an analysis of variance into persons, places, and error was carried out. This makes it possible to say whether the technique was sufficiently accurate to detect differences, due to whatever cause, between different people and whether there were significant differences due to technique between one laboratory and another.

Table XLIX gives the summary of the results. Out of the 15 sets only 3 for total carotenoids, but 13 for vitamin A, show significant discrimination between the different subjects within diet groups ; 12 for total carotenoids and 1 for vitamin A show a significant discrimination which is due to diet only, mainly to the difference between presence and absence of a carotene supplement in the diet.

Thus, 20 per cent. of the sets show significant discrimination for total carotenoids in the blood among subjects in the same diet group ; the corresponding figure for vitamin A is 87 per cent. Absence of significant discrimination implies, in practice, that the laboratories are not agreeing in the order in which they place the subjects.

Where the mean values in the three laboratories differ significantly for the same set of determinations, their descending order of magnitude also is given in Table XLIX. The first line on each date gives the order for the group on basal diet only, the second gives the order for all subjects. Where differences in order occur on the same date, they are due to the special behaviour of the group receiving the carotene supplement ; the blood values for total carotenoids are so much higher in this group that they influence considerably the pooled result. For vitamin A the two orders nearly always agree.

In general, there is no tendency for any one laboratory to be highest more frequently than any other.

* This group has been kept separate because both the mean value as shown on p. 127 and the standard deviation are significantly larger than in the other two groups.

TABLE XLIX

Analysis of Significance of Personal and Laboratory Differences in Plasma Values for Total Carotenoids and Vitamin A

Date of laboratory determinations	Plasma total carotenoids			Plasma vitamin A		
	Significant differences between individuals due to dietary group only	Significant differences present (at 5 per cent. level) between individuals in the same dietary group	Significant differences present between the means at the three laboratories*	Significant differences between individuals due to dietary group only	Significant differences present (at 5 per cent. level) between individuals in the same dietary group	Significant differences present between the means at the three laboratories*
3. 5.43	—	Yes	CLO } CLO } LOC } LOC } LCO } LOC } LCO } LOC } LOC } OLC } OLC } OLC } OCL }	—	Yes	OLC } OLC } LCO } LCO }
31. 5.43	Yes	—	—	—	Yes	—
28. 6.43	—	Yes	—	—	Yes	—
19. 7.43	Yes	—	—	—	Yes	—
16. 8.43	Yes	—	—	—	Yes	OLC } OLC }
13. 9.43	—	Yes	—	—	—	—
11.10.43	Yes	—	—	Yes	—	COL } COL } OCL } OCL } OCL } OCL }
8.11.43	Yes	—	—	—	Yes	—
6.12.43	Yes	—	—	—	Yes	—
3. 1.44	Yes	—	OLC } OLC } OCL } OCL }	—	Yes	—
31. 1.44	Yes	—	—	—	Yes	OCL } COL } COL } COL } COL } COL }
28. 2.44	Yes	—	—	—	Yes	—
27. 3.44	Yes	—	OLC } LOC }	—	Yes	—
17. 4.44	Yes	—	—	—	Yes	COL } CLO } CLO } CLO }
16. 5.44	Yes	—	—	—	Yes	—

* Where the means for the different laboratories are significantly different their names (C = Cambridge, L = Liverpool, O = Oxford) are arranged in descending order of magnitude of these means, the upper line for each date being for the group on the unsupplemented basal diet only, and the lower line for all the subjects.

