

Vitamin content of
Australian, New Zealand
And English Butters.

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**VITAMIN CONTENT OF
AUSTRALIAN, NEW ZEALAND
AND ENGLISH BUTTERS**

**By M. E. F. Crawford, E. O. V. Perry
and S. S. Zilva**

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PREFACE

THIS Report gives an account of investigations forming part of a scheme of research into the vitamin content of foodstuffs, particularly fruits, vegetables, and dairy products, and into the effects upon it of different methods of cultivation, preparation, transport, and storage. These studies are made by Dr. S. S. Zilva and his assistants, working in the service of the Medical Research Council at the Lister Institute, London, with valuable collaboration by workers in the Dominions. A grant for the cost of the work during a period of years was made to the Council by the Empire Marketing Board.

Accounts of other parts of these investigations have already been published, either in monograph form or as papers contributed to scientific journals, and the present report deals only with the work done on the vitamin content of butters of Empire origin. The subject has importance in that butter, among ordinary foodstuffs, is at its best a particularly good source of the fat-soluble accessory food factors, vitamins A and D, which are essential for the normal growth of children and for the maintenance of good health. Vitamin A plays a part in maintaining resistance to infections, while vitamin D is necessary for the proper formation of the bones and teeth, and a deficiency of it causes rickets in children and gives increased liability to dental decay. Potency in respect of these two factors is therefore an extremely important quality in butter, and the fact that butters marketed in this country come from many different sources made it desirable to investigate the influence upon their vitamin content brought by the varying conditions of production, transport, and distribution.

From a practical point of view, it is gratifying to find from the Report that the butters imported from Australia and New Zealand show a high and uniform potency in vitamins A and D. They are equal in this respect to the best summer butters produced at home, and even to butter made from the milk of cows whose diet has been fortified by an artificial supply of the vitamins. The finding is the more important in that these butters from the southern hemisphere are available in our winter, when it is known that the vitamin content of milk and butter produced in northern latitudes falls below its summer level. Butter from the southern Dominions, moreover, constitutes an increasing proportion of our total supply: in 1913 some 20 per cent. of the United Kingdom imports of butter came from the southern Dominions, while in 1927 the proportion had increased to 30 per cent., and last year to no less than 44 per cent.

It is also satisfactory to note that the fat-soluble vitamins in butter have great stability in cold-storage. There is, indeed, no fear of sig-

CONTENTS

	PAGE
C. Packing and Storage	41
D. Results of Tests	42
(a) <i>Vitamin A</i>	42
(b) <i>Vitamin D</i>	45
VII. GENERAL DISCUSSION OF RESULTS	46
VIII. SUMMARY	47
REFERENCES	48
APPENDIX	49

I. INTRODUCTION

THE importance of dairy products in the nutrition of the human race need not be stressed. There is scarcely a people, whether primitive or civilized, which has not included them in its dietary from time immemorial, and few articles of food meet so many of the nutritional requirements of the human organism.

Milk contains all the known vitamins, and of these vitamins A and D are present in the fat. As no very significant loss of the fat-soluble vitamins is incurred in the usual process of separating and churning milk-fat, almost the entire fat-soluble vitamin content of the milk is to be found in the butter (Crawford, Golding, Perry, and Zilva, 1930).

Butter even at its best cannot rival cod-liver as a source of vitamins A and D, or mammalian liver as a source of vitamin A, or still less irradiated ergosterol as a source of vitamin D. It must, however, be borne in mind that it is consumed in moderately large quantities and at regular intervals; its function is therefore preventive rather than curative, and as such its value as a vitamin food is high. As far as this country is concerned this dietetic property of butter assumes special importance in the winter when the amount of the fat-soluble vitamins, especially D, consumed in the general diet may often fall short of the minimum requirements, and thus the consumption of a potent butter can be instrumental in making such diet adequate in this respect.

The fact that butters consumed in this country are of many origins and are prepared under different conditions suggested the necessity of investigating the influence of the various factors connected with their manufacture, transit, and distribution on their vitamin content. Our present knowledge concerning this problem is based mainly on theoretical considerations and scattered items of experimental evidence obtained by the use of a variety of methods at different periods of the development of the subject. Such information obviously cannot be considered entirely satisfactory from the point of view of preventive medicine.

In this investigation an attempt was made to study the various points systematically, and the results dealing with English, Australian, and New Zealand butters only are reported. From the beginning of the work it was realized that many difficulties were associated with it, especially as regards the methods of determination of the vitamins. It was considered that the methods suitable for an investigation of this character should be biological and of an objective character so that the results when recorded would be open to the interpretation of the reader as well as of the experimenter. Such methods are laborious and slow, and even when practised under the most carefully controlled conditions do not yield results of very high accuracy. They have the further disadvantage that the number of doses that can conveniently be employed in each test is limited, so that the minimum dose, which in

this inquiry was intended to serve to a great extent as the basis for comparison, may sometimes not be attained. Nevertheless, although small differences in vitamin activity cannot thus be established, any great disparity in potency such as might be indicative of a serious loss in the vitamins, can undoubtedly be registered by the methods employed. It is also to be pointed out that owing to some factors still unknown and consequently not yet controlled, the response of the experimental animals to substances of the same potency may fluctuate somewhat when tested at different times. The magnitude of such fluctuations, in the case of tests carried out in the same laboratory under carefully controlled conditions, would, in our opinion, most probably fall outside the range of accuracy which could justifiably be aimed at by the methods used. It might even be considered as doubtful whether the utilization of a notation of units based on standards such as have recently been adopted by the League of Nations and which were not available at the inception of the inquiry, would have served as a better basis of comparison in these circumstances.

II. HISTORICAL SURVEY

A more complete understanding of the results reported in this publication will be obtained if an historical survey of the development of our knowledge of the fat-soluble vitamins A and D in butter is briefly outlined. It will be sufficient for this purpose to mention the salient facts, starting with the discovery of the fat-soluble complex which is now known to contain vitamins A and D. This physiological entity was revealed by the work of McCollum and Davis (1913) and of Osborne and Mendel (1913, 1 and 2). The former workers discovered that when rats ceased to grow on an artificial diet of caseinogen, lactose, lard, and inorganic salts a resumption of growth could promptly be induced by the introduction into the diet of an ether extract of egg-yolk or butter. As a result of their researches they postulated the existence in these products of some organic complex indispensable for growth, which they designated later as the 'fat-soluble A factor'. Osborne and Mendel arrived independently at a similar conclusion. The discovery of the 'fat-soluble A factor' or the 'growth-promoting factor', or 'vitamin A' as it was afterwards called, led to an extensive study by various workers of its distribution in nature. Further facts concerning its physiological function also came to light. It was revealed very soon after its discovery, for instance, that rats subsisting on a diet deficient in this factor were subject to respiratory infection and to an infection of the eye known as xerophthalmia.

A few years later evidence emanating from an inquiry carried out along entirely different lines from those employed in the researches on the fat-soluble A factor showed that another physiological function, namely, that of preventing rickets, might often be ascribed to this principle. Mellanby (1918, 1919, 1920, 1921), experimenting with puppies, demonstrated that rickets was a disease caused by dietetic

deficiency. He was particularly struck by the fact that, although there were some discrepancies, the distribution of the factor which prevented rickets in puppies was very similar to that of the fat-soluble A factor as estimated on rats, an agreement sufficiently marked to warrant the assumption that a relationship existed between the two principles. The actual relationship was eventually disclosed. It was found that the antixerophthalmic or anti-infective function of the 'growth-promoting fat-soluble A factor' or 'vitamin A' was due to a principle distinct from the rickets-curing factor, although the two were closely associated in nature. After this differentiation, the former retained the original name of vitamin A, the latter became known as vitamin D. Each is capable of inducing growth but not in the absence of the other, and therefore all positive results obtained in investigations previous to the differentiation of the two vitamins, in which growth was the criterion, signify the presence of vitamins A and D together, whilst failure of growth in these cases might mean either the absence of both or one of these vitamins. Quantitative tests which disclose the presence of one fat-soluble vitamin, even if the other be absent, are now employed for vitamins A or D.

Butter, which is capable of inducing growth in rats fed on a diet deficient in both fat-soluble vitamins, contains therefore vitamins A and D, but in early investigations these two principles were assessed together as the 'growth-promoting fat-soluble A factor' or 'vitamin A'; only during the last few years were these vitamins studied in it differentially. Being an important article in the human dietary, butter naturally received the attention of many investigators in the field of vitamin research, and from these researches it soon became evident that there was a significant variation in the vitamin potency of butters. Steenbock, Boutwell, and Kent (1918), observing variations in the amount of the 'fat-soluble A factor' present in butter-fat, suggested that the ration of the cow might be responsible for these differences. Drummond and Coward (1920) and Drummond, Coward, and Watson (1921) showed that the variation was due to the difference in the diet of the cow and that butters produced during the winter months from stall-fed cows on dry feeds of hay, roots, and cake were of a low 'vitamin A' potency. Their experiments also indicated that the drying up of pasturage in a hot summer may lower the value of butter in this respect. Steenbock, Sell, and Buell (1921) found also that there was a seasonal variation in the vitamin content of butters; thus butters prepared at the end of March or April were markedly poorer in the 'fat-soluble A factor' than butters churned from milk of the same origin at the end of May or June. Hughes, Fitch, and Cave (1921) established a higher 'fat-soluble vitamin' content in the milk and butter from cows fed on a diet rich in this vitamin than in the milk produced by cows receiving a diet which was low in it. Kennedy and Dutcher (1922) observed that the presence of 'vitamin A' in cow's milk is entirely regulated by its occurrence in the ration of the animal and that stall-fed cows could produce a milk rich in vitamin if a proper combination of grain and leafy food was used.

Drummond *et al* (1923 and 1924) made similar observations and showed that the 'vitamin A' (i.e. vitamins A and D complex) could be raised by administering cod-liver oil, which is rich in vitamins A and D, to cows on winter rations. The administration of coco-nut oil or arachis oil, which are poor in the 'vitamin A complex' did not enhance the vitamin value of the butter. Luce (1924, 1 and 2) brought forward evidence that the diet of the cow was the principal factor in determining the growth-promoting and antirachitic properties of the milk. Chick and Roscoe (1926), estimating vitamin A (the anti-xerophthalmic or anti-infective vitamin) and vitamin D (the antirachitic vitamin) separately, found that the vitamin A content of the milk depended on the diet of the cow, being at a maximum when the animal was fed upon fresh, green fodder and least when the animal received a winter ration of cereals and roots. The milk contained the maximum amount of both fat-soluble vitamins A and D when the cow was at pasture in the summer. Golding, Soames, and Zilva (1926) have shown that the vitamin A, but not the vitamin D, content of the butter from cows fed on a winter ration, very poor in both fat-soluble vitamins, could be raised by the addition of kale. When cod-liver oil, however, was added to the diet of the cows instead of kale there was a very marked rise in both vitamins (A and D) in the butter. Wachtel (1929) and Steenbock, Hart, Hanning, and Humphrey (1930) found independently that the antirachitic potency of milk could be increased by feeding irradiated yeast to cows.

Another factor which possibly conduces to an increase of vitamin D but not vitamin A in milk is exposure of the animal to sunlight or to the ultra-violet radiation of a mercury quartz lamp. On this point the evidence is conflicting. Luce (1924) obtained no clear-cut evidence that irradiation of the cow increased the antirachitic properties of its milk. Chick and Roscoe (1926), however, found that the antirachitic value of milk depended on the degree of exposure of the cow to the sun. This finding was supported by the observation of Dutcher, Honeywell, and Dahle (1927), who reported that butter from cows subjected to sunlight possessed higher calcifying properties, and consequently antirachitic properties, than butter from cows kept in the dark. Falkenheim and co-workers (1926) did not observe an improvement in the antirachitic potency by insulating the cow, but found that its exposure to ultra-violet radiation increased the vitamin D content of the milk. The beneficial effect of ultra-violet irradiation of the cow on the vitamin D content of the milk was also recorded by Gowan and co-workers (1926) and by Bruck-Biesok, Pirquet, and Wagner (1927). On the other hand, Steenbock, Hart, Riising, Hoppert, Basherov, and Humphrey (1930) found that daily exposure of cows to sunlight or to artificial ultra-violet radiation had no demonstrable effect on the vitamin D of the milk. The antirachitic value of dried milk was also studied in this connexion by Hess and Unger (1921) and by Supplee and Dow (1927). Whilst the former workers found that dried summer milk was no better than dried 'stall-fed' milk in preventing the development or in decreasing the incidence of rickets in infants during

the winter, the latter observers established the superiority of the antirachitic property, as tested on rats, of dried summer milk over dried winter milk.

In spite of the conflicting nature of the above results, there is a general recognition that summer-produced milk and butter-fat is superior to the winter products. The primary cause of this may, as Steenbock and his collaborators suggest, lie in other factors than sunlight acting *directly* on the cow.

Concerning the stability of vitamins A and D in butter, the available evidence suggests that under satisfactory conditions of storage these vitamins do not deteriorate quickly. Osborne and Mendel (1916) observed that butter-fats kept at 18° C. in the dark and in the light and at 8° C. in the dark retained their 'vitamin A' activity for about 15 months. Steenbock, Boutwell, and Kent (1918) found that heated butters and butters kept in poor storage do not contain 'vitamin A'. Drummond, Coward, and Watson (1921) examined a number of samples of butter which had been in storage for various lengths of time (not longer than 8 months), usually at low temperatures, and found that they did not possess a lower average 'vitamin A' content than fresh samples. They concluded that butters stored below 10° C. do not suffer any appreciable loss of 'vitamin A' if undue exposure of surface to air is prevented and if other conditions of storage are good. The experiments of Chick and Roscoe (1926) showed that butter made from milk possessing antirachitic potency retained its activity after storage in a frozen condition for more than 2 years.

III. METHODS USED FOR ESTIMATING VITAMINS A AND D

A standard strain of the black and pied variety of the Norwegian rat was used in this investigation. The animals were bred at the Lister Institute by Miss H. H. Smith under conditions already described (Smith and Chick, 1926). A minor alteration in the diet was instituted in 1929, brown bread and marmite being replaced by dried yeast.

The cages in which the animals were kept during the test period consisted of a galvanized-iron, wire top 10×10×4 in. and a tray 10½×10½×1 in. The trays were filled with finely-broken peat over which rested a sheet of fine perforated zinc which served as a 'false-bottom'. In vitamin A tests each cage contained a glass jar which acted as a 'kennel' for the animal. Water was offered in inverted glass bulbs which, while making the water accessible to the rat, did not allow it to leak out. The bulbs were sterilized at the beginning of each test and the water was changed at least every other day. The peat was changed weekly and the 'false bottom' and trays were washed and disinfected at similar intervals. The temperature of the animal house was regulated by a thermostat and varied in the winter between 64° F. and 70° F. In the summer the temperature rose

occasionally to 80° F. but never fell below 65° F. The windows were coated with white paint and stippled, direct sunlight being scrupulously kept out.

