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STUDIES ON FATS, OILS AND VANASPATIS

By

N. D. Kehar and Associates

Division of Animal Nutrition

Indian Veterinary Research Institute, Izatnagar



INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI

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PREFACE

Fat is an essential constituent of human dietary. An average Indian prefers *ghee* (clarified heated butter) to oil or any other type of fat. But during the second World War, most of the articles of human consumption were in short supply and so was *ghee*. The shortage of *ghee* and its high price had made it out of the reach of the bulk of the populace. Hydrogenated vegetable oils, commonly known as *vanaspati*, became popular in the early forties, as they resembled *ghee* and were cheaper.

In view of the divergent opinion of scientific workers on the nutritive value of different fats and oils and as hydrogenated vegetable oil was often sold in the market as 'vegetable *ghee*' it became imperative to study in detail, the nutritive value of 'vegetable *ghee*' in relation to the *ghee* prepared from butter.

The Indian Council of Agricultural Research appreciated the significance of this important problem both from the point of view of animal husbandry and public health and financed a broad based and extensive six-year scheme beginning from April 1944.

These investigations deal with the nutritive value of different oils and fats in relation to growth, reproduction and lactation, and their effect upon the absorption and utilization of vitamins (A and B), calcium, phosphorus and protein. They also provide information on the chemical constants of both heated and unheated *ghee* and raw, refined and hydrogenated oils.

The exploratory experiment of Part I of this project was initiated by my predecessor, Dr K. C. Sen in April, 1944. But, soon after, he was appointed Director, Indian Dairy Research Institute, Bangalore and left this Institute. I took over charge of this investigation in August 1944 and had been associated with it till it was completed in April, 1950. The staff engaged in this scheme had been undergoing frequent changes, and during the course of six years, four Research Officers and six Research Assistants had changed hands. Their names appear under the chapters with which their work is connected.

Though portions of the work, comprised in this project extending over a period of six years, could normally have been published earlier, it was considered desirable from the administrative point of view that investigations on all aspects of this important problem should be completed and considered *in toto* before releasing for publication.

I take this opportunity of expressing my gratitude and also of my associates, to the Indian Council of Agricultural Research for financing this investigation. I am especially indebted to Sir Datar Singh, Vice President, Indian Council of Agricultural Research and Shri P. N. Nanda, Animal Husbandry Commissioner with the Government of India for their keen interest in the development of this project.

To Dr S. Datta and Dr F. C. Minett, the present and former Directors, Indian Veterinary Research Institute, Izatnagar, I tender most sincere thanks for the sustained interest evinced by them in this work and for the kind support and encouragement extended during the period of this investigation.

I am also grateful to the Hindustan Vanaspati Manufacturing Company Ltd., Bombay and the Modi Vanaspati Manufacturing Company Ltd., Modinagar for supplying part of our requirements of hydrogenated fats, free of cost.

In the end, I express my grateful thanks to the Statistical Adviser, Indian Council of Agricultural Research, for analysing and interpreting these results and also to Mr. M. R. Mahajan, Assistant Animal Husbandry Commissioner, for his continued interest in the execution of this investigation and for constructive and helpful criticism in the presentation of these results.

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CHAPTER I

INTRODUCTION

FATS are important in the dietary for more than one consideration. They are (1) the most economical sources of energy, (2) carriers of important fat-soluble vitamins and (3) suppliers of the essential fatty acids. Fats ingested by humans can generally be classified into two types, viz. of animal origin like butter, *ghee* (clarified butter fat) and lard, etc. and of vegetable origin like olive, groundnut, coconut, *til* and other oils. While butter-substitutes in the form of margarines are more current in Western countries, *vanaspatis* (hydrogenated vegetable oils) are generally common in India.

Very little is known, however, about the vitamin A content and other physical and chemical characteristics of *ghees* as well as oils and *vanaspatis* obtained from different parts and sources in India. Moreover, there is a lot of controversy regarding the comparative nutritive value of animal and vegetable fats, particularly of butter-fat as compared to vegetable oils, both in natural as well as in hydrogenated form. The literature on the subject has been reviewed by several workers, more especially by Deuel, *et al.* [1944], Cowgill [1945] and Smith [1948]. A large number of papers on this subject have been published after our work had been completed but for the sake of giving an overall picture of the whole subject, such references have also been included in this introduction. The salient observations of different workers in this field can be classified under the following three broad groups:

(1) The Dutch workers Boer, Jansen, *et al.* [1941, 1942, 1947], claim that summer butter contains a specific growth promoting factor and as such, is superior to other fats. By fractionating the fatty acids of butter and carrying out feeding experiments with them the growth promoting factor was believed to be vaccenic acid, and since addition of this acid was found to increase the growth promoting value of rape seed oil, it was supposed that vaccenic acid was the growth promoting factor. But later work led them to the conclusion that the growth promoting factor was not vaccenic acid but some impurity associated with it. Attempts to absorb it on Fuller's earth were not successful [Boer, *et al.*, 1947]. Kentie [1947], however, stated that it could be absorbed but not separated from Fuller's earth. That the pure vaccenic acid has no growth promoting value has been conclusively proved by the exhaustive experiments of Deuel, *et al.* [1948] and Nath, *et al.* [1948].

(2) The second group of investigators, working at Wisconsin, Schantz, Boutwell, Elvehjem, Hart, *et al.* [1940, 1943], carried out a series of experiments and concluded that when lactose was the sole carbohydrate in the diet, butter-fat was superior to most of the vegetable-fats tested. They found that the factor responsible for the superior growth promoting value of butter was in the saturated fraction of the fatty acids. It was also found that the effect was best seen when it was fed during the first few weeks of growth of the rat. But it disappeared later or

when lactose was replaced by other carbohydrates. It would be seen that the views of these two groups of workers are not in agreement.