TABLE L

*Individual Variation over Time in Values for Total Carotenoids
(Expressed as "I.U." per 100 ml. plasma, the means being taken from Table XLVII)*

	Cambridge			Liverpool			Oxford		
	Standard deviation	Mean	Coefficient of variation (per cent.)	Standard deviation	Mean	Coefficient of variation (per cent.)	Standard deviation	Mean	Coefficient of variation (per cent.)
No supplement	18.44	25.45	72.46	18.30	29.22	62.63	19.17	31.39	61.07
Carotene supplement	43.33	140.58	30.82	46.56	149.59	31.13	54.93	165.33	33.32
Vitamin A supplement	9.79	24.57	39.85	11.49	29.67	38.73	12.75	28.77	44.32

*Individual Variation over Time in Values for Vitamin A
(Expressed as I.U. per 100 ml. plasma, the means being taken from Table XLVIII)*

	Cambridge			Liverpool			Oxford		
	Standard deviation	Mean	Coefficient of variation (per cent.)	Standard deviation	Mean	Coefficient of variation (per cent.)	Standard deviation	Mean	Coefficient of variation (per cent.)
No supplement	20.39	65.86	30.96	21.18	56.97	37.18	23.91	65.85	36.31
Carotene supplement	28.29	112.08	25.24	22.70	88.74	25.58	23.63	95.30	24.80
Vitamin A supplement	21.13	79.12	26.71	21.42	74.97	28.57	25.32	82.90	30.54

TABLE LI

Individual Variances (S^2) and Standard Deviations (S) over Time in Values for Total Carotenoids, Expressed as "I.U." per 100 ml. Plasma, at the Three Laboratories

Individuals	Cambridge			Liverpool			Oxford		
	S^2	S	Degrees of freedom	S^2	S	Degrees of freedom	S^2	S	Degrees of freedom
<i>No supplement :</i>									
Bloomfield	169.91	13.03	17	340.24	18.45	17	138.71	11.78	17
Brown	66.00	8.12	3	233.67	15.29	3	363.58	19.07	3
Garling	126.26	11.24	13	74.37	8.62	13	139.61	11.81	13
Golding	30.92	5.56	3	14.25	3.77	3	47.00	6.86	3
Hicken	167.03	12.92	8	67.25	8.20	8	215.53	14.68	8
Proctor	20.29	4.50	6	96.81	9.84	6	490.50	22.15	4
Russell	288.00	16.97	1	12.50	3.54	1	288.00	16.97	1
Saunders	223.00	14.93	2	1.34	1.16	2	48.00	6.93	2
Thompson	107.33	10.32	9	118.62	10.89	9	154.49	12.43	9
Watson	100.99	10.05	13	90.13	9.49	13	327.08	18.09	12
Whitehead, A.	33.90	5.81	5	19.10	4.37	5	431.77	20.78	5
Whitehead, D.	4125.24	64.23	6	3655.14	60.46	6	2826.57	53.17	6
Williams	181.61	13.48	8	156.50	12.51	8	166.28	12.89	8
Wodeman	124.00	11.14	7	35.43	5.95	7	228.41	15.11	7
Woodhouse	120.47	10.98	10	54.89	7.41	10	285.56	16.91	10
<i>Mean</i>	339.98	18.44	<i>Sum 111</i>	334.92	18.30	<i>Sum 111</i>	367.56	19.17	<i>Sum 108</i>
<i>Carotene supplement :</i>									
Bartley, W.	2219.70	47.11	14	2457.10	49.57	14	3230.41	56.84	14
Broderick	659.70	25.68	4	881.70	29.70	4	904.70	30.08	4
Drake	1883.33	43.40	15	2239.85	47.33	15	3380.67	58.14	15
<i>Mean</i>	1877.71	43.33	<i>Sum 33</i>	2167.39	46.56	<i>Sum 33</i>	3016.80	54.93	<i>Sum 33</i>
<i>Vitamin A supplement :</i>									
Drabble	91.11	9.55	13	95.14	9.75	13	138.31	11.76	13
Triggell	99.93	10.00	15	164.00	12.81	15	183.76	13.56	15
<i>Mean</i>	95.83	9.79	<i>Sum 28</i>	132.03	11.49	<i>Sum 28</i>	162.66	12.75	<i>Sum 28</i>