1. Vitamin A.

This vitamin was estimated by a method based on Steenbock's principle. Rats were kept on a diet free from vitamin A but containing vitamin D, and at a stage when the bodily reserves of vitamin A of the animals began showing signs of depletion the test doses of the butters were administered. The growth response was taken as the chief criterion of vitamin A activity.

Rats 19–25 days old, weighing about 35 g. were employed in these tests. Each litter was kept in a large cage for the first 14 days, after which time they were placed in separate cages of the type described above. During the first month the animals received a diet, free from both fat-soluble vitamins A and D, which was constituted as follows:

Inactivated caseinogen	20 parts by weight.
Wheat starch	50 " " "
Hardened oil (cotton seed, sesame, or arachis)	15 " " "
Yeast, dried and powdered	8 (later 16) "
*Salt mixture	5 parts by weight.
Lemon-juice	5 " " "
Water	50 " " "

* NaCl	51.9
MgSO ₄ ·7H ₂ O	164.0
NaH ₂ PO ₄ ·H ₂ O	104.1
K ₂ HPO ₄	286.2
CaH ₄ (PO ₄) ₂ ·H ₂ O	162.0
Ca(C ₂ H ₅ O ₂) ₂ ·5H ₂ O	390.0
Ferric citrate	35.4

The caseinogen, which was white and light in texture, was previously freed from traces of the fat-soluble vitamins by treatment in an electrically heated oven through which a current of air was circulating. The caseinogen was spread in thin layers on trays and heated for 51 hours at 115° C. During the working-hours of this period (about 21 hours), it was raked over hourly so that it was well exposed to the hot air. At the end of this treatment the caseinogen was buff-coloured and light. Care was taken to avoid overheating, since scorched, orange-coloured caseinogen causes diarrhoea in rats.

A month after the animals had been on the above experimental diet the hardened oil, before being incorporated, was irradiated in order to supply vitamin D. The fat was melted and poured into shallow, sheet, tin trays 30 × 50 cm. and exposed under a 'Pistolette' mercury-vapour quartz lamp (Hewittic Electric Co.) at a distance of 38 cm. for half an hour.

This diet was compounded about once a week and was kept at 0° C. in tightly closed containers. A weighed quantity of the diet was given daily to the individual rats. The quantity offered was always a few grammes in excess of that consumed the previous day, and the animals were thus supplied with the basal diet *ad lib*. Except in

vitamin A this basal diet was complete in all essential constituents, such as proteins, fats, carbohydrates, and salts; the yeast, lemon-juice, and irradiated oil supplied the vitamins B, C, and D respectively. Animals on this diet alone, after exhausting their bodily reserves of vitamin A, cease growing, lose weight, and ultimately die. A group of rats in each test was fed on the basal diet alone to test this for absence of vitamin A and served as negative controls. Another group, the positive controls, received the basal diet supplemented, during the test period, with 3 to 5 drops of cod-liver oil daily to provide an amount of vitamin A adequate to produce normal growth.

The rats were weighed three times a week; when they began showing premonitory symptoms of vitamin A depletion their weight was recorded daily until the depletion was well established, when the daily administration of the test dose began. This condition is usually manifested by a loss of appetite, muscular tone, glossiness of coat, &c. Though eye symptoms usually develop, they are not invariable. On the other hand, eye symptoms may be present while the animal is still growing. The rats were carefully watched for any of the above symptoms after having subsisted about 4 weeks on the basal diet. The exact time for starting the dosage with the test substance can only be gauged by experience and it is difficult to lay down hard and fast rules. The following main indications were, however, adopted: (a) when the weight had been stationary for some time, (b) when a sudden considerable loss of weight (5-10 g.), especially following on a short stationary period, occurred, and (c) when gradual loss of weight followed a short period of weight maintenance.

A quantity containing 7 doses of the test butter was weighed out weekly for each animal and kept in the dark at 0° C. From this store about one-sixth was removed daily, except on Sundays, and administered to the rat, precautions being taken that the butter was entirely consumed. The administration of the dose was continued for 4 weeks.

Each test group consisted of 6 animals, 3 of each sex, and as the growth response in rats varies not only with sex but also with litter, members of each family were distributed among the groups receiving the test doses and those acting as positive or negative controls; these groups were, therefore, as evenly balanced as possible.

2. Vitamin D.

The Chick, Korenchevsky, and Roscoe method (1926) was employed. In this method the rats receive a basal diet low in the anti-rachitic vitamin and in phosphorus, whilst the test doses are administered from the beginning of the test. The degree of calcification in the bone as determined by the ash content is used as a criterion for vitamin D activity.

The rats were started on the test when 21 to 25 days old (weight about 42 g.) and were uniformly distributed as regards sex and litter among the various groups receiving the different doses (at least 6

rats per group). They received McCollum diet 3143, which is composed as follows:

Wheat	33	parts	by	weight.
Maize	33	"	"	"
Wheat gluten	15	"	"	"
Gelatin	15	"	"	"
Calcium carbonate	3	"	"	"
Sodium chloride	1	"	"	"

The gelatin was heated in 60 c.c. of water and mixed thoroughly with the other components and the whole allowed to set in a cake. The diet was made up about once a week and stored in a tightly closed container at 0° C. The animals were offered the diet *ad lib.*; the administration of it and of the test-butter doses and the weighing of the uneaten food residues were carried out in the same way as in the vitamin A tests. The rats in the positive control groups subsisted on the basal diet plus 3 to 5 drops of cod-liver oil per day, and those in the negative control groups on the basal diet alone.

The animals were killed by coal gas after 28 days and both hind-legs were removed within a few hours of death and carefully cleaned from adhering flesh and the patellae. The bones (femur, tibia, and fibula) were weighed in weighing bottles, heated in an oven at 105° C. for 24 hours and the dry weight determined. They were then tied up in fine-meshed silk free from flaws and broken between the fingers in order to ensure better penetration of the solvent. The silk bags and the contents were next extracted in boiling absolute alcohol under a reflux condenser for 8 hours and allowed to remain in the alcohol overnight. After a 24-hours ether extraction in a Soxhlet apparatus and drying in an oven for 4 hours at 105° C., the 'fat-free' weight was obtained and the ash content determined by incineration. The degree of calcification of the bone was judged by the relative ash content or by the relative value of the ratio A/R, i.e. the ratio of bone ash to 'organic residue'. The latter was obtained by subtracting the weight of the ash from the weight of the dry, extracted bone.

3. The Statistical Criterion Employed.

The original intention was to determine the relative potencies of the butters by the minimum protective dose, but the small number of animals (6 per dose) and the relatively few doses employed in each test made this a not very reliable comparison. Recourse was, therefore, had to the additional aid of a statistical method. The statistical examination of our results was carried out by Miss H. M. Woods, F.S.S., from the Division of Epidemiology, London School of Hygiene and Tropical Medicine, to whom we should like to express our deep gratitude for the help she has rendered us.

The number of animals used in each experimental group was small, and for statistical examination it was thought that Fisher's Test (Fisher, 1930), known as the 't' test for measuring the significance of differences of means in small samples, was the most appropriate method to apply. In this method the standard deviation is estimated

by pooling the sum of the squares of the deviations from the mean in the two samples and dividing by the total number of degrees of freedom contributed by them. 't' is obtained by calculating the ratio of the differences between the means of the two samples to the standard error as estimated. Fisher has tabulated the 5 per cent. and 1 per cent. values of 't' such that fluctuations of sampling would only give rise to as large or larger values once in twenty and once in a hundred times respectively. The 5 per cent. test has been used as a criterion of significance throughout this study. For example, to test the significance of a gain in weight, the value of 't' was determined for the two samples, experimental and positive control, and if this was exceeded by the 5 per cent. value of 't' in Fisher's tables for N-2, i.e. allowing two degrees of freedom, the effect of diet on growth was regarded as not significantly different in the two samples. When the results from the two groups differ significantly it is very unlikely that this difference is due to chance, and probable (20 chances to 1) that the divergence is due to the difference of treatment of the groups, i.e. that the doses given were markedly different in potency. When the results from two groups although different are not significantly so, then this difference may have been due to chance and it would be rarely justifiable to assume a definite difference in the potency of the doses under comparison.

In each vitamin A experiment the mean daily gain in weight during the period before and after addition of butter to the diet has been compared with that of control animals receiving cod-liver oil, in order to see whether any difference in treatment had had a significant effect. In the few cases where in the preparatory period the average growth of a group differed significantly from the controls this showed that the two groups were not comparable as regards growth in the preparatory period, and it is difficult to draw definite conclusions from the growth of the rats after the dose of vitamin had been added to the diet.

In the vitamin D tests the statistical interpretation of the results relies on the A/R ratio as determined from the leg-bones of the rats at the end of the experiment.

IV. NEW ZEALAND BUTTERS

We are indebted to Mr. W. Wright, Inspector of Dairy Products of the Dominion of New Zealand, for the following general details concerning the preparation of butter in New Zealand.

In New Zealand the cows are on pasture all the year round. Practically the entire milk intended for butter-making is skimmed on the farm and the cream transported to the factory in the majority of cases every second day and in some instances every day. With the exception of 4 or 5 proprietary places these butter factories are worked on a co-operative basis. On arrival at the factories the cream is tested for acidity, which of course varies with the season and the prevailing temperature, and if necessary is neutralized with sodium bicarbonate.

It is then pasteurized and cooled. The churning takes place the following day, and the butter is packed and forwarded to the grading store. After grading it is transferred to cold-store where it remains until it is shipped, usually not longer than a fortnight. All butters must remain in cold store for at least four days before shipment. The transit to this country takes 32 to 42 days, during which time the butter is kept at about 15° F. The bulk of the consignments reach the consumer within a short period after the discharge of the goods, and those consignments which are not sold immediately after arrival are kept in cold store (about 15° F.) before reaching the retailer.

The butters investigated in these experiments were prepared at Lepperton and Mangorei, on the northern slopes of Mount Egmont, near New Plymouth, Taranaki, during the summer of 1927-8 and the winter of 1929. The following are the details of the origin and preparation of the experimental material. The method of preparation is representative of that adopted in New Zealand in the manufacture of creamery butter for export to the United Kingdom and also for local consumption.

1. Summer Butters, 1927-8.

A. METHODS OF PREPARATION.

At Mangorei the butters were prepared from mixed breeds and at Lepperton from Jersey cows only. They were made at the height of the grass period, namely, 22 and 24 December 1927. No food was offered to supplement the pasture. New Plymouth sunshine records covering 20 days prior to the cream being selected are as follows:

1927.		<i>Bright Sunshine.</i>	
		<i>Hours. Minutes.</i>	
December	2	12	50
"	4	12	55
"	5	13	5
"	6	0	50
"	7	5	5
"	8	9	35
"	9	12	35
"	11	11	0
"	12	10	55
"	13	9	0
"	14	5	40
"	15	8	20
"	16	9	5
"	18	5	20
"	19	9	10
"	20	7	0
"	21	10	15
"	22	8	5

The average 'bright sunshine' per day equalled 8 hrs. 56 min. The average lactation state of the herds from which the cream selected was drawn was 5 months. The soil and climatic conditions of the districts surrounding the factories selected were comparable. Details of cream selected and manufactured are as follows:

Lepperton butters.

The Lepperton factory was selected for making the butter from cream of a pure Jersey herd. The cream used was graded as 'finest'. The parcels of cream separated on the farm selected were thoroughly mixed together, and then halved.

Jersey not neutralized. The first half was made into 'not neutralized' butter; details of manufacture are as follows:

Acidity of cream before pasteurizing 0.18% as lactic acid.

The cream was pasteurized on the 'flash' system in two stages. The pasteurizing temperature was at the 1st stage 168° F., and the 2nd stage 204° F. The cream was immediately cooled by a water cooler.

Temperature of cream off cooler 74° F.

Acidity of cream off cooler 0.14%

No starter was used.

The cream was then held in a refrigerated vat overnight for 17 hours before churning.

Churning temperature 47° F.

Churning acidity at time of putting in cream 0.14%

Temperature of wash water 39° F.

A combined churn and worker was used.

The butter was graded 94½ points.

Jersey neutralized. The remaining half of the selected parcels of cream separated on the farm was made up into 'neutralized' butter; the details of manufacture are as follows:

Acidity of cream when halved from the selected bulk 0.18%

Acidity was raised by adding starter and allowing to ripen.

Acidity of cream before neutralizing 0.25%

Bicarbonate of soda was used as the neutralizing agent.

The cream was pasteurized on the 'flash' system in two stages, 1st stage 180° F., 2nd stage 204° F.

Temperature of cream off water cooler 63° F.

Acidity of cream off cooler 0.11%

Acidity reduction effected by neutralizing and pasteurizing 0.14%

The cream was held overnight in a refrigerated vat for 16 hours.

Churning temperature 46° F.

Churning acidity 0.12%

Temperature of wash water 39° F.

The butter was graded 94½ points.

Mangorei butters.

All cream used was graded as 'finest'. 3,242 lb. of cream were used. The percentages of cream used from the various breeds of cows are as follows:

Ayrshire	15%
Shorthorn Cross	15%
Holstein	34%
Jersey	36%

The parcels of cream separated on the farm selected were thoroughly mixed together and then halved.

Mixed breeds not neutralized. The first half was made up into 'not neutralized' butter, and details of manufacture were as follows:

Acidity of cream before pasteurizing 0.17%

The cream was pasteurized on the 'flash' system in two stages, 1st stage 180° F., 2nd stage 200° F. and was immediately cooled, first by a water cooler and secondly by a direct expansion refrigerated cooler.

Temperature of cream off refrigerated cooler 54° F.

Acidity of cream off cooler 0.14%

No starter was used.

The cream was then held in a refrigerated vat overnight for 16 hours before churning.

Churning temperature 46° F.

Churning butter-fat content 35%

Churning acidity 0.15%

Temperature of wash water 39° F.

A combined churn and worker was used.

The butter was graded 94 points.

Mixed breeds neutralized. The remaining half of the selected parcels of cream separated on the farm was made up into 'neutralized' butter; the details of manufacture are as follows:

Acidity of cream when halved from the selected bulk 0.17%

Acidity was raised by adding starter and allowing to ripen.

Acidity of cream before neutralizing 0.21%

Bicarbonate of soda was used as the neutralizing agent.

The cream was pasteurized on the 'flash' system in two stages, 1st stage 180° F., 2nd stage 200° F.

Temperature off refrigerated cooler 53° F.