(3) As opposed to both these groups, Deuel and co-workers [1944, 1945, 1946], at Los Angeles, carried out extensive experiments to compare butter-fat with different vegetable and animal fats and margarines but could not find any difference between them. Support for this view, that butter-fat is no better than many other fats, has also been forthcoming from different parts of the U. S. A. [Zialcita and Mitchell, 1944], England [Henry, *et al.* 1945] and Sweden [Euler and associates, 1947 and 1948].

In India, however, Datta [1945] and Basu and Nath [1946] found *ghee* to be better than *vanaspatis* and vegetable oils in growth promoting value. Mason, *et al.* [1945], however, found the addition of butter-fat to a poor rice diet similar to that eaten in South India by the poor people, to result in adverse growth. This has not been confirmed by work in this laboratory nor by other investigators.

In nearly all the experiments referred to in the above discussion, vitamins, minerals, proteins and other nutritive factors were supplied in adequate amounts to ensure that it was the nutritive value of the glycerides themselves which had been considered. Further, in most of the work, the experiments had been of short duration, limited to the first few weeks of active growth of the rat. However, Deuel and associates [1945] carried on the work over ten generations of rats and still found that there was no difference between butter-fat and margarine made from vegetable-fats. This experiment was also on a diet, adequate, if not superadequate, with regard to all the nutritive factors like vitamins, minerals, proteins, etc. The Council on Foods and Nutrition [1942], after a comparative study of the nutritive value of butter and oleo-margarine, concluded that at present there is no evidence that the use of fortified oleo-margarine in an average adult diet would lead to nutritional difficulties. A similar statement is probably justified in the case of growing children, but preliminary results from animal experiments indicate that more work is necessary before any specific conclusions can be drawn. They, therefore, recommended that the practice of clearly identifying butter and oleo-margarine should continue. However, Maynard [1946] subsequently stated that "according to the consensus of present scientific information, there is no nutritional disadvantage in substituting vegetable-fat for butter in the human mixed-diet if the vegetable fat is appropriately fortified with vitamin A". From consideration of all the evidences available, Smith [1948] has also come to the conclusion that, when the diet is nutritionally adequate, butter-fat is not superior to vegetable-fats and that, if in fact, any specific growth promoting factor is present in summer butter, it is of little or no importance. It will thus be seen that there is considerable difference of opinion on this important subject.

During the Second World War considerable scarcity was experienced in this country for pure *ghee*, and its price soared up so high as to make it out of the reach of the bulk of the populace. Hydrogenated vegetable-oils, commonly known as *vanaspatis*, became popular since they resembled *ghee*.

Since there was difference of opinion regarding comparative nutritive value of different fats and oils, and since the *vanaspatis* were often sold as 'vegetable *ghee*', it was considered imperative to study in detail the nutritive value of 'vegetable

ghee in relation to what we are accustomed to understand by *ghee* prepared from clarified heated butter.

Appreciating the immense importance of this problem, the Indian Council of Agricultural Research sponsored a scheme for carrying out detailed investigations into the nutritive value of fats and oils. The first comprehensive work on this line, which extended over six years, was started in India at this Institute in April 1944, when an investigation was undertaken to study in detail the following aspects of this important problem:

1. The chemical constants of pure *ghee* and raw, refined and hydrogenated oils. This study was also extended to *ghee* and oils when heated.
 2. The vitamin A and carotene content of fats and oils.
 3. The comparative nutritive value of *ghee* and crude, refined and hydrogenated oils when incorporated in synthetic or provincial dietaries with special reference to digestibility, growth, reproduction, lactation and general health.
 4. Effect of long period feeding of different fats on the morphological constituents of blood.
 5. Effect of different fats on calcium, phosphorus and protein metabolism.
 6. Effect of different fats on the requirement of vitamins A and B complex.
- The experimental results are given in the subsequent chapters.

CHAPTER II

CHEMICAL CONSTANTS OF PURE GHEE

By N. D. KEHAR, S. N. RAY, B. C. JOSHI, and B. C. RAISARKAR

GHEE forms one of the most important animal-fats in the Indian dietary. Manufacture of *ghee* is, therefore, one of the most important industries in this country, as about 43.3 per cent of the total milk produced in India is utilised for preparation of *ghee* [Datar Singh, 1949]. In spite of this, demand for this commodity far exceeds the production. As a result, large scale adulteration of *ghee* takes place. The importance of fixing physical and chemical standards for distinguishing genuine and adulterated *ghee* has, therefore, become imperative. The standards adopted by the Indian food analysts are, however, based mainly on the European or American figures, as sufficient work on the variability of these constants under Indian conditions is not forthcoming. In fact the only work that is available to us is by Doctor *et al.*, [1940] who studied the effect of different feeds and breeds on the physical and chemical constants of *ghee*. The data collected by them represented only one farm and are thus of limited value. The present study was undertaken to examine the effect of breed, season and locality on some of the chemical constants for *ghee*. It was felt that the data thus amassed could be utilised in setting up standards for grading of *ghee* in India.

Through the courtesy of the Imperial Dairy Expert, arrangements were made to collect *ghee* from 12 cattle farms distributed all over India and representing eight different breeds of cattle and one breed of buffalo. Three batches of samples, one in September 1944 and two others in January and June 1945 were examined. Only one sample from each farm in any season was received and analysed.

The *ghee* in all the farms was prepared according to one standard method. This was done to obviate the possibility of difference in the quality of *ghee* which is likely to occur when produced under divergent methods. Butter-fat produced by churning fresh cream was melted and the *ghee* was separated by decantation. Immediately after preparation, the samples were sealed in airtight containers and sent to Izatnagar. These were stored in the laboratory in a refrigerator and were analysed at the earliest opportunity. Standard methods of analysis approved by the Association of Official Agricultural Chemists [1940] were followed. The following constants were examined : (a) Reichert-Meissl number, (b) Polenske number, (c) Kirschner number, (d) saponification value, (e) iodine value. For iodine value, Rosenmund-Kuhn's pyridine sulphate dibromide method as modified by Yasuda [1931] was used. The results are given in Table I. The over-all averages given in this Table are, however, mostly influenced by the values for cow *ghee* as the number of samples of cow *ghee* examined were greatly in excess of those of buffalo *ghee*.