TABLE LII

Individual Variances (S^2) and Standard Deviations (S) over Time in Values for Vitamin A, Expressed as I.U. per 100 ml. Plasma, at the Three Laboratories

Individuals	Cambridge			Liverpool			Oxford		
	S^2	S	Degrees of freedom	S^2	S	Degrees of freedom	S^2	S	Degrees of freedom
<i>No supplement :</i>									
Bloomfield	638.77	25.27	16	640.69	25.31	16	580.02	24.08	16
Brown	273.00	16.52	3	7.33	2.71	3	700.33	26.46	3
Garling	454.99	21.33	13	339.63	18.43	13	667.17	25.83	13
Golding	73.00	8.54	2	10.34	3.22	2	2214.34	47.06	2
Hicken	277.12	16.65	7	558.84	23.64	7	72.27	8.50	7
Proctor	301.81	17.37	6	372.14	19.29	6	605.14	24.60	6
Russell	0.50	0.71	1	200.00	14.14	1	18.00	4.24	1
Saunders	85.34	9.24	2	117.00	10.82	2	244.34	15.63	2
Thompson	406.86	20.17	8	794.95	28.19	8	272.00	16.49	8
Watson	582.70	24.14	11	718.20	26.80	11	1379.84	37.15	11
Whitehead, A.	249.37	15.79	5	446.57	21.13	5	232.97	15.26	5
Whitehead, D.	334.25	18.28	3	162.00	12.73	3	828.33	28.78	3
Williams	406.55	20.16	7	444.12	21.07	7	516.86	22.73	7
Wodeman	723.50	26.90	5	245.10	15.66	5	131.87	11.48	5
Woodhouse	148.06	12.17	9	149.12	12.11	9	181.29	13.46	9
<i>Mean</i>	415.63	20.39	<i>Sum</i> 98	448.40	21.18	<i>Sum</i> 98	571.83	23.91	<i>Sum</i> 98
<i>Carotene supplement :</i>									
Bartley, W.	1375.61	39.09	13	452.07	21.26	13	341.60	18.48	13
Broderick	520.25	22.81	3	629.67	25.09	3	479.00	21.89	3
Drake	326.41	18.07	14	549.78	23.45	14	777.03	27.88	14
<i>Mean</i>	800.45	28.29	<i>Sum</i> 30	515.40	22.70	<i>Sum</i> 30	558.54	23.63	<i>Sum</i> 30
<i>Vitamin A supplement :</i>									
Drabble	600.53	24.51	13	338.49	18.40	13	675.02	25.98	13
Tridgell	312.78	17.69	15	563.20	23.73	15	611.90	24.74	15
<i>Mean</i>	446.38	21.13	<i>Sum</i> 28	458.87	21.42	<i>Sum</i> 28	641.20	25.32	<i>Sum</i> 28

Comparison between Laboratories in Respect of the Variation in Time of the Determinations for Individual Subjects

If the determinations for particular individuals tend to show a greater variation with time in one laboratory than in another, the fact suggests that the technique in the first laboratory is more erratic.

This point has been examined. For each place the standard deviations over time were calculated for each person. The results were then averaged by pooling sums of squares, in the usual way. Tables LI and LII show the results. The pooled values are shown in Table L. The figures show no significant differences between laboratories.

Summary

A statistical study was made of the values for vitamin A and total carotenoids in those samples of blood plasma on which all the three laboratories (Cambridge, Liverpool, and Oxford) carried out an analysis. On the average Cambridge obtained the lowest values for total carotenoids and Liverpool the lowest for vitamin A.

Variation from person to person in the same group at the same time was considerable. The coefficient of variation was of the order of 20 or 30 per cent. except for the values for total carotenoids in the group receiving a carotene supplement where it was about 15 per cent.

The Oxford values for total carotenoids were absolutely and relatively more variable than the others. The Liverpool values for vitamin A were absolutely and relatively less variable than the others. Otherwise there was no significant difference.