Acidity of cream off cooler 0.12%

Acidity reduction effected by neutralizing and pasteurizing 0.09%

The cream was held overnight in a refrigerated vat for 14 hours.

Churning temperature 44° F.

Churning butter-fat content 33%

Churning acidity 0.13%

Temperature of wash water 40° F.

The butter was graded 94 points.

Both Lepperton and Mangorei butters were held in the factory cool chambers at approximately 40° F. for a few days, before being forwarded with other lines of butter to the Moturoa grading stores.

These butters reached the laboratory on 10 March 1928. The test for vitamin A was carried out in March to June 1928. Vitamin D was determined in the 'Jersey neutralized' and 'Mixed breeds neutralized' butters in April to May and in the 'Jersey not neutralized' and 'Mixed breeds not neutralized' in May to June 1928.

B. RESULTS OF TESTS.

(a) *Vitamin A.*

In these tests 3.3 per cent. marmite, calculated on the wet weight, was used in the basal diet as a source of the vitamin B complex. Later researches (Collison, Hume, Smedley-Maclean, and Smith, 1929) have suggested, however, that this might not be quite adequate and consequently there is a possibility that the growth of the rats may have been somewhat limited. This should be borne in mind while considering the results of this test.

Jersey not neutralized (Table 1).

Although in the preparatory period the rate of growth was the highest in the positive control group, there was no significant difference in this respect between it and that of the animals receiving 0.6 g. and 0.1 g. doses. After dosing, the response by the rats receiving 0.6 g. or 0.2 g., although less marked than that of the control group, did not differ from it significantly. The lower rate of growth of the rats on the lowest dose did differ significantly. As the animals on the 0.2 g. dose were unsatisfactory, in so far as their growth in the preparatory period was low, it is only possible to say that the minimum curative dose did not lie below 0.1 g.

Jersey neutralized (Table 1).

As in the previous test the rate of growth in the preparatory period was highest in the positive control group; there was, however, no significant difference between it and the groups on the two highest doses. After dosing, the response in all groups was less marked than that of the control but the discrepancy was not statistically significant. As the animals in 0.1 g. group were unsatisfactory and as the difference between the 0.2 g. group and the positive controls was nearly significant, the minimum curative dose probably lies in the neighbourhood of 0.2 g.

Mixed breeds neutralized (Table 1).

In the preparatory period all groups did not grow as well as the control group, but only in the 0.2 g. group was the difference statistically significant. After dosage, the lower response was not significant in the 0.6 g. group, but was significant in the 0.2 g. and 0.1 g. groups. It is difficult to interpret the results obtained with 0.2 g. since the growth of this group also deviated from that of the control in the preparatory period. It is only possible to say that the minimum dose does not lie below 0.1 g.

Mixed breeds not neutralized (Table 1).

In this test the difference in rate of growth between the control group and the other groups in the preparatory period was statistically

significant only in the 0.2 g. group. After dosing, however, this difference was only significant in the 0.1 g. group. It is, therefore, only possible to give the same assessment as for the preceding butter.

TABLE 1.

Influence of method of preparation and herd on the vitamin A content of New Zealand butters, summer 1927-8.

	Average daily rate of growth.	N-2.	Value of t.	5 per cent. value of t.	
<i>Preparatory period.</i>					
Positive controls	1.04 g.				
<i>Jersey not neutralized.</i>					
0.6 g.	0.93 g.	14	-1.11	2.15	not sig.
0.2 ,,	0.77 ,,	15	-3.81	2.13	sig.
0.1 ,,	0.96 ,,	13	-0.85	2.16	not sig.
<i>Jersey neutralized.</i>					
0.6 g.	0.95 g.	13	-0.89	2.16	not sig.
0.2 ,,	0.92 ,,	14	-1.06	2.15	not sig.
0.1 ,,	0.86 ,,	14	-2.64	2.15	sig.
<i>Mixed breeds neutralized.</i>					
0.6 g.	0.95 g.	14	-1.00	2.15	not sig.
0.2 ,,	0.86 ,,	14	-2.48	2.15	sig.
0.1 ,,	0.96 ,,	14	-0.96	2.15	not sig.
<i>Mixed breeds not neutralized.</i>					
0.6 g.	0.88 g.	14	-1.79	2.15	not sig.
0.2 ,,	0.84 ,,	14	-2.49	2.15	sig.
0.1 ,,	0.90 ,,	14	-1.47	2.15	not sig.
<i>Dosing period.</i>					
Positive controls	1.23 g.				
<i>Jersey not neutralized.</i>					
0.6 g.	0.81 g.	14	-1.67	2.15	not sig.
0.2 ,,	0.99 ,,	15	-1.09	2.13	not sig.
0.1 ,,	0.37 ,,	13	-2.80	2.16	sig.
<i>Jersey neutralized.</i>					
0.6 g.	1.05 g.	13	-0.73	2.16	not sig.
0.2 ,,	0.78 ,,	14	-2.06	2.15	not sig.
0.1 ,,	0.74 ,,	14	-1.95	2.15	not sig.
<i>Mixed breeds neutralized.</i>					
0.6 g.	1.00 g.	14	-1.04	2.15	not sig.
0.2 ,,	0.62 ,,	14	-2.40	2.15	sig.
0.1 ,,	0.55 ,,	14	-3.12	2.15	sig.
<i>Mixed breeds not neutralized.</i>					
0.6 g.	0.97 g.	14	-1.16	2.15	not sig.
0.2 ,,	0.86 ,,	14	-1.50	2.15	not sig.
0.1 ,,	0.50 ,,	14	-3.02	2.15	sig.

(b) *Vitamin D.*

It will be seen (Table 2) that the maximum A/R ratio was obtained in each case with a daily dose of 0.6 g. of butter, whilst with the 0.2 g.

and still more with the 0.1 g. doses lower figures were registered. The differences recorded in the case of the lower doses between the various butters are, in our opinion, of such an order as to fall within the limits of the experimental error of the method and consequently they cannot be taken as a significant index of unequal vitamin D contents.

TABLE 2.

Influence of method of preparation and herd on the vitamin D content of New Zealand butters, summer 1927-8.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.76					
<i>Jersey neutralized.</i>						
0.6 g.	0.80	117	10	+0.93	2.23	not sig.
0.2 g.	0.70	74	10	-2.46	2.23	sig.
0.1 „	0.65	52	10	-2.17	2.23	not sig.
<i>Mixed breeds neutralized.</i>						
0.6 g.	0.79	113	10	+0.98	2.23	not sig.
0.2 „	0.70	74	10	-1.35	2.23	not sig.
0.1 „	0.67	61	10	-2.18	2.23	not sig.
Negative controls	0.53					
Positive controls	0.79					
<i>Jersey not neutralized.</i>						
0.6 g.	0.81	109	9	+0.32	2.26	not sig.
0.2 „	0.75	83	8	-0.98	2.31	not sig.
0.1 „	0.62	26	8	-4.71	2.31	sig.
<i>Mixed breeds not neutralized.</i>						
0.6 g.	0.80	104	9	+0.13	2.26	not sig.
0.2 „	0.69	57	9	-3.01	2.26	sig.
0.1 „	0.67	48	9	-2.67	2.26	sig.
Negative controls	0.56.					

In this and in all subsequent Tables of vitamin D tests:

A/R = Ash/ 'organic residue'

$$\text{Degree of protection} = \frac{D-N}{P-N} \times 100$$

where:

D = Average A/R of dose group.

N = „ „ „ negative control group.

P = „ „ „ positive control group.

2. Stored Summer Butters.

RESULTS OF TESTS.

(a) Vitamin A.

The summer butters were re-tested for vitamin A in March to July 1930, namely, 2 years after arrival in England. During this period the experimental material was kept in cold-store at 9-13° F.

The basal diet differed slightly from the one used when these

butters were first tested. As a source of the vitamin B complex, 5 per cent. and eventually 10 per cent. of dried yeast, calculated on the wet weight of the diet, was used instead of 3.3 per cent. marmite. This alteration, as already mentioned, was effected because, during the interval between the tests, indications were obtained that the source of the vitamin B complex used in the first test was not quite satisfactory.

TABLE 3.

Influence of storage on the vitamin A content of New Zealand butters, summer 1927-8.

	<i>Average daily rate of growth.</i>	<i>N-2.</i>	<i>Value of t.</i>	<i>5 per cent. value of t.</i>	
<i>Preparatory period.</i>					
Positive controls	1.09 g.				
<i>Jersey not neutralized.</i>					
0.2 g.	1.00 g.	11	-0.48	2.20	not sig.
0.1 „	1.15 „	11	+0.35	2.20	not sig.
<i>Jersey neutralized.</i>					
0.2 g.	1.25 g.	11	+0.73	2.20	not sig.
0.1 „	1.08 „	11	-0.06	2.20	not sig.
<i>Mixed breeds neutralized.</i>					
0.2 g.	1.26 „	11	+0.88	2.20	not sig.
0.1 „	1.10 „	12	+0.06	2.18	not sig.
<i>Mixed breeds not neutralized.</i>					
0.2 g.	1.07 g.	11	-0.11	2.20	not sig.
0.1 „	1.19 „	11	+0.59	2.20	not sig.
<i>Dosing period.</i>					
Positive controls	2.68 g.				
<i>Jersey not neutralized.</i>					
0.2 g.	1.69 g.	11	-2.44	2.20	sig.
0.1 „	0.69 „	11	-6.65	2.20	sig.
<i>Jersey neutralized.</i>					
0.2 g.	1.77 g.	11	-3.22	2.20	sig.
0.1 „	1.24 „	11	-5.40	2.20	sig.
<i>Mixed breeds neutralized.</i>					
0.2 g.	1.92 g.	11	-3.41	2.20	sig.
0.1 „	1.37 „	12	-5.47	2.18	sig.
<i>Mixed breeds not neutralized.</i>					
0.2 g.	1.74 g.	11	-2.61	2.20	sig.
0.1 „	1.38 „	11	-4.66	2.20	sig.

The response in growth of the positive control group was exceptionally high (Table 3). In these tests the growth response of the two doses tested (0.2 g. and 0.1 g.) was higher in every case than in the tests carried out 2 years previously (Table 1). Also, on the whole, the time taken to deplete the rats of vitamin A was shorter. This was most probably due to the change of the source and level of the

vitamin B complex in the basal diet. As will be seen from Table 3, in all cases the highest dose tested (0.2 g.), did not give as good a growth response as the cod-liver oil in the positive control group; moreover, this difference was statistically significant. It must be noted, however, that the response in growth of the positive control group was exceptionally high in this test. In the preparatory periods there was no significant difference between the rate of growth in the butter groups and in the positive control group. The general indication points to the fact that no loss of vitamin A took place during storage.

(b) *Vitamin D.*

The re-tests took place in March to May 1930, after nearly two years storage. The results are contained in Table 4. It is evident from these data that all the butters showed less protection in daily doses of 0.2 g. after storage than before (Table 2). The higher dose (0.6 g.) gave, within the limits of the experimental error of the method, complete protection.

TABLE 4.

Influence of storage on the vitamin D content of New Zealand butters, summer 1927-8.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.80					
<i>Jersey not neutralized.</i>						
0.6 g.	0.79	96	11	-0.28	2.20	not sig.
0.2 ,,	0.59	22	11	-3.76	2.20	sig.
<i>Jersey neutralized.</i>						
0.6 g.	0.80	100	11	-0.03	2.20	not sig.
0.2 ,,	0.60	26	11	-3.76	2.20	sig.
<i>Mixed breeds neutralized.</i>						
0.6 g.	0.77	89	11	-0.85	2.20	not sig.
0.2 ,,	0.59	22	11	-3.38	2.20	sig.
<i>Mixed breeds not neutralized.</i>						
0.6 g.	0.78	93	11	-0.34	2.20	not sig.
0.2 ,,	0.64	41	11	-2.91	2.20	sig.
Negative controls	0.53					

It is to be noted that the antirachitic potency of the four butters differed somewhat before but not after storage. There is little doubt that all these butters lost some vitamin D during storage.

3. Winter Butters, 1929.

A. ORIGIN AND METHODS OF PREPARATION.

As in the previous experiment, butters were prepared from mixed breeds at Mangorei and from Jersey cows only at Lepperton. The butters were made on 9 May and 16 May 1929, these dates being as

VITAMIN CONTENT OF BUTTERS

near to mid-winter as it was possible to go on account of the very small supply of cream available in the month of June. No food was offered to supplement the pasture feeding. The average lactation period of the supplying herds on 15 May was $9\frac{1}{2}$ months. Sunshine records for the district for 36 days prior to the butters being made are as follows:

1929.	Bright Sunshine. Hours.
April 1	10
" 2	9
" 3	10
" 4	10
" 5	10
" 7	11
" 8	5
" 9	1
" 10	10
" 11	1
" 12	1
" 14	Nil
" 15	6
" 16	Nil
" 17	6
" 18	2
" 19	9
" 21	8
" 22	4
" 23	5
" 25	2
" 26	9
" 28	3
" 29	1
" 30	6
May 1	7
" 2	8
" 3	1
" 5	9
" 6	8
" 7	9

The average sunshine per day was 6 hours. Climatic conditions were good for 6 weeks prior to the butter being made, moderate weather and warm rains being experienced. The climatic conditions were comparable, as the two factories are only a few miles apart. Details of cream selected and manufactured are as follows:

Lepperton butters.

Cream from a purely Jersey herd of cows was used at the Lepperton Factory. Approximately 95 per cent. of the cream used was graded as 'finest', the balance being '1st grade'. The parcels of cream were thoroughly mixed, then halved.

Jersey not neutralized. The first half was made up into 'not neutralized' butter; details of manufacture are as follows:

Acidity of cream before pasteurizing 0.17%
No neutralizing agent was used.

The cream was pasteurized by the 'flash' system in two stages to a final temperature of 204° F.

Temperature of cream off water cooler	64° F.
Acidity of cream off cooler	0.14%

The cream was held overnight in a refrigerated vat for 16 hours before churning.

Churning temperature	50° F.
Churning acidity	0.15%
Temperature of wash water	41° F.

Analysis of butter:

Butter-fat	81.7%
Water	15.3%
Salt	2.2%
Curd and ash	0.8%

The butter was graded 93½ points.

Jersey neutralized. The remaining half of the selected cream was made into 'neutralized' butter, the details of manufacture are as follows:

Initial acidity of cream	0.17%
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Sodium bicarbonate was used as the neutralizing agent. The cream was pasteurized by the 'flash' system in two stages to a final temperature of 204° F.

Temperature of cream off water cooler	65° F.
Acidity of cream off cooler	0.11%
Acidity reduction effected by neutralizing and pasteurizing	0.06%

The cream was held overnight in a refrigerated vat for 15 hours before churning.

Churning temperature	46° F.
Churning acidity	0.13%
Temperature of wash water	40° F.