TABLE I
Influence of breed, season and locality on the chemical constants of pure ghee

Breed	Locality	Saponification value			Iodine value			R. M. value		
		Autumn	Winter	Summer	Autumn	Winter	Summer	Autumn	Winter	Summer
Sindhi	Hosur	220.8	222.7	220.6	35.4	37.4	36.3	26.10	23.35	20.90
"	Bangalore	225.1	222.4	..	33.6	34.7	..	27.00	21.56	..
Kangayam	Hosur	221.4	222.2	219.9	35.6	40.5	35.9	25.19	24.42	20.46
Ongole	Guntur	218.8	224.2	220.4	34.5	38.9	36.2	20.79	20.14	18.04
Gir	Bangalore	224.4	220.9	..	35.2	32.0	..	29.23	27.89	..
"	Bankapur	214.2	218.5	..	38.8	34.5	..	17.65	20.28	..
"	Junagadh	..	222.7	215.4	..	37.9	36.3	..	21.29	16.01
Kankrej	Sanand	225.7	..	217.0	36.2	..	39.8	27.23	..	18.65
"	Chharodi	224.2	228.6	222.0	29.5	34.9	36.0	26.90	25.85	17.82
Sahiwal	Delhi	227.2	227.2	223.8	33.8	32.2	36.2	28.82	28.98	20.63
"	Montgomery	..	230.6	231.1	..	32.8	29.1	..	26.10	18.92
Haryana	Hissar	226.2	227.3	222.5	31.8	29.6	35.8	26.60	25.90	20.79
Tharparkar	Karnal	..	229.5	227.2	..	36.2	33.8	..	27.80	17.22
Murrah buffalo	Ambala	224.3	232.8	218.5	33.4	27.4	31.2	31.35	24.76	22.99
"	Bangalore	228.4	224.6	..	28.9	30.2	..	29.32	28.77	..
AVERAGE		223.4	225.3	221.7	33.9	34.2	35.1	26.35	24.76	19.31
OVER-ALL AVERAGE			223.6			34.4			24.29	

TABLE I—*contd.*
Influence of breed, season and locality on the chemical constants of pure ghee

Breed	Locality	Polenske value			Kirschner value		
		Autumn	Winter	Summer	Autumn	Winter	Summer
Sindhi	Hosur	1.10	1.45	1.40	21.60	17.90	16.89
"	Bangalore	1.30	1.80	..	22.10	20.31	..
Kangayam	Hosur	1.90	1.85	1.40	21.57	21.36	16.08
Ongole	Guntur	1.20	1.95	1.40	17.66	21.02	14.39
Gir	Bangalore	2.00	1.80	..	22.57	21.67	..
"	Bankapur	1.20	1.55	..	14.50	15.34	..
"	Junagadh	..	1.50	1.10	..	16.47	12.77
Kankrej	Sanand	2.60	..	1.15	22.12	..	14.89
"	Chharodi	2.00	1.80	1.85	22.10	19.60	15.23
Sahiwal	Delhi	2.10	2.30	1.95	23.61	20.06	15.26
"	Montgomery	..	1.80	2.40	..	18.44	14.21
Hariana	Hissar	2.30	2.30	1.85	21.70	19.09	15.23
Tharparker	Karnal	..	1.65	2.20	..	20.08	13.88
Murrah buffalo	Ambala	2.00	2.35	1.10	26.40	20.00	18.75
"	Bangalore	2.20	1.25	..	25.39	23.04	..
AVERAGE		1.87	1.61	1.62	21.78	20.31	15.24
OVER-ALL AVERAGE			1.77			19.28	

DISCUSSIONS

Saponification values

The values obtained lay within the range of 214.2 to 232.8, with an over-all average of 223.6. For the Western breeds, the range given by Davies [1939] is 219.7 to 232.6. It will thus be seen that while the maximum Indian values were similar to those found in the United Kingdom or the United States of America, the corresponding figures for the minimum values were definitely lower in some of our samples.

The values show some seasonal variation, as may be seen from the averages for the different seasons. The values increase from autumn to winter and then drop sharply in summer. The difference between the winter and summer samples was statistically significant ($P > 0.05$) i.e. at 5 per cent level. This lowered value in summer was probably due to the lowering of the volatile fatty acids, as will be discussed under the Reichert-Meissl and Kirschner values.

An interesting trend was found when *ghee* samples of different regions were examined. It will be seen in Table II that the saponification values of the *ghee* prepared from the milk of the North Indian cattle were significantly higher than those from the South Indian cattle. In this Table, the North Indian farms have been represented by Delhi, Montgomery, Hissar, Karnal and Ambala, whereas the South Indian farms comprise all the other farms in Central and South India, viz. Hosur, Bangalore, Guntur, Bankapur, Junagadh, Sanand and Chharodi farms. If the zonal and seasonal characteristics are considered separately (Table II), it will be seen that for the autumn samples, there was no significant difference between the two zones. As winter approached the differences became highly significant, the saponification values of the samples from North India being greatly increased. This is not probably due to differences in the feeding practice, as the farms studied in Northern India, though lacking good pastures in winter, compensate the scarcity by supply of green fodder from irrigated tracts. Further, it will be seen from the later discussions that the winter Reichert-Meissl values showed very little difference from the autumn values, showing thereby that there was not much scarcity of green fodder supply in winter at the North Indian farms. It, therefore, appears that the differences in certain values of the two zones for autumn and winter season can best be explained on climatic basis. Winter in North India, is more severe than in the South and this probably causes an increase in the saponification values of the butter-fat. In support of this, it may be pointed out that with the advent of summer, the values in both the zones dropped and as the South Indian summer is of longer duration, the values of samples from this zone still remained significantly below those from North India; though as may be predicted, the zonal difference in this season was much less than it was in the winter. These findings point out the importance of climate on the saponification values and explain why under the rigorous hot conditions prevalent for long periods in different parts of India, the saponification values of Indian butter-fat may be lower than those found in the Western countries [Davies, 1939].