The results of the determinations for the same people at the same time, carried out in all three places, were analysed statistically :

(a) Twenty per cent. of such sets of results showed significant discrimination for total carotenoids in blood among subjects in the same diet group ; the corresponding figure for vitamin A was 87 per cent.

(b) As a rule the three laboratories showed significant differences in mean values for the results of the same set of determinations, but there was no tendency for any one laboratory to be highest more frequently than any other.

An examination of the variability in time of the results for each individual was made. There were no significant differences between laboratories.

TABLE LIII (Part 1)
Monthly Average Bodyweight for Each Volunteer

Date	Bartley, M.*	Bartley, W.*	Bloom- field	Broderick*	Brown	Drabble†	Drake*	Garling	Garnett*	Golding	Hicken
	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.
1942											
July	—	—	68·7	66·9	69·2	—	—	—	—	—	64·0
August	65·5	62·1	71·0	67·1	68·7	—	—	59·2	—	54·4	65·3
September .. .	66·0	63·0	72·1	67·4	68·7	—	—	59·6	64·0	55·1	66·2
October	66·2	63·3	72·1	68·5	69·4	—	—	59·9	63·5	55·1	65·5
November .. .	66·2	62·6	71·0	68·9	69·2	65·3	—	60·1	63·5	55·3	64·9
December .. .	66·7	61·7	69·4	68·5	68·9	64·9	—	60·3	63·3	55·6	64·2
1943											
January	66·9	61·9	70·8	68·7	68·5	66·0	—	61·0	63·5	56·2	64·6
February .. .	67·6	62·4	70·8	68·7	67·8	66·9	—	60·8	—	56·7	64·0
March	66·5	62·8	71·0	68·9	66·2	66·5	52·6	61·2	—	56·0	63·5
April	65·3	61·9	70·1	68·5	—	67·4	51·5	61·0	—	55·3	64·2
May	—	63·0	70·1	69·2	65·8	67·1	51·5	61·5	—	54·2	64·4
June	—	63·5	69·9	68·5	65·8	66·2	51·9	61·0	—	54·9	63·7
July	—	63·7	70·3	68·7	66·7	66·7	52·2	61·7	—	55·1	63·5
August	—	63·7	70·3	69·4	66·5	67·1	52·4	62·4	—	55·1	63·3
September .. .	—	62·8	71·9	68·3	65·1	66·5	51·7	62·8	—	54·4	63·7
October	—	64·0	71·9	68·5	—	67·8	52·8	63·5	—	54·9	63·3
November .. .	—	63·7	71·9	70·3	—	67·1	53·3	63·3	—	56·5	63·0
December .. .	—	64·2	72·3	71·2	—	66·9	53·1	62·6	—	56·9	62·8
1944											
January	—	65·5	72·6	70·5	—	67·4	52·6	62·8	—	57·4	—
February .. .	—	66·0	73·7	70·8	—	69·2	52·8	63·0	—	57·8	—
March	—	66·0	74·2	—	—	70·8	53·3	64·2	—	58·3	—
April	—	65·3	72·8	—	—	69·9	53·1	63·7	—	58·1	—
May	—	—	73·0	—	—	68·7	52·6	63·3	—	57·2	—
June	—	—	—	—	—	—	52·4	62·6	—	—	—
July	—	—	72·1	—	—	—	52·4	62·8	—	55·8	—
1945											
January	—	67·6	—	—	—	—	52·2	62·8	—	55·8	—
July	—	66·7	—	—	—	—	50·6	62·1	—	54·0	—

* Received carotene supplement.

† Received vitamin A supplement from 28th January, 1943.

U.—Monthly Average Bodyweight for Each Volunteer.