Analysis of butter:

Butter-fat	82.5%
Water	15.4%
Salt	1.6%
Curd and ash	0.5%

The butter was graded 93½ points.

Mangorei butters.

Approximately 94 per cent. of the cream used was graded as 'finest', the balance being classed as '1st grade'. The following breeds of cows were well represented in the cream used: Ayrshire, Holstein, Shorthorn Cross and Jersey. The selected cream was divided into two equal batches.

Mixed breeds not neutralized. Half the selected cream was made up into 'not neutralized' butter; the details of manufacture are as follows:

Acidity of cream before pasteurizing and after division	0.16%
No neutralizing agent was used.	

VITAMIN CONTENT OF BUTTERS

The cream was pasteurized by the 'flash' system in two stages to a final temperature of 200° F.

Temperature of cream off refrigerated cooler	51° F.
Acidity of cream off cooler	0.13%

The cream was held overnight in a refrigerated vat for 15 hours before churning.

Churning temperature	43° F.
Churning acidity	0.14%
Temperature of wash water	40° F.

Analysis of butter:

Butter-fat	81.4%
Water	15.4%
Salt	2.4%
Curd and ash	0.8%

The butter was graded 93½ points.

Mixed breeds neutralized. The remaining batch of the selected cream was made into 'neutralized' butter; details of manufacture were as follows:

Acidity of cream (after division)	0.17%
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Sodium bicarbonate was used as the neutralizing agent. The cream was pasteurized by the 'flash' system in two stages to a final temperature of 200° F.

Temperature of cream off refrigerated cooler	51° F.
Acidity of cream off cooler	0.07%
Acidity reduction effected by neutralizing and pasteurizing	0.1%

The cream was held overnight in a refrigerated vat for 14 hours before churning.

Churning temperature	46° F.
Churning acidity	0.08%
Temperature of wash water	40° F.

Analysis of butter:

Butter-fat	81.7%
Water	15.4%
Salt	2.1%
Curd and ash	0.8%

The butter was graded 93½ points.

Both Lepperton and Mangorei butters were held in the factory cool chambers at approximately 40° F. for a few days before being forwarded to the freezing works for grading and storage, prior to shipment.

The butters reached the laboratory on 15 August 1929. The tests for vitamin A were carried out in October to November 1929. Vitamin D was determined during September to October, 1929.

B. RESULTS OF TESTS.

(a) Vitamin A.

Five per cent. dried yeast, calculated on the wet weight, as the source of the vitamin B complex, and hardened sesame oil were used in the basal diet in this test.

Jersey not neutralized (Table 5).

The small differences in the rate of growth between all groups and that of the positive control group before dosing were not significant. After dosage, the response in all groups was less than that of the control group and only in the 0.2 g. group was this difference not significant. One may, therefore, assume that the minimum dose was probably between 0.1 g. and 0.2 g. and consequently the activity of this butter is of the same order as that of the summer butter.

TABLE 5.

Influence of method of preparation and herd on the vitamin A content of New Zealand butters, winter 1929.

	<i>Average daily rate of growth.</i>	<i>N-2</i>	<i>Value of t.</i>	<i>5 per cent. value of t.</i>	
<i>Preparatory period.</i>					
Positive controls	1.12 g.				
<i>Jersey not neutralized.</i>					
0.2 g.	1.13 g.	13	+0.08	2.16	not sig.
0.1 "	1.38 "	13	+1.61	2.16	not sig.
0.05 "	1.22 "	13	+0.73	2.16	not sig.
<i>Jersey neutralized.</i>					
0.2 g.	1.24 g.	13	+1.00	2.16	not sig.
0.1 "	1.21 "	13	+0.63	2.16	not sig.
0.05 "	1.25 "	13	+1.07	2.16	not sig.
<i>Mixed breeds neutralized.</i>					
0.2 g.	1.14 g.	13	+0.14	2.16	not sig.
0.1 "	1.28 "	13	+1.15	2.16	not sig.
0.05 "	1.30 "	13	+1.35	2.16	not sig.
<i>Mixed breeds not neutralized.</i>					
0.2 g.	1.27 g.	13	+1.02	2.16	not sig.
0.1 "	1.28 "	13	+1.16	2.16	not sig.
0.05 "	1.29 "	13	+1.16	2.16	not sig.
<i>Dosing period.</i>					
Positive controls	1.17 g.				
<i>Jersey not neutralized.</i>					
0.2 g.	0.86 g.	13	-1.42	2.16	not sig.
0.1 g.	0.70 "	13	-4.22	2.16	sig.
0.05 "	0.40 "	13	-7.55	2.16	sig.
<i>Jersey neutralized.</i>					
0.2 g.	0.65 g.	13	-3.07	2.16	sig.
0.1 "	0.73 "	13	-3.72	2.16	sig.
0.05 "	0.15 "	13	-7.44	2.16	sig.
<i>Mixed breeds neutralized.</i>					
0.2 g.	0.09 g.	13	-3.35	2.16	sig.
0.1 "	0.71 "	13	-1.70	2.16	not sig.
0.05 "	0.10 "	13	-4.02	2.16	sig.
<i>Mixed breeds not neutralized.</i>					
0.2 g.	0.96 g.	13	-1.29	2.16	not sig.
0.1 "	-0.11 "	13	-4.76	2.16	sig.
0.05 "	-0.50 "	13	-4.80	2.16	sig.

Jersey neutralized (Table 5).

As in the preceding test the differences between the butter groups and the positive control group were not significant before dosing, but after dosing there was in every butter group a lower response than that of the control group. It would appear that 0.2 g. was not sufficient to induce optimum growth. This butter was, therefore, approximately of the same potency as the summer sample.

Mixed breeds neutralized (Table 5).

There was no significant difference between the rate of growth of the butter groups and of the positive control group in the preparatory period. After dosing the only group which showed a lower response than the positive control group, which was not significant, was the 0.1 g. group. In the case of the 0.2 g. group this difference was significant. The discrepancy can be ascribed to the fact that the animals in the 0.2 g. group were unsatisfactory. The minimum dose in this case lay probably in the neighbourhood of 0.1 g., but owing to the unsatisfactory batch of rats in both groups no definite conclusion can be drawn.

Mixed breeds not neutralized (Table 5).

The difference in the preparatory period in all cases was here insignificant. After dosing, the only not significant difference was observed in the 0.2 g. group and consequently the minimum dose may be assumed to be between 0.1 g. and 0.2 g.

(b) *Vitamin D.*

It will be seen from Table 6 that 0.6 g. of butter was not sufficient to produce the maximum A/R ratio except in the case of the 'Mixed breeds neutralized'. The other three butters show, within the limits of the experimental error, about the same activity. This activity is decidedly lower than that of the summer butters (Table 2), an observation which was to be expected from theoretical considerations. The results obtained with the 'Mixed breeds neutralized' butter were rather ambiguous. The degree of antirachitic protection given by a daily dose of 0.1 g. of butter was about the same as that given by a similar dose of the other butters. On the other hand, the higher doses (0.2 g.) of the 'Mixed breeds neutralized' butter afforded more protection than the corresponding doses of the other samples and consequently it might be considered possible that this butter contained more vitamin D than the others.

TABLE 6.

Influence of method of preparation and herd on the vitamin D content of New Zealand butters, winter 1929.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.82					
<i>Jersey not neutralized.</i>						
0.6 g.	0.74	73	14	-2.20	2.15	sig.
0.2 "	0.67	50	12	-3.51	2.18	sig.
0.1 "	0.58	20	13	-5.94	2.16	sig.
<i>Jersey neutralized.</i>						
0.6 g.	0.73	70	14	-2.35	2.15	sig.
0.2 "	0.60	27	14	-4.83	2.15	sig.
0.1 "	0.59	23	14	-6.95	2.15	sig.
<i>Mixed breeds neutralized.</i>						
0.6 g.	0.81	97	14	-0.21	2.15	not sig.
0.2 "	0.74	73	12	-1.08	2.18	not sig.
0.1 "	0.58	20	14	-5.25	2.15	sig.
<i>Mixed breeds not neutralized.</i>						
0.6 g.	0.76	80	14	-1.90	2.15	not sig.
0.2 "	0.65	43	14	-3.27	2.15	sig.
0.1 "	0.56	13	14	-5.58	2.15	sig.
Negative controls	0.52					

4. Stored Winter Butters.

RESULTS OF TESTS.

The tests were carried out during October to December 1930, about a year, therefore, after the first test.

(a) Vitamin A.

In these tests 10 per cent. of dried yeast, calculated on the wet weight of the diet, was introduced instead of the 5 per cent. used in the tests of these butters before storage. The general growth response was somewhat better than in the first tests (Table 7). In all cases there was no significant difference in the preparatory period between the rate of growth in the butter groups and in the positive control group. On the other hand, after dosing, this difference became significant for the animals receiving 0.1 g. of any of the butters. As in the stored summer butters there is no indication that a loss of vitamin A took place during storage.

(b) Vitamin D.

Only two butters, namely, 'Jersey not neutralized' and 'Jersey neutralized', were re-tested for their antirachitic content after storage in the case of the winter butters. These tests were carried out during November to December 1930, which was 1 year and 2

TABLE 7.

Influence of storage on the vitamin A content of New Zealand butters, winter 1929.

	<i>Average daily rate of growth.</i>	<i>N-2.</i>	<i>Value of t.</i>	<i>5 per cent. value of t.</i>	
<i>Preparatory period.</i>					
Positive controls	1.18 g.				
<i>Jersey not neutralized.</i>					
0.2 g.	1.30 g.	13	+0.62	2.16	not sig.
0.1 g.	1.27 g.	13	+0.55	2.16	not sig.
<i>Jersey neutralized.</i>					
0.2 g.	1.59 g.	13	+2.22	2.16	barely sig.
0.1 „	1.23 „	13	+0.27	2.16	not sig.
<i>Mixed breeds neutralized.</i>					
0.2 g.	1.43 g.	13	+1.13	2.16	not sig.
0.2 „	1.20 „	13	+0.11	2.16	not sig.
<i>Mixed breeds not neutralized.</i>					
0.2 g.	1.30 g.	13	+0.68	2.16	not sig.
0.1 „	1.36 „	13	+0.96	2.16	not sig.
<i>Dosing period.</i>					
Positive controls	1.80 g.				
<i>Jersey not neutralized.</i>					
0.2 g.	0.69 g.	13	-2.03	2.16	not sig.
0.1 „	0.69 „	13	-3.25	2.16	sig.
<i>Jersey neutralized.</i>					
0.2 g.	0.75 g.	13	-2.19	2.16	barely sig.
0.1 „	0.90 „	13	-2.58	2.16	sig.
<i>Mixed breeds neutralized.</i>					
0.2 g.	1.64 g.	13	-0.44	2.16	not sig.
0.1 „	0.46 „	13	-2.95	2.16	sig.
<i>Mixed breeds not neutralized.</i>					
0.2 g.	1.41 g.	13	-1.14	2.16	not sig.
0.1 „	0.36 „	13	-3.43	2.16	sig.

months after the first test. Owing to the fact that full antirachitic protection was not originally obtained with 0.6 g. of butter, no lower dose was included in the test and it was inadvisable to introduce higher doses as the amount of fat administered in these circumstances might have complicated the determination. It will be seen from Table 8 that the response in the case of both butters was rather lower than that obtained in the first test. This difference cannot, however, be considered significant in view of the limitations of the method.

It might also be pointed out that in the case of the 'Jersey not neutralized' butter the figures obtained with 0.6 g. after storage were nearly significant, while before storage the corresponding figures were significant.

TABLE 8.

Influence of storage on the vitamin D content of New Zealand butters, winter 1929.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.93					
<i>Jersey not neutralized.</i>						
0.6 g.	0.83	71	10	-2.15	2.23	not sig.
<i>Jersey neutralized.</i>						
0.6 g.	0.76	50	10	-2.81	2.23	sig.
Negative controls	0.59					

V. AUSTRALIAN BUTTERS

The following are the general details of the preparation of Australian butters, for which we are indebted to Major J. R. King, D.S.O., of the Australian Dairy Produce Board. The cows are on pasture all the year round throughout Australia and about 90 per cent. of the butter is manufactured on the co-operative basis. The cream is skimmed on the farms and generally reaches the factory, mostly in an acid condition, within 24-36 hours. It is churned after being neutralized with sodium bicarbonate within twenty-four hours of its arrival at the factory. The graded butter is then shipped 1-7 days later and during transit to this country, which takes 6-7 weeks, it is stored at 10°-15° F. Only about 10-14 days elapse between the time of arrival of the butter here and its reaching the retailer. In the meantime in winter it is stored in the warehouse at Hays Wharf and in summer it is kept in refrigerator chambers until required for consumption.

1. Summer Butters, 1927-8.

A. ORIGIN.

As it was not possible to obtain samples of Australian butters in 1928, specially prepared under controlled conditions, two random samples, one from New South Wales (Bega) and the other from Queensland (Coroy), prepared during the middle of the Australian summer (1927-8) were investigated. These samples were kindly supplied to us by Major King, to whom we are also indebted for the following information.

New South Wales.

The District is about 70 miles north of the Victorian border and 25 miles inland from the sea; it is beautifully undulating, somewhat similar to Surrey, and very well watered. Originally it was fairly heavily timbered country, but was cleared about 100 years ago, and is now one of the most prosperous districts in New South Wales. The

rock formation of the country is granite and the soil a fine, granite, sandy loam.

The grasses are largely natural, interspersed with cocksfoot, clover, and English rye-grass. Artificial feeding is resorted to if the weather should be dry, and the food supplied then is mostly green maize or a plant called 'sorghum' (allied to the sugar-cane family) in the summer-time and, towards autumn, green oats or barley. When the season is satisfactory this green maize and sorghum, also the oats and barley, are put into silos and fed to the stock during the winter season.

The district itself gets a large percentage of sunshine and is fairly well sheltered from the winds by the mountains to the west.

Cattle are largely Ayrshire, Milking Durham, Jersey, and Jersey Cross.

Queensland.

Coroy is about 150 miles north of Brisbane, between the ranges in the west and the sea in the east, south of Gympie some 40 miles. The country is undulating, well watered by several rivers and streams, and was up till a few years ago covered with light scrub on the lower lands and heavily timbered with hardwood trees on the higher ground. It is only within the last 25 years that dairying was taken up; prior to this the country north of Coroy was a gold-field, of which Gympie was the head-quarters, and as the gold reefs gave out dairying was taken on; a very satisfactory settlement has grown up right throughout the district.

The grass is largely *Paspalum dilatatum*, and cocksfoot with a sprinkling of rye-grass, but owing to the heat the rye-grass soon disappears.

Cattle are largely a cross between Milking Durham, Ayrshire, Jersey, and Guernsey.