The saponification values of buffalo *ghee* were found to be greater than those of the cow *ghee*, the respective values being 225.7 and 223.3. The difference was not, however, statistically significant.

TABLE II

Comparison of the values of chemical constants of ghee samples from the South and North Indian zones in different seasons

Constants	Total average	t	Autumn	t	Winter	t	Summer	t
Saponification value	221.9 (S) 226.9 (N)	8.89 (35)**	222.6 (S) 225.9 (N)	1.27 (10)	223.0 (S) 229.5 (N)	4.42 (12)**	219.3 (S) 224.6 (N)	2.39 (9)* ..
Iodine value	35.4 (S) 32.6 (N)	2.84 (35)**	34.2 (S) 33.0 (N)	0.62 (10)	35.7 (S) 31.6 (N)	2.20 (12)*	36.7 (S) 33.2 (N)	2.52 (9)*
R. M. value	23.66 (S) 25.45 (N)	1.16 (35)	25.49 (S) 23.92 (N)	1.41 (10)	25.17 (S) 23.71 (N)	2.13 (12)	19.65 (S) 20.11 (N)	
Polen's value	1.61 (S) 2.06 (N)	3.30 (35)**	1.72 (S) 2.30 (N)	1.66 (10)	1.66 (S) 2.08 (N)	2.30 (12)*	1.38 (S) 1.90 (N)	2.23 (9)*
Kirschner value	19.02 (S) 19.75 (N)		21.07 (S) 23.90 (N)	1.4 (10)	19.63 (S) 21.53 (N)		15.04 (S) 15.46 (N)	

(a) Figures in paranthesis denote degrees of freedom
 (S) denotes South Indian Zone, (N) the North Indian Zone
 * Significant at 5 per cent level, ** Significant at 1 per cent level

Iodine values

The iodine values ranged from 27.4 to 40.5, the average value being 34.4. These figures are very similar to those found in other countries, the range for English butter-fat being from 25.7 to 37.9 [Davies, 1939].

The values did not differ significantly from one season to another (Table I). This probably indicates that the lowering of the iodine values due to lack of green fodder in summer was prevented by supplying sufficient quantity of oil-cakes containing fats of high iodine values.

When, however, the influence of locality was examined, the South Indian samples of *ghee* were found to have higher iodine values all through the year, as compared to the values for the North Indian samples. These differences in the two zones were statistically significant both during summer and winter. The differences in the same zone in different seasons, however, did not show any significant variation, thus indicating that change in season perhaps did not much affect the values. In the Northern zone, the values dropped sharply in winter, pointing towards a differential utilisation of unsaturated fatty acids by the animals in this zone. This was probably due to the heightened metabolism through cold, when the unsaturated fatty acids are more liable to be oxidised preferentially. That this drop in values was not due to the lack of green fodder in winter, is explained by the rise in the values in summer when green fodder is much less available in all parts of North India. It may be assumed, therefore, that the higher iodine values of *ghee* samples from South India are probably characteristic of the breeds studied in those regions. The number of samples examined from each breed was, however, too small to differentiate one breed from another.

In order to explore further the nature of the unsaturated acids prevalent in the *ghee* samples from the two zones, the thiocyanogen values of the autumn samples were determined as well. From the iodine and thiocyanogen values, the linoleic and oleic acid contents were calculated [Jacobs, 1938]. The results are given in Table III. It will be seen that while the iodine values of the samples of the two zones in this season did not differ significantly, the linoleic glyceride contents of the *ghee* samples from the South zone were significantly higher ($t = 3.27$ for a degree of freedom of 10, i.e. significant at 1 per cent level). No such difference in the oleic glyceride contents was noticeable. Unfortunately, similar analysis on samples in other seasons could not be carried out for want of reagents; otherwise it was possible that much more significant differences could have been obtained when the iodine values themselves were significantly higher in the cases of the South Indian samples. It may, however, be concluded that the tendency of milk-fat, of cattle breeds grouped under the South Indian zone, showing higher iodine values, is due to their power of excreting greater quantities of unsaturated fatty acids like linoleic acid.

The iodine values for buffalo *ghee* were definitely lower than those for cow *ghee*, the respective values being 30.2 and 35.1. This difference is highly significant statistically, the value of 't' being 3.77 for a degree of freedom of 35. This indicates that buffaloes can utilise the unsaturated fatty acids much more efficiently than cows.

TABLE III

*Lionoleic and oleic glyceride contents of ghee from different parts of India
(South Indian Zone)*

Breed	Locality	Iodine value	Thiocyano- gen value	Linoleic glycerides per cent	Oleic glycerides per cent
Sindhi	Hosur	35.4	31.65	4.34	32.41
Kangayam	"	35.6	30.49	5.90	29.48
Ongole	Guntur	34.5	29.90	5.31	29.39
Sindhi	Bangalore	33.6	28.71	5.64	27.69
Gir	"	35.2	31.50	4.27	32.30
Murrah-buffalo	"	28.9	26.30	3.00	27.54
Gir	Bankapur	38.8	33.98	5.58	33.65
Kankrej	Sanand	36.2	33.06	3.63	34.77
AVERAGE		34.8	30.70	4.71	30.93

North Indian Zone

Sahiwal	Delhi	33.8	30.90	3.46	32.30
Haryana	Hissar	31.8	29.76	2.35	32.21
Haryana	Izatnagar	32.0	29.46	2.90	31.26
Murrah-buffalo	Ambala	33.4	30.95	2.82	33.13
AVERAGE		32.8	30.24	2.88	32.23

Reichert-Meissl, Polenske and Kirschner values

The ranges for the Reichert-Meissl, Polenske and Kirschner values for all the samples lay between 16.01 and 34.76 (average 24.29), 1.1 and 2.8 (average 1.77) and 12.77 and 30.0 (average 19.28) respectively. The corresponding values for butter-fat of the Western cattle are between 20.63 and 33.15, 1.4 and 3.0 and 19.26 respectively [Davies, 1939]. It will thus be noticed that the minimum values found by us are definitely below those given by Davies. It may be argued that this might have been caused by the loss of volatile fatty acids during the process of preparing ghee from butter, but Doctor *et al.*, [1940] have shown that no such loss occurred when ghee was prepared by decantation process, the technique adopted in manufacturing the ghee samples examined by us.