TABLE LIII (Part 2)
Monthly Average Bodyweight for Each Volunteer

Date	Proctor	Russell	Saunders	Stuart	Thompson	Tridgell†	Watson	White-head, A.	White-head, D.	Williams	Wodeman	Wood-house
	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.	kg.
1942												
July ..	57.4	63.3	60.1	63.5	68.3	—	57.8	66.0	53.5	66.0	75.1	64.6
August ..	57.6	63.0	60.8	64.0	70.1	59.9	58.5	67.1	54.0	66.7	75.3	65.1
September ..	58.3	62.6	61.7	64.4	69.4	61.0	59.0	66.5	54.4	66.9	75.1	65.5
October ..	58.1	61.9	62.4	64.4	69.6	61.5	59.2	66.7	53.5	66.9	75.3	66.0
November ..	58.3	61.2	62.6	64.4	70.1	61.7	60.1	67.1	53.3	67.1	75.8	66.0
December ..	57.6	61.5	62.6	62.6	70.8	61.5	60.3	67.1	53.5	66.9	75.3	65.8
1943												
January ..	56.9	62.1	62.4	64.6	70.3	61.0	61.0	67.1	52.2	67.1	75.3	65.8
February ..	57.6	62.4	63.3	64.4	69.9	61.2	61.2	67.4	52.4	67.4	75.8	66.7
March ..	57.6	62.6	63.3	64.6	69.6	61.7	61.5	68.3	53.1	66.7	75.1	66.7
April ..	57.2	62.8	61.9	64.2	69.2	59.9	59.9	67.6	53.1	—	74.6	66.7
May ..	56.5	62.6	60.3	63.0	69.2	60.6	60.1	68.7	53.3	66.2	74.6	66.9
June ..	56.0	—	59.2	62.6	69.2	60.6	60.1	69.9	53.8	66.2	75.3	66.5
July ..	56.5	—	58.7	63.5	69.2	61.2	60.3	69.9	53.8	66.0	76.0	65.8
August ..	56.2	—	59.9	—	68.5	61.7	60.8	70.3	53.3	65.5	76.4	66.0
September ..	57.4	—	—	—	68.9	62.4	60.8	70.8	53.3	66.2	76.2	65.3
October ..	57.8	—	—	—	69.9	62.1	61.0	—	—	67.1	77.3	66.2
November ..	57.4	—	—	—	71.0	62.1	61.0	—	—	66.9	78.7	66.5
December ..	57.8	—	—	—	70.8	62.6	61.5	—	—	66.7	80.3	66.5
1944												
January ..	58.3	—	—	—	71.2	62.1	61.5	68.3	—	66.2	80.3	65.5
February ..	58.5	—	—	—	70.3	61.9	61.0	68.3	—	66.5	81.0	65.5
March ..	58.3	—	—	—	70.3	62.1	61.5	—	—	66.2	81.9	66.0
April ..	57.8	—	—	—	70.8	61.7	61.0	—	—	66.7	81.6	66.2
May ..	56.9	—	—	—	—	60.8	60.8	—	—	—	81.2	66.5
June ..	56.9	—	—	—	—	61.0	61.5	—	—	—	—	—
July ..	55.8	—	—	—	—	61.5	60.3	—	—	—	—	—
1945												
January ..	55.8	—	—	—	—	62.1	60.6	—	—	60.1	79.8	65.3
July ..	55.1	—	—	—	—	62.1	57.2	—	—	—	80.3	63.0

† Received vitamin A supplement from 28th January, 1943.

GENERAL SUMMARY

Twenty men and three women received a diet deficient in vitamin A and carotene but complete in all other respects for periods ranging from 6½ to 25 months.

The diet consisted of natural foodstuffs. It was found by chemical tests to supply no more than 70 I.U. β -carotene daily. By biological tests with rats it proved to be very low in vitamin A potency.

Sixteen of the subjects received the diet unsupplemented until they showed unmistakable signs of deficiency or were obliged to leave the experiment for various extraneous reasons, the only useful criteria of deficiency being the plasma level for vitamin A and the dark adaptation values. They were then dosed with vitamin A or carotene.