The soil ranges from volcanic chocolate to sandy loam. Temperature rarely gets over 90° F. and the rainfall is exceedingly heavy, but occasionally there are dry spells. The average hours of sunshine are estimated at 8 or 9 hours a day, and in the summer-time considerably longer.

B. RESULTS OF TESTS.

These butters reached the laboratory on 22 February 1928, and were tested for vitamin A during June to August 1928.

(a) *Vitamin A.*

In the first part of the test 3.3 per cent. marmite, calculated on the wet weight, was employed as the source of the vitamin B complex. During the last 10 days the marmite was supplemented with 0.25 to

some extent vitiated. It will be seen from Table 9 that there was no significant difference before and after dosing even in the case of the

TABLE 9.
Vitamin A content of Australian butters, summer 1927-8.

	Average daily rate of growth.	N-2.	Value of t.	5 per cent. value of t.	
<i>Preparatory period.</i>					
Positive controls	0.80 g.				
<i>New South Wales.</i>					
0.6 g.	0.73 g.	10	-1.11	2.23	not sig.
0.2 ,,	0.77 ,,	10	-0.40	2.23	not sig.
0.1 ,,	0.90 ,,	10	+1.42	2.23	not sig.
<i>Queensland.</i>					
0.6 g.	0.75 g.	10	-0.76	2.23	not sig.
0.2 ,,	0.76 ,,	10	-0.60	2.23	not sig.
0.1 ,,	0.81 ,,	10	+0.13	2.23	not sig.
<i>Dosing period.</i>					
Positive controls	0.91 g.				
<i>New South Wales.</i>					
0.6 g.	0.99 g.	10	+0.18	2.23	not sig.
0.2 ,,	1.26 ,,	10	+1.13	2.23	not sig.
0.1 ,,	1.07 ,,	10	+0.50	2.23	not sig.
<i>Queensland.</i>					
0.6 g.	0.92 g.	10	+0.02	2.23	not sig.
0.2 ,,	0.69 ,,	10	-0.37	2.23	not sig.
0.1 ,,	1.00 ,,	10	+0.25	2.23	not sig.

groups which received only 0.1 g. of butter. The weight curves showed further that the rate of growth obtained with this dose of either butter was of an order not much lower than that usually obtained in the positive control group. This most probably was the minimum dose.

(b) *Vitamin D.*

The results of the vitamin D tests of the 1927-8 butters, carried out during March to April 1928, are given in Table 10. In the case of both

TABLE 10.
Vitamin D content of Australian butters, summer 1927-8.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.76					
<i>New South Wales.</i>						
0.6 g.	0.79	113	10	+0.55	2.23	not sig.
0.2 ,,	0.73	87	10	-0.65	2.23	not sig.
0.1 ,,	0.59	26	10	-4.27	2.23	sig.
<i>Queensland.</i>						
0.6 g.	0.76	100	10	0	2.23	not sig.
0.2 ,,	0.68	65	10	-1.68	2.23	not sig.
0.1 ,,	0.56	13	10	-5.64	2.23	sig.
Negative controls	0.53					

butters, the maximum antirachitic protection was obtained with a daily dose of 0.6 g. The 0.2 g. dose afforded, in the case of the New South Wales butter, 87 per cent. of the maximum protection and 65 per cent. in the case of the Queensland butter. A slightly better antirachitic protection was also registered with the former in the 0.1 g. dose test. It is, however, impossible to say with certainty whether the vitamin D content of this butter was higher than that of the Queensland sample, considering the experimental error of the method.

2. Summer Butters, 1928-9.

A. ORIGIN AND METHODS OF PREPARATION.

New South Wales.

(a) Soil, &c.	Granite foundation and rich alluvial flats.
Climate.	Moderate.
Geographical features.	Undulating to mountainous.
(b) State of pastures.	At the time of butter manufacture very short and dry, grasses chiefly natural.
(c) Foods given to supplement pastures.	Lucerne and maize.
(d) Seasonal conditions at time of sample being taken.	Extremely dry for a number of months; rain had just commenced, but had not had time to alter conditions.
(e) Information on Breeds.	90% Jersey and Jersey Cross with remainder Shorthorn and Ayrshire.
(f) Average Lactation.	Nine months.
(g) Neutralizing agent.	Soda-bicarbonate.
Acidity before neutralization.	0.41%
Acidity at churning.	0.2%
Pasteurization temperature.	185° F.-187° F.
Churning temperature.	45° F.
Churning time.	25 min.
Working time.	11 min.
Moisture (by quick method).	15.8%

Queensland.

(a) Soil, &c.	Varying classes, brown and black soil, sandy loam.
Climate.	Semi-tropical.
Geographical features.	Alluvial flats, ridges and slopes of brown and black soil, sandy loam and sandy country. Undulating.
(b) State of pastures.	Pastures fresh at time of production, with some approaching maturity stage.
(c) Foods given to supplement pastures.	Sorghum, maize, and lucerne.
(d) Seasonal conditions at time of sample being taken.	High temperature and somewhat humid.
(e) Information on breeds.	Milking Shorthorns, Jersey, Ayrshire and grades of such breeds.
(f) Average lactation.	Four to five months in profit.
(g) Neutralizing agent.	Soda-bicarbonate.
Acidity before neutralization.	0.45%
Acidity at churning.	0.25%
Pasteurization temperature.	185° F.
Churning temperature.	48° F.
(h) Not cold-stored in Brisbane; dispatched from factory to boat.	

Victoria.

The Mirboo factory, Gippsland, was chosen as corresponding in climate, soil conditions, and character of supplies, with the factories in the districts understood to have been chosen for New South Wales and Queensland. It is within a herd-testing zone.

In this State and in the district in which Mirboo is situated, the month of February can be regarded as mid-summer, and the commercial manufacture from which this box was taken was made on 20 February.

Soil. Soil throughout the district is a grey loam 6 to 8 inches overlying good clay sub-soil.

Climate. Climate is mild in summer but usually rather extreme in cold during the winter months. Country varies from undulating to hilly. Distance from coast approximately 30 miles.

Pastures. Consist of rye and cocksfoot grasses and mixed clovers, principally cow-grass, alsyke and white clover, a fair proportion of which is now in a dry state. Top dressing of pasture not very extensive.

Concentrate and food given to supplement pastures. Very little concentrate used but pastures in nearly all cases are supplemented with green maize, millet, and oats, cut and fed to the cows.

Seasonal conditions. Seasonal conditions for dairying are very fair at the time of year but supplies are falling off on account of the drying of the pastures, and the period cows have been milking. Weather hot and muggy. Average temperature for 4 days during week of manufacture 90° F. including the day on which the cream for manufacture of the butter, of which the box forwarded is a sample, was received at the factory.

Breeds. The cream supply on the day of manufacture was from 20 herds and approximately 400 cows, comprising approximately 60 per cent. Jersey and 40 per cent. mixed cross-breeds with a preponderance of Jersey strain.

Lactation period. The majority of the cows supplying calved in September or early October, and are, consequently, slightly past their mid-lactation period.

Pasteurizing Details.

Quantity of cream	1681 lb.
Temperature of cream	85° F.
Acidity of cream	0.54%
Reduced to	0.18%
Bicarbonate of soda used	5.5 lb.
Temperature of cream when added	85° F.
Heated to	150° F.
Time taken	30 min.
Time held	30 min.
Cooled to (cold water)	95° F.
Cooled to (brine)	42° F.
Total time taken	2.25 hrs.
Check acidity	0.18%

Churning Details.

Time held in pasteurizer	16 hrs.
Temperature of cream 20/2/29	54° F.
Temperature of cream at churning	46° F.
Acidity of cream at churning	0.18%
Temperature of break water	46° F.
Churning time	60 min.
Temperature of wash water	46° F.
Size of butter granules	wheat size
Quantity of salt added	12 lb.

Churning Details (continued).

Time of working butter	20 min.
Moisture contents at churn	16%
Temperature in churning-room	80° F.
Temperature in cool room	40° F.
Time held at factory	144 hrs.

Consigned to Melbourne in ice truck.

Analysis of butter sample taken when packed.

Moisture	15.7%
Fat	82.5%
Salt	1.1%
Curd	0.7%

Remarks.

Weather conditions: sultry, oppressive, thundery.

Temperature in factory: 85° F.

All cream collected twice weekly.

Commenced week 19 February 1929.

Quality of cream used: 'Choicest'.

Butter graded 92 points at Government Cool Stores by Commonwealth Graders.

Held at Cool Stores: 6 days.

Temperature of Cool Store Room: 8 to 10° F.

Temperature of butter leaving Cool Store for ship: 20° F.

Left Melbourne: 5/3/1929.

Arrived at London: 11/4/1929.

Arrived at Lister Institute: 15/4/29.

B. RESULTS OF TESTS.

(a) Vitamin A.

The butters were received at the laboratory on 15 April 1929, and were tested for vitamin A during April to June 1929. Dried yeast (5 per cent.) was used in the basal diet.

As can be seen from Table 11, the rate of growth of the butter groups did not differ significantly from that of the positive controls in the preparatory period. In each case after dosing, the 0.2 g. groups were the only ones which did not differ significantly from the positive control group. Considering that the positive controls in this test grew unusually well, it is probable that the minimum dose for all these butters lay between 0.1 g. and 0.2 g.

(b) Vitamin D.

The tests were carried out during April to May 1929. The results are given in Table 12. In all the three butters the maximum antirachitic protection was obtained with the daily dose of 0.4 g. of butter. The lower doses did not afford this high protection, judged from the percentage A/R figures, although there was no significant deviation between the results of the 0.2 g. groups of the Queensland and Victoria butters and the positive controls. No satisfactory explanation can be given for the apparently inconsistent results obtained with these doses in the case of the New South Wales and Queensland

TABLE 11.
Vitamin A content of Australian butters, summer 1928-9.

	Average daily rate of growth.	N-2.	Value of t.	5 per cent. value of t.	
<i>Preparatory period.</i>					
Positive controls	1.01 g.				
<i>New South Wales.</i>					
0.2 g.	1.01 g.	11	0	2.20	not sig.
0.1 "	1.09 "	10	+0.67	2.23	not sig.
0.05 "	1.11 "	10	+0.48	2.23	not sig.
<i>Queensland.</i>					
0.2 g.	0.89 g.	10	-1.12	2.23	not sig.
0.1 "	1.04 "	10	+0.19	2.23	not sig.
0.05 "	1.07 "	9	+0.50	2.26	not sig.
<i>Victoria.</i>					
0.2 g.	1.08 g.	10	+0.01	2.23	not sig.
0.1 "	0.98 "	10	0	2.23	not sig.
0.05 "	1.06 "	10	0	2.23	not sig.
<i>Dosing period.</i>					
Positive controls	2.28 g.				
<i>New South Wales.</i>					
0.2 g.	1.79 g.	11	-1.52	2.20	not sig.
0.1 "	1.59 "	10	-2.84	2.23	sig.
0.05 "	0.54 "	10	-5.71	2.23	sig.
<i>Queensland.</i>					
0.2 g.	1.09 g.	10	-2.18	2.23	not sig.
0.1 "	0.84 "	10	-2.74	2.23	sig.
0.05 "	0.89 "	9	-4.80	2.26	sig.
<i>Victoria.</i>					
0.2 g.	1.60 g.	10	-2.09	2.23	not sig.
0.1 "	1.15 "	10	-4.47	2.23	sig.
0.05 "	0.73 "	10	-6.26	2.23	sig.

TABLE 12.
Vitamin D content of Australian butters, summer 1928-9.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.87					
<i>New South Wales.</i>						
0.4 g.	0.88	103	11	+0.25	2.20	not sig.
0.2 "	0.70	50	11	-4.90	2.20	sig.
0.1 "	0.70	50	11	-4.97	2.20	sig.
<i>Queensland.</i>						
0.4 g.	0.88	103	11	+0.30	2.20	not sig.
0.2 "	0.73	59	11	-1.29	2.20	not sig.
0.1 "	0.69	47	11	-3.92	2.20	sig.
<i>Victoria.</i>						
0.4 g.	0.86	97	11	-0.46	2.20	not sig.
0.2 "	0.79	76	10	-1.53	2.23	not sig.
0.1 "	0.71	53	11	-4.27	2.20	sig.
Negative controls	0.53					

butters, which show almost the same antirachitic protection with the 0.1 g. as with the 0.2 g. dose. We feel inclined to attribute this discrepancy to the limitations of the method.

3. Stored Butters.

RESULTS OF TESTS.

(a) *Vitamin A.*

Two of the above butters, namely, New South Wales 1927-8 and New South Wales 1928-9, were re-tested for vitamin A after storage, during February to March 1931, so that the former was stored for 2 years and 8 months and the latter for 1 year and 9 months.

No significant differences appeared in the preparatory period in this test (Table 13). During the dosing period one group only, New

TABLE 13.

Influence of storage on the vitamin A content of Australian butters.

	<i>Average daily rate of growth.</i>	<i>N-2.</i>	<i>Value of t.</i>	<i>5 per cent. value of t.</i>	
<i>Preparatory period.</i>					
Positive controls	1.70 g.				
<i>New South Wales, 1927-8.</i>					
0.1 g.	1.50 g.	10	-1.00	2.23	not sig.
0.05 ,,	1.55 ,,	10	-0.66	2.23	not sig.
<i>New South Wales, 1928-9.</i>					
0.1 g.	1.45 g.	11	-1.30	2.20	not sig.
<i>Dosing period.</i>					
Positive controls	1.76 g.				
<i>New South Wales, 1927-8.</i>					
0.1 g.	0.84 g.	10	-1.17	2.23	not sig.
0.05 ,,	-0.16 ,,	10	-2.26	2.23	sig.
<i>New South Wales, 1928-9.</i>					
0.1 g.	1.01 g.	11	-1.04	2.20	not sig.

South Wales 1927-8, 0.5 g. of butter, differed significantly from the positive controls. The stored New South Wales 1928-9 butter was only tested in 0.1 g. doses. As will be seen, the group of rats receiving this dose did not differ significantly in its rate of growth from the positive control group. The minimum curative dose probably lay in the neighbourhood of 0.1 g. for both butters and consequently no marked loss can be registered (see Tables 9 and 11).

(b) *Vitamin D.*

The same two butters were also re-tested in February to March 1931 for their antirachitic potency (after storage periods of 1 year and 10 months and 2 years and 11 months respectively), and as will be seen

(c) *Sweet.*

The milk was collected over a period of 4½ days, evening and morning, from 24 March to 29 March. After separation, the cream was pasteurized in bulk at 190° F., cooled, and held overnight in the cold-store before churning. No starter was used in this case.