As may be expected from the experience of other workers, the Reichert-Meissl values (Table I) varied widely in different seasons. The summer values were far

below those of autumn and winter, the differences being highly significant statistically. That this drop is caused mainly by a diminution in the amount of butyric acid secreted, was seen from the corresponding decrease in the Kirschner values. The amount of volatile acids, other than butyric remained more or less constant, as seen from the Polenske value data. This diminution in butyric acid was most surely caused by a paucity of green fodder in summer. Similar lowering of the Reichert-Meissl values due to rigorous weather and a possible low plane of nutrition had been observed in the Irish cattle by Arup [1930] and Smith and Dastur [1938] in their experimental animals.

Unlike the saponification and iodine values, the differences as regards the Reichert-Meissl and Kirschner values, were not significant, between the *ghee* samples of the North and South zones. There seemed to be a tendency for higher Reichert-Meissl values in the North Indian samples, but the differences were not statistically significant (Table II). On the other hand, however, the North Indian cattle appeared to produce butter-fat having significantly higher Polenske values. The difference was not statistically significant in autumn, but became significant in summer and more so in winter. The information at hand does not, however, enable us to assess how far this contrast in the Polenske values of the *ghee* samples from the two zones was due to feeding practices, environment or breed difference.

When buffaloes were compared with cows it was seen that the Reichert-Meissl values for samples of buffalo *ghee* were much higher than those for the cow *ghee*. The average values for the two fats were 29.44 and 23.49. The 't' value was 3.05 for a degree of freedom of 35, indicating that the difference was highly significant. This difference was entirely due to the higher butyric acid content in the buffalo *ghee*, as the Kirschner values for the buffalo and cow *ghee* were 24.72 and 18.43 respectively ($t = 3.96$, i.e. highly significant). It will be seen that when the Kirschner values were subtracted from the Reichert-Meissl values, the figures obtained for buffalo and cow *ghees* were 4.72 and 5.06 respectively. These values denote the quantities of volatile fatty acids other than butyric acid. The similarity of the values of these two last figures again emphasises the fact that the higher Reichert-Meissl values of buffalo *ghee* were caused by its greater content of butyric acid. The significance of this species difference and its bearing on the metabolic activities are difficult to explain.

There were no differences in the Polenske values of the butter-fat from the two species, the average values for buffalo and cow *ghee* being 1.78 and 1.77 respectively.

It will thus be seen that for the standardisation and grading of Indian *ghee* samples it will be necessary to adopt constants which will be different from those used in the Western countries. The saponification number, Reichert-Meissl and Kirschner values found in certain samples prepared during the summer were definitely low, when viewed from the standpoint of Western standards and they might thus be classed as impure products. It is, therefore, necessary that much more detailed work should be undertaken on the effect of climate, feed, breed and methods of management on the chemical constants of *ghee* to ascertain the proper ranges for Indian standards.

CHAPTER III

VITAMIN A AND CAROTENE CONTENT OF FATS AND OILS

By N. D. KEHAR, S. N. RAY, T. S. KRISHNAN, B. C. JOSHI and B. C. RAISARKAR

THE vitamin A potency of butter-fat produced from the milk of cattle under diverse conditions of feeding and of different breeds has been the subject of a large number of investigations carried out in Europe and America. It has been established thereby that, as measured by the biological rat-growth method, there is no difference in the total vitamin A potency of butter-fats from different breeds of cattle under identical conditions of feeding. The ratio between carotene and vitamin A present in butter-fats from different breeds may, however, show wide variation even under identical feeding conditions. Thus the butter-fat from Guernseys has twice as much carotene as that from Ayrshire [Wilbur *et al.*, 1933] and thrice as much carotene as that from Shorthorns [Kon and Booth, 1933]. It is also recognised that access to fresh green pasture yields butter of high vitamin A value, whereas cows fed on poor dried up pasture or on badly cured hays and straws yield butter-fat low in vitamin content [Davies, 1940].

Very little work has been done in India to evaluate the effect of breed or season on the vitamin A potency of *ghee* (clarified butter-fat). Doctor *et al.*, [1940] observed that under similar conditions of feeding and management, foreign breeds (Ayrshire) secreted into milk more carotene and vitamin A than the Indian breeds like Sindhi or Gir. No difference, however, was found to exist in the milk of these two latter breeds. They also showed that buffaloes (Murrah breed) yielded butter-fat having a lower vitamin A potency than even the Indian breeds of cattle. They also confirmed the findings of other workers that buffalo-milk fat was practically free from carotene. No other comparative work on the effect of breed or climate on the vitamin A content of *ghee* or butter has yet been carried out in India.

There also exists diversity of opinion about the presence or absence of vitamin A in *ghee*, as sold in Indian markets. Banerjee and Sunawala [1935] failed to detect any vitamin A in four samples of *bazar ghee*. On the other hand, Grewal [1933] in Lahore found 18 I.U. per gram of *ghee*. Muthana and Seshan [1941] found eight micrograms of vitamin A per gram of Bengal *bazar ghee* and 12 micrograms per gram of Sindhi *ghee*. Majumdar [1941] found a very low value for market buffalo-*ghee*, i.e. 1.1 microgram per gram. Ahmed *et al.*, [1946] found that six out of the seven market samples of *ghee*, examined by them, had a vitamin A content of less than 24 I.U. per gram.