Five of the subjects received throughout about 5,000 I.U. β -carotene daily in different forms (β -carotene in oil or margarine, carrots, spinach, cabbage) at different times, and two similarly received 2,500 I.U. vitamin A as a concentrate.

Vitamin A and total carotenoids were estimated by several different laboratories, sometimes by as many as four.

Vitamin A was estimated in blood by the antimony trichloride method with use of a Hilger Nutting visual spectrophotometer or a photo-electric colorimeter.

The plasma values for vitamin A obtained by three different laboratories often varied much for the same sample, but the average of a sufficient number yielded consistent results. The three laboratories carrying out the analyses agreed that no great reliance could be placed on the results of a single estimation of vitamin A in plasma.

The vitamin A content of concentrates used as supplement was determined from the extinction coefficient at 325 $m\mu$ with a Hilger E3 quartz spectrograph.

Total carotenoids, and α - and β -carotene after chromatography, were estimated in blood, foods, and faeces by measurement of the intensity of yellow colour in a Hilger Nutting visual spectrophotometer or in a photo-electric colorimeter.

Capacity for dark adaptation was measured with the adaptometer of Wald and the adaptometer of Craik, and later also by the rod scotometry method of Livingston. Improvements in technique are described.

The measurements made most frequently, at about fortnightly intervals, were of the "final" rod threshold after 20 minutes, but the cone-rod transition time and full curve of dark adaptation were measured on many occasions also.

Clinical examinations were made regularly, including blood counts, skin biopsies, audiometry tests, psychological examinations, and slit-lamp examinations of the eye, together with many other tests.

Faeces were collected for fixed periods and analysed for total carotenoids, and for α - and β -carotene.

The first result of the deficient diet was a marked drop in the carotenoid content of the blood. Within three months, the initial average value of about 150 "I.U." fell to about 40 "I.U." per 100 ml. Most, if not all, of this residual carotenoid content was made up of substances other than

α - and β -carotene. No other effect which could definitely be ascribed to the deficient diet was encountered during the first eight months.

Unfortunately, for various reasons, an insufficient number of observations was made to establish the vitamin A content of the blood of the subjects before they began to receive the experimental diet. Data for establishing the value for a normal level have, therefore, been assembled from the literature and from observation of other subjects. The value thus arrived at was about 120 I.U. per 100 ml. (p. 67).

For the first two months on the diet, the average plasma value for vitamin A of the deprived group was 88 I.U. per 100 ml. After about eight months a definite downward trend was shown in ten out of the sixteen volunteers in the group. The fall became very pronounced in four men who eventually reached levels below 50 I.U. vitamin A per 100 ml. (Fig. 1, p. 13). One man, however, showed no appreciable drop even after twenty-two months of deprivation.

In determining the capacity for dark adaptation, normal values for the final rod threshold, measured with the adaptometer of Wald, were between 1.37 and 2.30 log. μ m lamberts.

Variability obscured any but the most definite trends but, when values were averaged for one or two months, less pronounced trends became apparent. The final rod threshold showed no change during the first four months of deprivation. During the subsequent five winter months a slight but definite deterioration occurred in all the deprived subjects but, in the following two months, the initial threshold was restored in all but two of them.

During the succeeding 16 months of the experiment a large rise in threshold occurred in three subjects only, about 10, 12, and 20 months, respectively, from the start of the experiment.

The normal cone-rod transition time varied between 5 and 12 minutes, and the values remained throughout the experiment almost completely within this range except in the three subjects referred to and possibly one other. The highest value was 33½ minutes.

The three subjects whose dark adaptation deteriorated significantly had low average plasma vitamin A values at the critical time. A fall of the average plasma value to below 50 I.U. per 100 ml. occurred a few weeks earlier.