Churning details:

Quantity of cream	61 lb.
Acidity before churning	0.10%
Churning temperature	55° F.
Time of churning	30 min.
Brine temperature	45° F.
Yield	33½ lb.
Time held at Reading in cold-store	14 days

C. PACKING AND STORAGE.

After churning, each type of butter was packed in two parcels, large and small, and dispatched to London.

Large (28 lb.) were packed in wooden boxes, remade from New Zealand butter-boxes, after being wrapped in two layers of thick parchment paper.

Small (6 lb.) were wrapped in parchment paper only.

The large parcels were placed, soon after arrival in London, in cold-storage at 9–13° F., small samples being removed from storage for testing from time to time. The small parcels were kept at a temperature of 30–35° F. during testing and at 9–13° F. during the intervals between the tests.

In these butters the series of tests carried out is rather complicated owing to the subdivision of the various samples into small and large parcels; for convenience of discussion they are, therefore, summarized in Table 15.

TABLE 15.

*First test. April to July 1928.**Vitamins A and D.*

<i>Description of butter.</i>	<i>Doses.</i>
Normal	0.6, 0.2, 0.1 g.
Acid neutralized	" " "
Sweet	" " "

Source of vitamin B = 3.3% marmite.

*Second test. February to June 1929.**Vitamins A and D.*

<i>Description of butter.</i>	<i>Doses.</i>
Normal (small parcel)	0.6, 0.2, 0.1 g.
Acid neutralized (small parcel)	" " "
Sweet (small parcel)	" " "

Source of vitamin B = 5% yeast.

VITAMIN CONTENT OF BUTTERS

Third test. December 1929 to May 1930.

<i>Description of butter.</i>	<i>Vitamin A only.</i>		
	<i>Doses.</i>		
Normal (large parcel)	.	.	0.6, 0.2, 0.1 g.
Acid neutralized (large parcel)	.	.	" " "
Acid neutralized (small parcel)	.	.	" " "
Sweet (large parcel)	.	.	" " "
Sweet (small parcel)	.	.	" " "

Source of vitamin B = 5% yeast until
20 March 1930, then 10% yeast.

D. RESULTS OF TESTS.

(a) *Vitamin A.*

First test (Table 16). In this test only 3 animals were used in the positive control group; as this number was insufficient for statistical

TABLE 16.

Influence of method of preparation on the vitamin A content of English butters.

	<i>Average daily rate of growth.</i>	<i>N-2.</i>	<i>Value of t.</i>	<i>5 per cent. value of t.</i>	
<i>Preparatory period.</i>					
Positive controls	0.97 g.				
<i>Normal.</i>					
0.6 g.	0.62 g.	12	-3.93	2.18	sig.
0.2 "	0.95 "	12	-0.17	2.18	not sig.
0.1 "	0.92 "	12	-0.42	2.18	not sig.
<i>Acid neutralized.</i>					
0.6 g.	0.85 g.	12	-1.10	2.18	not sig.
0.2 "	0.83 "	12	-1.40	2.18	not sig.
0.1 "	0.90 "	12	-0.71	2.18	not sig.
<i>Sweet.</i>					
0.6 g.	0.85 g.	12	-1.04	2.18	not sig.
0.2 "	0.87 "	11	-0.89	2.20	not sig.
0.1 "	0.85 "	12	-1.13	2.18	not sig.
<i>Dosing period.</i>					
Positive controls	1.65 g.				
<i>Normal.</i>					
0.6 g.	0.75 g.	12	-2.51	2.18	sig.
0.2 "	1.32 "	12	-0.98	2.18	not sig.
0.1 "	0.90 "	12	-2.02	2.18	not sig.
<i>Acid neutralized.</i>					
0.6 g.	0.59 g.	12	-1.64	2.18	not sig.
0.2 "	0.75 "	12	-2.41	2.18	sig.
0.1 "	1.03 "	12	-1.76	2.18	not sig.
<i>Sweet.</i>					
0.6 g.	1.16 g.	12	-1.02	2.18	not sig.
0.2 "	1.16 "	11	-1.27	2.20	not sig.
0.1 "	0.63 "	12	-2.10	2.18	not sig.

purposes, we were obliged to include some positive controls from contemporary tests.

In most of the butter doses the rates of growth, although lower than in the control group, were not significantly different either before or after dosing. The 0.6 g. group on 'Normal' butter both in the preparatory and dosing period and the 0.2 g. group on 'Acid neutralized' in the dosing period showed significant differences from the positive controls. As, however, the lower doses of the same butters gave results which did not significantly differ from the positive control group, this discrepancy can evidently not be attributed to the lower vitamin content of the dose. The minimum dose for each butter was, therefore, probably 0.1 g. or lower.

Second test (Table 17). The source of vitamin B was 5 per cent. yeast in the basal diet. Little loss in vitamin A seems to have taken place on storage (10 months) in this case. Since in both the preparatory and the dosing periods the rate of growth of the butter groups was not

TABLE 17.

Influence of storage on the vitamin A content of English butters.

	Average daily rate of growth.	N-2.	Value of t.	5 per cent. value of t.	
<i>Preparatory period.</i>					
Positive controls	1.00 g.				
<i>Normal.</i>					
0.6 g.	0.99 g.	10	-0.10	2.23	not sig.
0.2 ,,	1.14 ,,	11	+0.93	2.20	not sig.
0.1 ,,	1.12 ,,	11	+0.75	2.20	not sig.
<i>Acid neutralized.</i>					
0.6 g.	1.13 g.	8	+1.10	2.31	not sig.
0.2 ,,	1.23 ,,	9	+1.25	2.26	not sig.
0.1 ,,	1.02 ,,	11	+0.16	2.20	not sig.
<i>Sweet.</i>					
0.6 g.	1.04 g.	10	+0.41	2.23	not sig.
0.2 ,,	1.14 ,,	11	+1.07	2.20	not sig.
0.1 ,,	1.07 ,,	11	+0.51	2.20	not sig.
<i>Dosing period.</i>					
Positive controls	1.44 g.				
<i>Normal.</i>					
0.6 g.	1.81 g.	10	+0.93	2.23	not sig.
0.2 ,,	1.66 ,,	11	+0.55	2.20	not sig.
0.1 ,,	1.45 ,,	11	+0.03	2.20	not sig.
<i>Acid neutralized.</i>					
0.6 g.	1.00 g.	8	-0.67	2.31	not sig.
0.2 ,,	1.54 ,,	9	+0.28	2.26	not sig.
0.1 ,,	1.11 ,,	11	-1.04	2.20	not sig.
<i>Sweet.</i>					
0.6 g.	1.53 g.	10	+0.18	2.23	not sig.
0.2 ,,	1.63 ,,	11	+0.56	2.20	not sig.
0.1 ,,	1.36 ,,	11	-0.29	2.20	not sig.

significantly different from that of the control group, the minimum dose lay at or below the lowest butter dose employed (0.1 g.).

Third test (Table 18). After a storage period of 20 months the test

TABLE 18.

Influence of an additional period of storage on the vitamin A content of English butters.

	Average daily rate of growth.	N-2.	Value of t.	5 per cent. value of t.	
<i>Preparatory period.</i>					
Positive controls	1.07 g.				
<i>Normal (large parcel).</i>					
0.6 g.	0.91 g.	15	-0.31	2.13	not sig.
0.2 „	1.02 „	15	-0.11	2.13	not sig.
0.1 „	0.97 „	15	-0.20	2.13	not sig.
<i>Acid neutralized (large parcel)</i>					
0.6 g.	1.13 g.	15	+0.12	2.13	not sig.
0.2 „	0.94 „	15	-0.24	2.13	not sig.
0.1 „	1.06 „	15	-0.02	2.13	not sig.
<i>Acid neutralized (small parcel).</i>					
0.6 g.	1.03 g.	15	-0.08	2.13	not sig.
0.2 „	1.12 „	15	+0.09	2.13	not sig.
0.1 „	0.96 „	15	-0.21	2.13	not sig.
<i>Sweet (large parcel).</i>					
0.6 g.	0.96 g.	15	-0.21	2.13	not sig.
0.2 „	1.02 „	15	-0.09	2.13	not sig.
0.1 „	1.01 „	14	-0.10	2.15	not sig.
<i>Sweet (small parcel).</i>					
0.6 g.	1.16 g.	15	+0.19	2.13	not sig.
0.2 „	1.18 „	15	+0.21	2.13	not sig.
0.1 „	1.02 „	15	-0.10	2.13	not sig.
<i>Dosing period.</i>					
Positive controls	2.47 g.				
<i>Normal (large parcel).</i>					
0.6 g.	1.70 g.	15	-1.78	2.13	not sig.
0.2 „	0.67 „	15	-2.83	2.13	sig.
0.1 „	1.20 „	15	-3.70	2.13	sig.
<i>Acid neutralized (large parcel).</i>					
0.6 g.	1.53 g.	15	-2.07	2.13	not sig.
0.2 „	1.01 „	15	-2.22	2.13	sig.
0.1 „	1.34 „	15	-3.27	2.13	sig.
<i>Acid neutralized (small parcel).</i>					
0.6 g.	1.95 g.	15	-1.55	2.13	not sig.
0.2 „	0.84 „	15	-2.88	2.13	sig.
0.1 „	0.63 „	15	-3.85	2.13	sig.
<i>Sweet (large parcel).</i>					
0.6 g.	1.90 g.	15	-1.68	2.13	not sig.
0.2 „	1.24 „	15	-2.29	2.13	sig.
0.1 „	1.36 „	14	-2.95	2.15	sig.
<i>Sweet (small parcel).</i>					
0.6 g.	2.09 g.	15	-0.97	2.13	not sig.
0.2 „	1.51 „	15	-2.43	2.13	sig.
0.1 „	1.31 „	15	-3.68	2.13	sig.

was repeated, 5 per cent. and later 10 per cent. of yeast being used in the basal diet as a source of vitamin B instead of the marmite used in the first test. In all cases the rate of growth of the rats during the preparatory period did not differ significantly from that of the positive control group. After dosing, however, in the case of each butter, the rates of growth obtained with the two lower doses (0.2 g. and 0.1 g.) differed significantly from that of the positive control group. Although the rates of growth of the groups receiving 0.6 g. of any of the butters were lower than that of the positive controls, the difference was not significant. The minimum dose probably lay in the neighbourhood of 0.6 g., which indicates a loss in potency. The above experiments show that the size of the parcel does not affect the vitamin potency of the butters on storage.

(b) *Vitamin D.*

The results of the determination of vitamin D in the original butters and in the butters stored in small parcels for about 5 months are given in Tables 19 and 20. The 'degree of protection' shows that full

TABLE 19.

Influence of method of preparation on the vitamin D content of English butters.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.75					
<i>Normal.</i>						
0.6 g.	0.69	81	10	-1.90	2.23	not sig.
0.2 „	0.63	63	10	-2.39	2.23	sig.
0.1 „	0.58	47	9	-4.74	2.26	sig.
<i>Acid neutralized.</i>						
0.6 g.	0.68	78	10	-1.91	2.23	not sig.
0.2 „	0.62	59	10	-4.91	2.23	sig.
0.1 „	0.60	53	10	-5.11	2.23	sig.
<i>Sweet.</i>						
0.6 g.	0.67	75	10	-2.42	2.23	sig.
0.2 „	0.61	56	10	-3.54	2.23	sig.
0.1 „	0.59	50	10	-3.67	2.23	sig.
Negative controls	0.43					

antirachitic protection was nearly obtained with 0.6 g. of butter and that there was apparently no marked difference in the antirachitic potency of the butters prepared under different conditions. The deviation of the 0.6 g. dose in the case of the 'Normal' and 'Acid neutralized' butters from the positive controls was not significant; on the other hand, in the case of the 'Sweet' butter this deviation was significant. The stored butters produced about the same response with the highest dose, but with the lower doses the protection was not as good as that obtained in the case of the butters before storage. This

is possibly due to the imperfection of the method. It is to be noted that there was no significant deviation in the results of the 0.6 g. groups of all the butters after storage from that of the positive control, although a deviation was established in the case of the 'Sweet' butter before storage.

TABLE 20.

Influence of storage on the vitamin D content of English butters.

	Average A/R.	Degree of protection.	N-2.	Value of t.	5 per cent. value of t.	
Positive controls	0.83					
<i>Normal.</i>						
0.6 g.	0.78	83	12	-1.51	2.18	not sig.
0.2 "	0.60	23	12	-4.74	2.18	sig.
0.1 "	0.60	23	12	-5.19	2.18	sig.
<i>Acid neutralized.</i>						
0.6 g.	0.78	83	12	-1.47	2.18	not sig.
0.2 "	0.64	37	11	-4.72	2.20	sig.
0.1 "	0.56	10	12	-9.78	2.18	sig.
<i>Sweet.</i>						
0.6 g.	0.82	97	11	-0.23	2.20	not sig.
0.2 "	0.65	40	12	-6.10	2.18	sig.
0.1 "	0.51	—	12	-10.61	2.18	sig.
Negative controls	0.53					

VII. GENERAL DISCUSSION OF RESULTS

The result of chief public interest which emerges from the investigations just described is the high and uniform vitamin potency of Australian and New Zealand butters. The systematic observations recorded here, together with the published results of isolated tests by others, leave no doubt that the Australian and New Zealand butters when they reach the consumer in this country contain both vitamin A and vitamin D to a value as high as that of butters produced in Great Britain or elsewhere in Europe. It is known that the vitamin content, and especially the vitamin D content, of milk and butter produced in northern latitudes declines in winter, when sunlight is deficient and the herds are stall-fed, and that it rises again in the summer. The Australian and New Zealand butters do not fall short of the best summer butters here, or even of butters produced from cows whose diet has been fortified by an artificial supply of the fat-soluble vitamins A and D. The results now presented, moreover, show that the methods of production and handling, and the delay in transit, have a negligible influence upon the vitamin content of the butters as they reach the consumer.

This good and uniform potency of the Australian and New Zealand butters makes them a particularly valuable source of the vitamins A and D for the British population, and especially during the winter

season when the vitamin potency of home or other European butters may be low.

It has been shown that the racial origin of the dairy cows providing the butter has no significant effect upon the vitamin content, and it has been shown also that the butters from different parts of Australia are closely equivalent in value.

It is satisfactory to find that these vitamins in butter have remarkable stability during cold-storage. Not only is there no appreciable loss of potency during the weeks of transit by sea, but in several cases no notable loss could be detected even after periods up to 2 years, and this stability was found whether the butter was stored in large or small quantities, or again, whether the butter had been prepared from sweet or acid cream. No fear need be entertained, therefore, that the vitamin content of Australian and New Zealand butters is likely to diminish during the period of 2 or 3 months which usually elapses between their first preparation and their final consumption in this country.