The present work was taken in hand to find out, firstly, whether there exists any breed difference in the vitamin A content of milk and hence in the butter or *ghee* prepared from it and secondly, whether the *ghee* produced from the milk of the same breed of cattle in different regions of India, at different seasons shows any variation in the vitamin A content. It was also proposed to purchase *ghee* from

the open market in the various provinces of India and to analyse those samples for their vitamin A content. The analysis of *bazar ghee* was undertaken to ascertain the amount of vitamin A reaching the actual consumer through this medium. These findings were assumed to be of value to the Public Health Authorities as the *ghee* forms the most important animal fat in the Indian dietary and differs from the vegetable oils used as food ingredients, in its vitamin A content, this vitamin being absent from the vegetable oils. In fact, the *ghee* may be taken to be the only source of vitamin A in the vegetarian dietary, as the rest of the food contains only carotene. In human beings, vitamin A, owing to its greater digestibility and greater biological potency, is four to five times as active as the same quantity of carotene [Booher, 1938]. Hence even the small quantity of vitamin A ingested with *ghee* will be of great value in bringing up the low levels of vitamin A potency of the Indian dietaries.

Side by side with these, studies were carried out on the carotene and vitamin A content of common vegetable oils, different brands of *vanaspatis* and lard. It is generally believed that the vegetable oils except red palm oil, contain no carotene or vitamin A. Detailed data regarding this point is, however, lacking. Many *vanaspatis* manufacturers advertise their products as having been fortified with vitamins without giving any indication of the quantities present. Hence, an attempt was made to determine the quantity of vitamin A available to consumers of these products. The vitamin D content of a few samples of *vanaspatis* was also estimated.

A. *Vitamin A potency of fresh pure ghee*

Ghee samples were obtained from twelve different farms all over India. Eight different breeds of cattle and one breed of buffalo were represented. Three batches of samples, one in September, 1944 and two others in January and June, 1945, were examined. A standardised process was used in all the farms to prepare the *ghee*. The *ghee* samples were despatched to our laboratory immediately after preparation, packed in small air-tight containers which were filled in a way that very little air space was left inside them. The samples on receipt were immediately kept in a refrigerator and analysed at the earliest opportunity. Carotene and vitamin A were extracted after saponification, according to a modified method of Gillam *et al.*, [1927]. Carotene was determined colorimetrically after removal of xanthophyll. The unsaponifiable matter of *ghee* was then dissolved in pure ethyl alcohol and the vitamin A in the solution was estimated in a Hilger vitameter. The results of these findings are incorporated in Table IV.

It will be seen that under identical feeding conditions, i.e. in the same farm, the vitamin A potencies of *ghee* made from the butter-fats of cows of different breeds were more or less the same. Thus *ghee* from Kangayam and Sindhi breeds from Hosur Cattle Farm had nearly the same values, and the ratio between carotene and vitamin A in such fats was also more or less of the same order. Similar findings were also seen in the Sindhi and Gir breeds of the Indian Dairy Research Institute Farm at Bangalore. On the other hand, the *ghee* from Murrah buffaloes in Bangalore had a lower vitamin A potency than the *ghee* from cows in the same farm. These data support the findings of Doctor *et al.*, [1940] that the buffalo *ghee* contains less vitamin A than cow *ghee*.

TABLE IV

Influence of breed and season on the vitamin A content of ghee from cows and buffaloes

(The amounts are given in micrograms per gram of ghee)

Breed	Locality	September 1944		January 1945		June 1945	
		Carotene	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A
Cows :							
Sindhi	Hosur	6.3	15.0	6.46	14.7	5.8	13.9
Sindhi	Bangalore	3.9	9.9	2.80	10.3
Kangayam	Hosur	6.9	13.4	7.54	14.4	6.7	13.3
Ongole	Guntur	1.1	9.75	1.4	6.6	1.2	8.04
Gir	Bangalore	3.9	11.3	2.59	10.7
Gir	Bankapur	2.0	11.3	trace	5.35
Gir	Junagadh	1.01	7.30	1.3	8.03
Kankrej	Sanand	5.4	12.3	1.64	6.0
Kankrej	Chharoi	5.6	15.0	1.9	7.23	1.38	7.81
Sahiwal	Delhi	2.4	6.2	2.82	9.33	2.91	9.82
Sahiwal	Montgomery	3.94	12.32	trace	9.07
Haryana	Hissar	3.2	12.5	3.1	13.0	2.35	12.31
Haryana	Izatnagar	3.6	12.0	1.6	3.2	2.4	9.1
Tharparkar	Karnal	4.34	11.3	1.46	8.04
Average		4.03	11.77	3.01	10.13	2.56	9.53
Buffaloes :							
Murrah	Ambala	nd	6.1	nd	10.7	nd	6.25
Murrah	Bangalore	nd	11.3	nd	6.1	nd	7.3

The vitamin A potency varied markedly in the same breed from place to place, e.g. Sindhi cows in Hosur yielded *ghee* much richer in vitamin A than the same breed at Bangalore. The explanation lies in the fact that whereas Hosur cattle get extensive grazing throughout the whole year, no grazing is possible in Bangalore. Moreover, the cattle at the latter place receive only cut green fodders and, thus, probably do not get their optimum quota of carotene.

The seasonal variations were rather irregular in most places, except in Hosur and Hissar where nearly constant values were obtained all through the year. This was due to the all-year grazing facilities available at both the farms. In most of the other farms, the winter and summer values of the vitamin A content of *ghees* tended to be lower than the autumn values. This trend is brought out in the average values of the different seasons (Table IV). More marked diminution was seen in the carotene values for winter and summer. Here again the carotene values of samples from Hosur and Hissar were found to be more or less the same all through the year. The low values for June samples from the other places were probably caused by a lack of fresh green fodder. This would chiefly affect the carotene excretion in the milk, as this pigment is seldom stored in the body as such. On the other hand, vitamin A values of the samples did not show wide changes as the vitamin A of the milk in summer was probably obtained to a great extent from the vitamin A stored in the body during periods of high levels of green feeding. Among cows, the highest values of vitamin A content of *ghee* approached 15 micrograms per gram, which may represent the peak values reached after supplying plenty of green fodder. This value is similar to the figures obtained by Ray, Sarkar and Sen [1943] in the butter-fat of cows under intensive green feeding conditions. The lowest values found by us were in the region of six micrograms per gram.