Measurements of the capacity for dark adaptation with Craik's adaptometer confirmed the above findings concerning the cone-rod transition time, but the Wald apparatus was more sensitive in recording changes of the final rod threshold. Only one of the three subjects showing a raised threshold with the Wald instrument gave abnormal readings with the Craik adaptometer. The latter always recorded changes in the cone-rod transition time as the first abnormal feature of the curve in vitamin A deficiency, while the adaptometer of Wald, in two subjects, recorded the changes of cone-rod transition time and final rod threshold simultaneously. These apparent discrepancies are discussed (p. 47).

Livingston's method of rod scotometry was applied from the tenth month of the experiment onwards. Two of the subjects whose final rod threshold was definitely raised showed also grossly abnormal fields of vision. In some subjects abnormal fields of vision seemed to be the only feature associated

with vitamin A deficiency. The observations are taken to suggest that rod scotometry may reveal slighter defects in night vision than other techniques.

The great majority of clinical examinations revealed no significant differences between the deprived and non-deprived group, or in the same person before and after deprivation of vitamin A. This applied to the biomicroscopical appearance of the cornea and conjunctiva, the blood picture including platelet counts, gastro-intestinal abnormalities, and the incidence of colds and coughs. The only signs and symptoms which seemed to be commoner in the deprived group, though their significance was doubtful, were dryness of the skin and eye discomfort. Audiometry, however, showed a significant worsening of hearing in the deprived group.

In several of the subjects follicular hyperkeratosis was present at the start and varied during the experiment, but the variations bore no relation to the vitamin A intake (p. 120).

Four of the volunteers contracted major illnesses during or after the period of deprivation; these illnesses were impetigo contagiosa, migraine, tuberculous pleurisy, and tuberculous disease of the spine (p. 23).

The scope of the therapeutic tests which could be carried out on the depleted subjects was limited because the number of them to become measurably deficient was much smaller than had been expected. Only three out of sixteen showed signs which could be used as criteria of the effectiveness of graded doses of vitamin A and carotene.

With a daily dose of about 1,300 I.U. vitamin A in oil, one adequately depleted subject showed gradual restoration of capacity for dark adaptation to a level which could not be subsequently improved with larger doses. The plasma value for vitamin A rose also, but could be further increased by large doses of vitamin A.

With a daily dose of about 1,250 I.U. carotene in oil, one adequately depleted subject showed an increase in the plasma value for vitamin A but a deterioration of the rod threshold. With a daily dose of 2,500 I.U. carotene in oil, the same, and another, adequately depleted subject showed improvement by both these criteria. In one, recovery was slow and not quite complete when, after 5½ months, the supplemented diet was replaced by an unrestricted one. In the other, in whom the state of depletion was less advanced, recovery was prompt and was complete within three weeks. Some other therapeutic tests on inadequately depleted subjects were made also (p. 26).

In prophylactic tests with a vitamin A concentrate, a daily dose of 2,500 I.U. appeared to maintain two subjects, though the value for vitamin A in the plasma was below that subsequently attained by these subjects on an unrestricted diet. This test lasted for 14 months in one subject and 17 months in the other.

In connection with the prophylactic and therapeutic tests with carotene, the term "maximum effective dose" is introduced. This is the part of a given dose of carotene which is not excreted in the faeces. How much of it is actually absorbed and how much is destroyed is uncertain.

The maximum effective dose was determined by analysis of the faeces for carotene administered in different materials, the period of sampling being seven days. There were considerable variations between individuals, and the same individual showed considerable differences at different times (Table XXVIII, p. 72).

The average amount of carotene excreted, expressed as a percentage of the intake, was 76 for sliced carrots, 75 for sliced carrots made into purée, 59 and 73 for cabbage (two different doses), 59 for spinach purée, 44 for commercially homogenized carrots, 57 for homogenized spinach, 29 for carotene in margarine, and 26 for carotene in arachis oil. Thus the maximum effective dose was highest when carotene was given in oil or margarine. It was higher for green vegetables than for carrots, except when the latter were homogenized. Homogenization had no effect on the maximum effective dose of carotene in leafy vegetables, but greatly increased it in the case of carrots. Converting sliced carrots into purée in a "Latapie" grinder or adding margarine to the dose of carrots did not change the maximum effective dose.