VIII. SUMMARY

1. The examination of samples of summer butters prepared under carefully controlled conditions in Australia (1927-8, 1928-9) and in New Zealand (1927-8) showed that all the samples had a vitamin A and D content closely approaching that of the best butters available from any other sources.

2. There was no significant difference between the vitamin potencies of the Australian butters from New South Wales, Queensland, and Victoria respectively.

3. The vitamin D content of samples of butter prepared in New Zealand during the winter of 1929 was definitely lower than that of the corresponding summer samples (1927-8). The results of the vitamin A tests for these butters were not decisive, but they gave indication that there was no significant change due to season.

4. The neutralization of acid creams before churning, such as is practised in Australia and New Zealand, has no destructive effect upon the vitamin A and D content of the resulting butters.

5. There was no significant loss of either vitamin A or D when butters were in cold-storage for a short period (5 to 10 months) whether in small or large bulk or whether prepared from sweet or from sour cream. Australian and New Zealand butters, even when stored in the cold for much longer periods (1 to 3 years), retained most of their original potency. Definite losses in vitamin A were detected only in some samples of English origin stored for nearly 2 years.

6. No notable differences were found between the vitamin A and D potency of New Zealand butters from a Jersey herd and that of the butters from mixed herds in New Zealand and Australia. The breed of the dairy herd seems to have no significant influence upon the vitamin content of the butters.

In conclusion we wish to acknowledge our best thanks to:

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APPENDIX

REPORT FROM THE AGROSTOLOGIST TO THE DIRECTOR OF THE FIELDS DIVISION, DEPARTMENT OF AGRICULTURE, WELLINGTON, NEW ZEALAND, MADE ON 31 JANUARY 1928 IN CONNEXION WITH VITAMIN EXPERIMENTS CARRIED OUT ON THE NEW ZEALAND SUMMER BUTTERS 1927-8.

The following is my report on the botanical composition of the pastures from which the trial butter was made:

The cream was taken from 57 farms and on an average 3 to 4 paddocks on each farm were open to the dairy herd about the period when the cream was taken. Thus some 200 paddocks are involved.

To botanically analyse all these pastures is obviously out of the question and hence farms were selected from various soil types, of varying farm management, particularly in regard to top dressing, and of varying altitudes. By eye examination of the respective fields on each farm an average pasture was selected and this sward analysed by the point-quadrat method. Details of these analyses are given in table herewith.

From the table it will be seen that most heterogeneous types of pasture furnished the food for the cream produced. There are approximately 7 types:

Group 1. Perennial rye-grass and white clover dominant. Virtually no second-rate grasses present. Inferior grasses and weeds subdominant.

Fields, Nos. 1, 2, 3, 5, and 6.

Group 2. Perennial rye-grass and white clover dominant; second-rate grasses, inferior grasses, and weeds subdominant.

Fields Nos. 4, 7, 8, and 9.

Group 3. Second-rate grasses, inferior grasses, and weeds dominant; perennial rye-grass, and white clover subdominant.

Fields Nos. 10, 11, 12, 13, 14, 15, 16, 17, and 18.

Group 4. White clover and Yorkshire Fog dominant. First-class grasses and weeds subdominant.

Field No. 19.

Group 5. White clover, Yorkshire Fog, and Sweet Vernal dominant; weeds subdominant; first-class grasses in small amounts.

Field No. 20.

Group 6. Cocksfoot dominant; second-rate grasses and weeds subdominant.

Field No. 21.

Group 7. Paspalum and first-class grasses dominant; weeds and inferior grasses subdominant.

Field No. 22.

No uniformity exists in regard to top dressing, but includes:

(a) various kinds of manures, chiefly superphosphate, basic slag, basic super, lime and super and potash.

(b) heavy dressings, moderate dressings, scanty dressings, annual dressings, twice-yearly dressings, spasmodic dressings.

The farms vary in altitude from sea-level to 1,260 feet, which means seasonal variation in regard to pasture growth and in amount of rain and sunshine.

It would appear before correlation between vitamin content and botanical type were possible a very different method of selection of cream will have to be adopted.

If vitamin content of butter is influenced by feed consumed then I must most certainly say that I consider that many of the pastures from which the cream was produced were below the standard of good, dairying grass-land in New Zealand, and this fact should be taken into consideration.

The pastures were analysed between the 13th and 20th of January. Thus some 3 to 4 weeks of dry weather intervened between the time of making of the butter and the analysing of the pasture sward. Thus the figure for bare ground given in the table is probably higher than in December and the amount of suckling clover would be greater than at the time when the analyses were done. The analyses,

however, as they stand are strictly comparable and show how large a number of pasture type variants were present over the area from which the cream was secured.

1929 REPORT ON PASTURES PREVIOUSLY REPORTED ON IN CONNECTION WITH VITAMIN CONTENT OF BUTTER MADE IN THE LEPPERTON AND MANGOREI DISTRICTS.

Weather. The weather prior to June 1929 was very favourable to pasture growth, the autumn being mild with occasional showers, and pasture growth was probably above normal. Early in June excessive rain was experienced and the fall for June was about a quarter of the annual average rainfall. Very few frosts were experienced and wind was the greatest factor against pasture growth.

Generally speaking, clovers were not so prominent as they were the previous season and in some districts the clover growth was decidedly poor. Cocksfoot and in some parts paspalum made great growth during the late summer and early autumn and later on in the autumn; the ryes again made splendid growth but, as before mentioned, white-clover growth was not up to the usual standard.

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No. 86. The Estimation of Foetal Age, the Weight and Length of Normal Foetuses, and the Weights of Foetal Organs. By J. N. Cruickshank, M. J. Miller, and F. J. Browne. [1924.] 2s. 6d. (2s. 7½d.).

No. 101. Poverty, Nutrition, and Growth: Studies of Child Life in Cities and Rural Districts of Scotland. By D. Noël Paton, Leonard Findlay, and others. [1926.] 10s. (10s. 4½d.).

No. 109. A Clinical and Pathological Study of 1,678 Cases of Dead-Births and Neo-natal Deaths. Compiled by E. L. Holland and J. E. Lane-Claypon. [1926.] 3s. 6d. (3s. 7½d.).

No. 114. Social Conditions and Acute Rheumatism. [1927.] 2s. 6d. (2s. 8d.).

No. 117. The Toxaemias of Pregnancy: A Clinical and Biochemical Study. By J. N. Cruickshank, J. Hewitt, and K. L. Couper. [1927.] 4s. (4s. 2d.).

No. 118. The Cause of Foetal Death in 144 Cases. By A. C. Palmer. [1928.] 3s. (3s. 2d.).

No. 145. The Causes of Neo-natal Death. By J. N. Cruickshank. [1930.] 1s. 6d. (1s. 7½d.).

No. 157. Nutritional Anaemia in Infancy: The Influence of Iron Deficiency on Infant Health. By H. M. M. Mackay, L. Goodfellow, and A. Bradford Hill. [1931.] 2s. (2s. 2d.).

No. 162. Intelligence and Disease. By Shepherd Dawson assisted by J. C. M. Conn. [1931.] 1s. (1s. 1½d.).

No. 171. Facial Growth in Children, with Special Reference to Dentition. Part I by Corisande Smyth. Part II by Matthew Young. [1932.] 1s. 6d. (1s. 8d.).

See also NUTRITION; RICKETS.

Dental Disease :

No. 70. The Structure of Teeth in relation to Dental Disease. By J. Howard Mummery. [1922.] 2s. (2s. 1d.).

Special Reports—continued.

No. 97. The Incidence of Dental Disease in Children. By the Committee for the Investigation of Dental Disease. [1925.] 1s. 6d. (1s. 7½d.).

No. 140. Diet and the Teeth: An Experimental Study. Part I. Dental Structure in Dogs. By May Mellanby. [1929.] 17s. 6d. (18s.).

No. 153. Diet and the Teeth: An Experimental Study. Part II. A. Diet and Dental Disease. B. Diet and Dental Structure in Mammals other than the Dog. By May Mellanby. [1930.] 2s. 6d. (2s. 8½d.).

No. 159. The Influence of Diet on Caries in Children's Teeth (Interim Report). By the Dental Committee. [1931.] 6d. (7d.).

See also CHILD LIFE (No. 171).

Diphtheria :

No. 115. The Prevention of Diphtheria. By J. Graham Forbes. [1927.] 2s. (2s. 1½d.). (Book). Diphtheria: its Bacteriology, Pathology, and Immunology. By the Bacteriological Committee. [1928.] 12s. 6d. (13s. 3d.).

See also EPIDEMIOLOGY (No. 75), STATISTICS (No. 187).

Dysentery :

Reports upon Investigations in the United Kingdom of Dysentery Cases received from the Eastern Mediterranean:—

No. 4. I. Amoebic Dysentery and the Protozoological Investigation of Cases and Carriers. By Clifford Dobell. [1917.] *Out of print.*

No. 5. II. Report upon 878 Cases of Bacillary Enteritis. By L. Rajchman and G. T. Western. [1917.] *Out of print.*

No. 6. III. Report upon recovered Cases of Intestinal Disease in the Royal Naval Hospital, Haslar, 1915-16. By Paul Fildes and others. IV. Report upon combined Clinical and Bacteriological Studies of Dysentery Cases from the Mediterranean. By S. R. Douglas and L. Colebrook. [1917.] 4s. 6d. (4s. 7½d.).

No. 7. V. Report upon 2,360 Enteritis 'Convalescents' received at Liverpool from various Expeditionary Forces. By E. Glynn and others. [1918.] 2s. (2s. 2d.).

No. 15. A Study of 1,300 Convalescent Cases of Dysentery from Home Hospitals: with special reference to the Incidence and Treatment of Amoebic Dysentery Carriers. By Clifford Dobell, H. S. Gettings, Margaret W. Jepps, and J. B. Stephens. [1918.] 1s. 3d. (1s. 4d.).

No. 29. A Contribution to the Study of Chronicity in Dysentery Carriers. By W. Fletcher and Doris L. Mackinnon. [1919.] 9d. (10d.).

No. 30. An Investigation of the Flexner-Y Group of Dysentery Bacilli. By H. S. Gettings. [1919.] 1s. (1s. 1d.).

No. 40. Studies of Bacillary Dysentery occurring in the British Forces in Macedonia. By L. S. Dudgeon and others. [1919.] 3s. (3s. 1½d.).

No. 42. A Study of the Serological Races of the Flexner Group of Dysentery Bacilli. By F. W. Andrewes and A. C. Inman. [1919.] 2s. (2s. 1½d.).

See also FOOD POISONING.

Encephalitis :

No. 108. The Sheffield Outbreak of Epidemic Encephalitis in 1924. [1926.] 1s. 9d. (1s. 10½d.).

Enteric Infections :

No. 9. A Report upon the Use of Atropine as a Diagnostic Agent in Typhoid Infections. By H. F. Marris. [1917.] 1s. (1s. 1d.).

No. 48. A Report on the probable Proportion of Enteric Infections among Undiagnosed Febrile Cases invalided from the Western Front since October 1916. By W. W. C. Topley, S. G. Platts, and C. G. Imrie. [1920.] 3s. (3s. 1½d.).

See also BACTERIOLOGY ; FOOD POISONING.

Epidemiology :

No. 75. The Schick Test, Diphtheria and Scarlet Fever. By S. F. Dudley. [1923.] 1s. (1s. 1½d.).

Special Reports—*continued.*

No. 111. The Spread of Droplet Infection in Semi-isolated Communities. By S. F. Dudley. [1926.] 1s. 6d. (1s. 7½d.).

No. 120. An Inquiry into the Relationship between Housing Conditions and the Incidence and Fatality of Measles. By J. L. Halliday. [1928.] 1s. (1s. 1d.).

See also SMALL-POX ; STATISTICS ; TUBERCULOSIS ; etc.

Flying, Medical Problems of :

Reports of the Air Medical Investigation Committee :—

No. 28. The Sense of Balance and Stability in the Air. By Henry Head. [1919.] 9d. (10d.). (Included in No. 53.)

No. 37. The Effects of Diminished Tension of Oxygen, with especial reference to the Activity of the Adrenal Glands. By C. H. Kellaway. The Ear in relation to certain Disabilities in Flying. By S. Scott. [1919.] 1s. (1s. 1d.).

No. 53. The Medical Problems of Flying (including reports on oxygen want, selection of candidates for flying, sense of balance, and flying strain). [1920.] 6s. (6s. 4d.).

No. 84. The Application of the Air Force Physical Efficiency Tests to Men and Women. By L. D. Cripps. [1924.] 1s. 6d. (1s. 7½d.).

Food Poisoning :

No. 24. A Report on the Investigation of an Epidemic caused by *Bacillus aertrycke*. By H. Marrian Perry and H. L. Tidy. [1919.] 9d. (10d.).

No. 91. An Investigation of the Salmonella Group, with Special Reference to Food Poisoning. By W. G. Savage and P. Bruce White. [1925.] 3s. 6d. (3s. 8d.).

No. 92. Food Poisoning : a Study of 100 Recent Outbreaks. By W. G. Savage and P. Bruce White. [1925.] 2s. 6d. (2s. 8d.).

No. 103. Further Studies of the Salmonella Group. By P. Bruce White. [1926.] 5s. (5s. 2½d.).

Goitre :

No. 154. Iodine Supply and the Incidence of Endemic Goitre. By J. B. Orr. [1931.] 4d. (5d.). See also NUTRITION (No. 123).

Haemoglobin: see BLOOD.

Hearing:

No. 166. Reports of the Committee on the Physiology of Hearing. I. The Localization of Sounds in the Median Plane. By J. H. Shaxby and F. H. Gage. II. Some Factors in Auditory Localization. By H. E. O. James and Marion E. Massey. [1932.] 1s. (1s. 1½d.).

Heart:

No. 8. Report upon Soldiers returned as Cases of 'Disordered Action of the Heart' (D.A.H.), or Valvular Disease of the Heart. By Sir Thomas Lewis. [1917.] 1s. (1s. 1d.).

No. 147. The Electrocardiogram. By W. H. Craib. [1930.] 1s. 8d. (1s. 4½d.).

Industrial Health :

The Annual Reports of the Industrial Health (formerly Fatigue) Research Board, and special reports on particular subjects, are published for the Council in separate series.

- The subjects dealt with include accident causation, rest pauses, spells of work, movement study, vocational selection, and problems of particular industries. A list can be supplied on application to the Secretary of the Board, 38 Old Queen Street, Westminster, S.W. 1.

Influenza:

No. 36. Studies of Influenza in Hospitals of the British Armies in France, 1918. [1919.] 3s. 6d. (3s. 8d.).

No. 63. Studies in the Aetiology of Epidemic Influenza. By J. McIntosh. [1922.] 2s. 6d. (2s. 7d.).

Jaundice:

No. 113. Spirochaetal Jaundice. By G. Buchanan. [1927.] 4s. (4s. 2d.).