One point of interest may be brought to notice here. The carotene content of *ghee* obtained from the milk of Ongole breed of cattle never rose to more than 1.5 microgram all through the year. The vitamin A contents were, however, quite high, i.e. at or below which, other breeds were giving *ghee* containing carotene at the rate of 2.5 to 4 micrograms per gram. In fact, such low values make this breed similar to buffalo, in which species, it may be noted, carotene is excreted in the milk in slight traces only.

It may, therefore, be concluded that none of the Indian breeds examined gave milk as rich in carotene as that of the Jersey breed. On the contrary, Ongoles secrete very little carotene in the milk and thus appear to resemble the buffalo. Under an intensive green feeding regime, the butter-fat of Indian breeds of cattle is very rich in vitamin A, the values being similar to those of the European breeds under similar heavy green feeding, as reported by foreign workers [Atkenson *et al.*, 1937].

Unlike Doctor *et al.*, (*loc. cit.*), we did not examine *ghee* from foreign breeds of cattle under Indian conditions of feeding and environment, but it is possible that the higher vitamin values which they obtained were due to the lower fat contents of the milk of these breeds, and thus the total potency in equal volumes of liquid milk of both foreign and indigenous breeds was of the same magnitude. This explanation also serves for the low vitamin A value of buffalo *ghee*. The fat content of buffalo milk is very high, so that the concentration of the vitamin A per gram

of butter-fat becomes lower, assuming that equal amounts of vitamin A are present in equal volumes of cow and buffalo milk.

B. Vitamin A content of bazar ghee

Thirty-one samples of *bazar ghee* collected during winter months, and 27 samples collected in summer from various parts of India, were examined. The average value for the winter samples was 1.26 micrograms per gram of *ghee* and for the summer samples 1.44. The detailed results are given in Table V.

The analyses were done both spectroscopically as well as tintometrically. The tintometric method followed was that used by Booth *et al.* [1933]. Most of the samples were more or less rancid, as was evident from the high acid and peroxide values. As rancidity was found to interfere with the spectroscopic determination of vitamin A, giving much higher figures, the tintometric results only are incorporated in the Table, according to the recommendations of Oser *et al.* [1943]. For calculating the vitamin A content in micrograms, Moore's blue units, as determined in the tintometric method, were converted into micrograms of vitamin A, using the conversion factor of 1 μ g. vitamin A=4.0 blue units.

It will be seen that, judged by the vitamin A content, most of the samples were of poor quality. There was, however, scarcely any difference in the average values for summer and winter samples.

No carotene could be detected in any one of the samples. This might have been due to two causes, firstly; most of these samples were perhaps prepared from buffalo milk which contains no carotene and secondly, it is also possible that even if some of the samples were of cow *ghee*, the carotene originally present might have been destroyed during storage.

In order to find out the rate of destruction of vitamin A and carotene in *ghee* samples during storage, a sample of pure cow *ghee* was kept in a glass bottle. The container was stoppered lightly and was left on the laboratory shelf. The percentage of loss in storage during the periods of 140 and 240 days was as follows: (1) vitamin A—20 per cent and 50 per cent respectively, and (2) carotene—35 per cent and 100 per cent respectively.

C. Vitamin A and D and carotene content of vegetable oils, vanaspati and lard

In the case of oils, both crude and refined samples of each oil were separately analysed. The oils were refined in the laboratory as follows:

Raw oil was treated with a requisite quantity of caustic soda to neutralise the free acidity, warmed and allowed to stand. The soaps and suspended impurities were filtered off and the oil washed free of soaps, etc. with hot water. The oil was then dried by shaking with anhydrous sodium sulphate. Bleaching was carried out by one treatment with animal charcoal.

The carotene content was determined by the method described earlier and the vitamin A was estimated by the Spectroscopic method. Vitamin D in a few samples of *vanaspati* was assayed against a standard sample of cod-liver oil by the A.O.A.C. official chick assay method [1940]. The results are given in Table VI.

TABLE V
Vitamin A content of bazar ghee

Locality	WINTER			SUMMER		
	Acid value	Peroxide value	Vitamin A y/g.	Acid value	Peroxide value	Vitamin A y/g.
	Average	Average	Average	Average	Average	Average
Patna	3.35	8.10	0.5	3.10	2.26	trace
Arrah	7.85	11.10	nil	2.50	2.80	[2.9] 0.97
Muzaffarpur	5.80	13.20	nil	3.95	3.16	trace
Bangalore	3.45	8.00	nil	12.60	3.00	1.7
Bangalore	5.60	1.68	1.1	10.40	5.61	2.1
Bangalore	4.80	3.80	0.3	4.10	1.93	0.9
Karachi	1.65	14.70	1.3	5.90	2.92	2.1
Karachi]	5.70	7.35	7.5	10.20	7.64	1.3
Karachi]	7.45	7.95	7.0	1.15	38.31	0.9
Calcutta	2.55	2.15	7.0	4.30	1.37	trace
Calcutta	0.90	1.90	nil	3.80	0.64	trace
Calcutta	3.00	4.30	nil	4.05	1.56	trace
Madras	14.75	0.60	4.7	13.60	1.29	3.8
Madras	34.75	1.00	0.3	10.50	1.49	4.3
Madras	1.90	4.20	0.9	15.85	1.19	3.9