In the prophylactic tests with carotene from different sources the maximum effective dose ranged between 1,250 and 3,700 I.U. daily, and, on the whole, the capacity for dark adaptation and the values for vitamin A and carotene in the blood were maintained on this dose for periods of 6½ to 22 months, with the possible exception of a period when sliced carrots with an average maximum effective dose of 1,250 I.U. daily provided the main supplement (p. 31).

In the liver of seventy-one civilians between the ages of 15 and 59 who died in Britain through accident in the years 1941-44, the "central" value for the reserves of vitamin A was determined as being 324 I.U. per gramme. This value represents a total reserve of about 500,000 I.U. for the whole liver. The relationship of this value to the requirement is considered (p. 63).

The requirement of vitamin A in terms of vitamin A and of carotene is discussed on the basis of the available evidence (p. 39). An amount of about 1,300 I.U. daily was found to be a minimum protective dose of vitamin A. The figure of 2,500 daily is recommended as an estimate of the requirement to cover individual variations and to leave a margin of safety.

It is stressed that, for carotene, the value for the minimum protective dose must be based on that for the maximum effective dose and not on that for intake. The conclusion is reached that the minimum protective dose of carotene is about 1,500 I.U. daily, and the figure of 3,000 I.U. daily is put forward as the value for the requirement to cover individual variations and to leave a margin of safety, on the supposition that 100 per cent. of the carotene is available. If this is accepted the following amounts represent the corresponding requirements in terms of the intake necessary from various sources :

<i>Source</i>	<i>Requirement (I.U. daily)</i>	<i>Divisor for calculating available potency in terms of vitamin A</i>
Carrots, boiled, sliced or purée	12,000	4.0
Carrots, homogenized	5,500	1.8
Cabbage, spinach	7,500	2.5
β-Carotene in fat	4,000	1.3

If a single value is put forward for the daily requirement for carotene, it is suggested that this should be 7,500 I.U., as being three times the daily requirement of vitamin A, and as being a figure roughly representative for the different sources of carotene in the diet.

A warning should be given that the figures of 2,500 I.U. of vitamin A and 7,500 I.U. of carotene for the daily requirement must not be used without discretion. The present work was carried out mainly on healthy males, 20 to 30 years of age. No suggestion is made as to how the values should be modified to apply to children, pregnant or lactating women, and diseased persons. They should not be relied on as an infallible standard for assessing the adequacy of the diet in nutritional surveys.

Certain other conclusions, apart from the main theme of the experiment, were reached :

The effect of alcohol consumption on the plasma level for vitamin A and on the capacity for dark adaptation was tested. The plasma level was not affected ; the capacity for dark adaptation was not improved, and in some cases was reduced, by alcohol intake. These findings are discussed with reference to the contrary results of other workers. The results lend no support to the suggestion of Clausen *et al.* (1940), that the response of the plasma level for vitamin A to administration of alcohol might act as an index of the magnitude of the liver reserves of vitamin A (p. 58).

In vitamin A deficient subjects the time required for maximum dark adaptation was found to be much longer than the 30 minutes usually sufficient for reaching the true final rod threshold of normal subjects, and might continue to improve for many hours (p. 47).

The final rod threshold of the deprived subjects, as measured by the adaptometer of Wald, showed a slight but definite " seasonal " deterioration during the winter seasons of 1942-43 and 1943-44. The significance of this phenomenon is discussed (p. 54).

The possibility of the synthesis of carotene in the human intestine was considered. It is confirmed that the pigments of *Staphylococcus aureus* include α - and β -carotene but the available evidence is against a synthesis of carotene by the micro-organisms of the intestine in the subjects of the experiment (p. 71).

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