Light Treatment :

No. 131. Irradiation and Health. Two Experimental Studies. By Dora Colebrook. [1929.] 1s. 6d. (1s. 7d.).

Malaria : see QUININE.

Special Reports—*continued.*

Maternal Mortality: *see* CHILD LIFE and STREPTOCOCCAL INFECTIONS.

Measles: *see* EPIDEMIOLOGY (No. 120).

Miners' Diets: *see* NUTRITION.

Miners' Diseases, etc.:

No. 89. Report on Miners' 'Beat Knee', 'Beat Hand', and 'Beat Elbow'. By E. L. Collis and T. L. Llewellyn. [1924.] 1s. 6d. (1s. 7d.).

See also JAUNDICE (No. 118).

Miners' Nystagmus: *see* VISION.

Nephritis:

No. 48. Albuminuria and War Nephritis among British Troops in France. By H. MacLean. [1919.] 2s. 6d. (2s. 8d.).

No. 142. A Classification of Bright's Disease. By Dorothy S. Russell. [1929.] 8s. 6d. (8s. 10d.).

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Reports of the Committee upon Injuries to the Nervous System:—

No. 54. The Diagnosis and Treatment of Peripheral Nerve Injuries. [1920.] 2s. (2s. 1½d.).

No. 88. Injuries of the Spinal Cord and Cauda Equina. [1924.] 1s. 6d. (1s. 7½d.).

Nutrition:

No. 13. An Enquiry into the Composition of Dietsaries, with special reference to the Dietsaries of Munition Workers. By Viscount Dunluce and Major Greenwood.

Out of print.

No. 87. Report on the Nutrition of Miners and their Families. By the Committee upon Quantitative Problems in Human Nutrition. [1924.] 1s. 3d. (1s. 4d.).

No. 105. Diets for Boys during the School Age. By H. C. Corry Mann. [1926.] 2s. 6d. (2s. 7½d.).

No. 128. Iodine in Nutrition: a Review of Existing Information. By J. B. Orr and I. Leitch. [1929.] 2s. 6d. (2s. 8d.). *See also* GOITRE (No. 154).

No. 135. The Carbohydrate Content of Foods. By R. A. McCance and R. D. Lawrence. [1929.] 2s. (2s. 1½d.).

No. 136. The Antiscorvy Vitamin in Apples. By Mary F. Bracewell, E. Hoyle, and G. W. H. Whittall. [1930.] 9d. (10d.).

No. 137. A Study in Nutrition. An Inquiry into the Diet of 154 Families of St. Andrews. By G. W. H. Whittall and A. M. T. Murray, assisted by M. Shanks. [1931.] 1s. (1s. 1½d.).

No. 138. Nutrition: The Physique and Health of Two African Tribes. By J. B. Whittall and M. Shanks. [1931.] 2s. (2s. 2d.).

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No. 140. Nutrition. An Inquiry into the Diet of Families in Cardiff and Newport. By G. W. H. Whittall and A. M. T. Murray, assisted by M. Shanks. [1932.] 6d. (7d.).

No. 141. Nutrition: Present Knowledge. By a Committee appointed jointly by the British Medical Association, the Royal Society, and the Council. [1932.] 6s. 6d. (7s. ½d.).

No. 142. Nutrition: Butter. The Composition of Irish, Danish, and English Butters. By G. W. H. Whittall and M. Shanks. [1932.] 6s. 6d. (7s. ½d.).

Special Reports—continued.

Quinine :

No. 96. Clinical Comparisons of Quinine and Quinidine. By the Committee upon Cinchona Derivatives and Malaria. [1925.] 1s. (1s. 1d.).

Radium :

No. 62. Medical Uses of Radium : Studies of the Effects of Gamma Rays from a large Quantity of Radium. By various authors. [1922.] 5s. (5s. 3d.).

No. 90. Medical Uses of Radium : Summary of Reports from Research Centres for 1923. [1924.] 1s. (1s. 1d.).

No. 102. Ditto for 1924. [1926.] 1s. 6d. (1s. 7d.).

No. 112. Ditto for 1925. [1926.] 1s. 3d. (1s. 4d.).

No. 116. Ditto for 1926. [1927.] 1s. (1s. 1½d.).

No. 126. Ditto for 1927. [1928.] 1s. (1s. 1½d.).

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Rheumatism : *see* CHILD LIFE (No. 114).

Rickets :

No. 20. A Study of Social and Economic Factors in the Causation of Rickets, with an Introductory Historical Survey. By L. Findlay and Margaret Ferguson. [1918.] *Out of print.*

No. 61. Experimental Rickets. By E. Mellanby. [1921.] 4s. (4s. 2d.).

No. 68. Rickets : the Relative Importance of Environment and Diet as Factors in Causation. By H. Corry Mann. [1922.] 2s. 6d. (2s. 7½d.).

No. 71. The Aetiology and Pathology of Rickets from an experimental point of view. By V. Korenchevsky. [1922.] 4s. (4s. 3d.).

No. 77. Studies of Rickets in Vienna, 1919-22. [1923.] 7s. 6d. (7s. 10½d.).

No. 93. Experimental Rickets : The Effect of Cereals and their Interaction with factors of Diet and Environment in producing Rickets. By E. Mellanby. 3s. 6d. (3s. 8d.).

Salvarsan : *see* VENEREAL DISEASES ; STREPTOCOCCAL INFECTIONS ; STAN-
LOGICAL (No. 128).

Scarlet Fever : *see* STATISTICS (No. 137).

Scurvy : *see* NUTRITION (No. 146).

Shock, Surgical :

Reports of the Committee on Surgical Shock and Allied Condi-

No. 25. Wound-Shock and Haemorrhage. [1919.] 4s. (4s. 6d.).

No. 26. Traumatic Toxaemia as a Factor in Shock.

No. 27. Blood Volume Changes in Shock.

N. M. Keith. [1919.] 9d. (10d.).

Small-pox :

Special Reports—continued.

Statistics (MISCELLANEOUS).

No. 16. A Report on the Causes of Wastage of Labour in Muniton Factories. By Major Greenwood. [1918.] 1s. 6d. (1s. 7d.).

No. 60. The Use of Death-rates as a Measure of Hygienic Conditions. By John Brownlee. [1922.] 3s. (3s. 1½d.).

No. 65. Internal Migration and its Effects upon the Death-rates: with Special Reference to the County of Essex. By A. B. Hill. [1925.] 3s. 6d. (3s. 8d.).

Scarlet Fever, Diphtheria, and Enteric Fever, 1895-1914: A Clinical-Statistical Study. By E. W. Goodall, M. Greenwood, and W. T. Russell. [1929.] 2s. (2s. 1½d.).

Bacterial Infections:

Study of some Organic Arsenical Compounds with a view to their Use in the Treatment of Bacterial Infections. By L. Colebrook. [1928.] 1s. 3d. (1s. 4d.).

Study of Intracranial Surgery. By H. Cairns. [1929.] 3s. (3s. 2½d.).

Preparation of Catgut for Surgical Use. By W. Bulloch, L. H. Lampitt, and J. H. Sushill. [1929.] 4s. (4s. 3d.).

The Intervertebral Discs. Observations on their Normal and Morbid Anatomy and their Relation to certain Spinal Deformities. By O. A. Beadle. [1931.] 2s. (2s. 2d.).

BURNS; SHOCK, SURGICAL.

T. Poisoning:

No. 11. The Causation and Prevention of Tri-nitro-toluene (T.N.T.) Poisoning. By Benjamin Moore. [1917.] 1s. (1s. 1½d.).

No. 58. T.N.T. Poisoning and the Fate of T.N.T. in the Animal Body. By W. J. O'Donovan and others. [1921.] 3s. (3s. 1½d.).

Tuberculosis:

No. 1. First Report of the Special Investigation Committee upon the Incidence of Phthisis in relation to Occupations.—The Boot and Shoe Trade. [1915.] 3d. (3½d.).

No. 18. An Investigation into the Epidemiology of Phthisis Pulmonalis in Great Britain and Ireland. Parts I and II. By John Brownlee. [1918.] 1s. 3d. (1s. 4½d.).

No. 22. An Inquiry into the Prevalence and Aetiology of Tuberculosis among Industrial Workers, with special reference to Female Muniton Workers. By Major Greenwood and A. E. Tebb. [1919.] 1s. 6d. (1s. 7d.).

No. 33. Pulmonary Tuberculosis: Mortality after Sanatorium Treatment. By Noel D. Bardswell and J. H. R. Thompson. [1919.] 2s. (2s. 2d.).

No. 46. An Investigation into the Epidemiology of Phthisis in Great Britain and Ireland. Part III. By John Brownlee. [1920.] 2s. 6d. (2s. 7½d.).

No. 67. Report on Artificial Pneumothorax. By L. S. T. Burrell and A. S. MacNalty. [1922.] 2s. 6d. (2s. 8d.).

No. 76. Tuberculosis in Insured Persons accepted for Treatment by the City of Bradford Health Committee. By H. Vallow. [1923.] 6d. (7d.).

No. 83. Tuberculosis of the Larynx. By Sir St. Clair Thomson. [1924.] 2s. 6d. (2s. 8d.).

No. 85. An Inquiry into the After-Histories of Patients treated at the Brompton Hospital Sanatorium at Frimley, during the years 1905-14. By Sir P. H.-S. Hartley, R. C. Wingfield, and J. H. R. Thompson. [1924.] 1s. 6d. (1s. 7d.).

No. 94. Tuberculin Tests in Cattle, with special reference to the Intradermal Test. By the Tuberculin Committee. [1925.] 3s. (3s. 3d.).

No. 122. The Intradermal Tuberculin Test in Cattle: Collected Results of Experience. By J. B. Buxton and A. S. MacNalty. [1928.] 1s. 6d. (1s. 7½d.).

No. 149. Tuberculosis in Man and Lower Animals. By H. H. Scott. [1930.] 4s. (4s. 4d.).

No. 152. Studies of Protection against Tuberculosis: Results with B. C. G. Vaccine in Monkeys. By A. Stanley Griffith. [1931.] 9d. (10½d.).

No. 164. The Value of Tuberculin Tests in Man with special reference to the Intracutaneous Test. By P. D'Arcy Hart. [1932.] 2s. (2s. 2d.).

No. 172. Tuberculous Disease in Children: Its Pathology and Bacteriology. By J. W. S. Blacklock. [1932.] 3s. (3s. 3d.).

Venereal Diseases:

No. 14. The Wassermann Test. By the Committee upon Pathological Methods. 1918. Reprinted. [1921.] 1s. (1s. 1d.).

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- No. 19. The Laboratory Diagnosis of Gonococcal Infections. Methods for the Detection of *Spironema pallidum*. By the Bacteriological Committee. *New Edition*. [1927.] 1s. 6d. (1s. 7½d.).
- No. 21. The Diagnostic Value of the Wassermann Test. By the Committee on Pathological Methods. [1918.] *Out of print.*
- No. 23. An Analysis of the Results of Wassermann Reactions in 1,435 Cases of or Suspected Syphilis. By Paul Fildes and R. J. G. Parnell. [1919.] 2s.
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- No. 44. Reports of the Special Committee upon the Manufacture, Biochemical and Clinical Administration of Salvarsan and of its Substitutes. I. [1920.] 1s. (1s. 1d.).
- No. 45. Unsuspected Involvement of the Central Nervous System in Syphilis. By Paul Fildes, R. J. G. Parnell, and H. B. Maitland. [1920.] 1s. (1s. 1d.).
- No. 47. The Accuracy of Wassermann Tests, applied before and after death. I. Necropsies. I. The Wassermann Test applied before death. By Paul Fildes and R. J. G. Parnell. [1920.] 2s. 6d. (2s. 7½d.).
- No. 55. (I.) Results of the Examination of Tissues from Eight Cases of Deep Injections of Salvarsan. By H. M. Turnbull. (II.) The Influence of Treatment on the Development and Persistence of Immunity, as indicated by the Presence of Agglutinins. By E. W. Ainley Walker. [1920.] 3s. (3s. 1½d.).
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- No. 129. The Wassermann Test. Technical Details of No. 1 Method M.R.C. (Modified). By E. J. Wyler. [1929.] 9d. (10d.).
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- No. 32. The Science of Ventilation and Open-air Treatment. Part I. By Leonard Hill. [1919.] 10s. (10s. 5½d.).
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- No. 65. First Report of the Miners' Nystagmus Committee. [1922.] *Out of print.*
- No. 80. Second Report of the Miners' Nystagmus Committee. [1923.] 9d. (10d.).
- No. 110. The Legibility of Print. By R. L. Pyke. [1926.] 4s. (4s. 2d.).
- Reports of the Committee on the Physiology of Vision :
- No. 104. I. Illumination and Visual Capacities. By R. J. Lythgoe. [1926.] 2s. 6d. (2s. 7½d.).
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Special Reports—continued.

186. VI. **Some Experiments on Peripheral Vision.** By Myer Salaman. [1922.] 6d. (2s. 7d.).
187. VII. **A Redetermination of the Trichromatic Mixture Data.** By W. D. [1929.] 1s. 3d. (1s. 4d.).
188. VIII. **The Movements of the Eyes in Reading.** By M. D. Vernon. [1930.] 9d. (10d.).
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178. X. **The Measurement of Visual Acuity.** By R. J. Lythgoe.

amins : see NUTRITION.

Wassermann Test : see VENEREAL DISEASES.

Wound Infections :

- No. 12. **The Classification and Study of the Anaerobic Bacteria of War Wounds.** By J. McIntosh. [1917.] *Out of print.*
- No. 39. **Report on the Anaerobic Infections of Wounds and the Bacteriological and Serological Problems arising therefrom.** By the Committee upon Anaerobic Bacteria and Infections. [1919.] 6s. (6s. 3½d.).
- No. 57. **Studies in Wound Infections.** By S. R. Douglas, A. Fleming, and L. Colebrook. [1920.] 4s. 6d. (4s. 8½d.).

The following books were published under the direction of the Medical Research Committee and are obtainable from the publishers named :

- Milk and its Hygienic Relations.** By Janet E. Lane-Claypon. 9s. net. [Longmans Green & Co.]
- The Amoebae living in Man.** By Clifford Dobell. 7s. 6d. net. [Bale, Sons & Danielsson, Ltd.]
- The Intestinal Protozoa of Man.** By Clifford Dobell and F. W. O'Connor. 15s. net. [Bale, Sons & Danielsson, Ltd.]

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NEW ZEALAND : Messrs. Whitcombe & Tombs, Ltd., Auckland, Christchurch, Dunedin, and Wellington.

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GOLD COAST : The Wesleyan Methodist Book Depot, P.O. Box 100, Cape Coast. Branches at Accra, Kumasi, and Sekondi.

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