TABLE V—*contd.**Vitamin A content of bazar ghee*

Locality	WINTER			SUMMER		
	Acid value	Peroxide value	Vitamin A γ /g.	Acid value	Peroxide value	Vitamin A γ /g.
	Average	Average	Average	Average	Average	Average
Delhi	0.35	0.40	nd
Delhi	0.35 } 0.35	0.65 } 2.35	nd } 0.00
Delhi	0.45	0.0	nd
Bombay	18.05	5.15	0.3	1.6	1.84	0.85
Bombay	18.80 } 22.15	4.15 } 4.75	0.3 } 0.50	7.8 } 8.70	10.19 } 25.78	2.15 } 1.00
Bombay	24.80	4.95	0.3	16.7	65.81	nd
Baroda	1.90	4.20	0.6	2.65	6.00	2.0
Baroda	4.30 } 3.98	2.25 } 3.37	0.4 } 0.48	1.00 } 1.88	2.28 } 3.99	trace } 2.10
Baroda	5.75	3.85	0.3	2.00	0.71	4.3
Lahore	4.55	2.75	0.2	0.80	2.70	trace
Lahore	2.80 } 3.55	1.85 } 2.05	1.0 } 0.67	0.85 } 0.82	0.76 } 1.72	nd }
Lahore	4.00	1.54	0.3	0.80	1.70	nd
Ambala	0.50	2.30	1.2
Nagpur	19.50	61.18	nd	4.20	7.89	4.3
Nagpur	12.80 } 12.97	9.91 } 22.33	1.1 } 0.63	1.70 } 3.63	9.36 } 15.66	1.5 } 1.98
Nagpur	7.10	1.90	0.3	5.00	29.70	nd
Average (Total)			1.28			1.44

TABLE VI

Vitamin A and D and carotene content of vegetable oils, vanaspati and lard

Oil or fat	Content of Vitamins per gm. of fat or oil		
	Vitamin A μg.	Carotene μg.	Vitamin D I. U.
Cow ghee]	6.0-15.0	tr-7.54	0.32
Groundnut oil	..	0.5	..
Cottonseed oil	..	5.4	..
Coconut oil
Mahua oil	..	3.2	..
Til oil	..	1.2	..
Mustard oil	..	3.6	..
Lard	0	0	..
Dalda	0	0	0
First quality, No. 1	0	0	..
Swastika	[0	0	0.06
Kotogem	[0	0	0.22
Cotex	[0	0	..
Binaula	0	0	..
Temple	0	0	..
Rajhans	0	0	0.02

N.B. On refining, the carotene content of vegetable oils was lost totally.

As would be seen from the Table, many of the crude oils contrary to the expectations, contained, small though appreciable quantities of carotene. The crude cottonseed oil was the richest, containing about 9 I.U./gm. while crude groundnut oil was the poorest, containing less than 1 I.U./gm. This has been recorded, so far as we know, for the first time in India, though Bertram [1937] had reported the presence of small amounts of carotene as the result of qualitative tests. Scheunert [1940] had found from biological assays small amount of vitamin A potency in rapeseed oil, but none in coconut and sesame oils. Our results are similar except that *til* (sesame) oil has been found by us to contain appreciable quantities of carotene. Refining led to a total loss of all the carotene in all the oils.

Most remarkable was the total absence of vitamin A in all the samples of *vanaspatis* and lard. All these samples were purchased from the local market in

sealed containers and kept in a refrigerator until they were analysed. The *vanaspati* samples contained three brands of hydrogenated groundnut oil ('Dalda', 'Swastika', 'First quality No. 1'), three of hydrogenated cottonseed oil ('Kotogem', 'Cotex', 'Binaula') and two of hydrogenated *til* oils ('Rajhans' and 'Temple'). Rao and Anantkrishnan [1945] had a similar experience. As vitamin A is rather unstable and is easily oxidised unless admixed with some anti-oxidants, it was believed that destruction of this vitamin must have taken place during storage. In order to test this hypothesis, it was decided to find the comparative rates of destruction of vitamin A added to *vanaspatis* as well as that naturally present in *ghee*. A sample of cow-*ghee* was analysed for its vitamin A content and was then kept in a glass bottle in a dark cupboard. Along with it, in similar bottles, samples of *vanaspatis* were also kept after being fortified with vitamin A. Radiostoleum was used as source of vitamin A concentrate. After intervals of four and eight months, the bottles were taken out and the samples analysed.

TABLE VII

Rate of destruction of added vitamin A in various brands of vanaspatis

Name	Initial concentration of added vitamin A in y/g.	Loss on storage (in per cent)	
		140 days	240 days
Dalda	18.4	26.6	52.7
First quality, No. 1	21.0	38.8	71.3
Swastika	19.4	17.0	52.0
Kotogem	13.1	39.7	60.7
Cotex	16.3	13.5	59.3
Binaula	17.5	20.7	34.0
Temple	20.3	34.0	59.3
Rajhans	15.0	8.0	38.0
Cow-ghee	10.0	10.0	25.0
	Carotene 2.96	32.1	61.3

The results are given in Table VII. It will be seen that, whereas the *ghee* had lost about 25 per cent of its potency only, the *vanaspati* had lost from 34 to 71 per cent of the added vitamin A. This shows that even under good storage conditions, i.e. even when protected from sunlight and excessive handling, the rate of destruction of vitamin A added to *vanaspatis* was much greater than that in cow-*ghee* under similar conditions. It is no wonder, therefore, that under usual storage conditions prevalent in India, the vitamin content of the market samples of

vanaspati was totally lost. It is, therefore, desirable for the protection of vitamin A added to *vanaspati*, that suitable antioxidants should also be incorporated simultaneously.

Low values for vitamin D were found in the *vanaspati* samples. It was totally absent from Dalda. If the *vanaspatis* had been fortified with vitamin D, this low value could not have been caused by loss of the vitamin during storage, as vitamin D is comparatively stable. Assuming that vitamin D had really been added, it is probable that other factors might have masked the calciogenic action of vitamin D. The higher fatty acids present in the *vanaspatis* might interfere with the absorption of feed-calcium with the result that calcium salts are drained off from the bones to other parts of the body and also perhaps excreted through the gut. Support for this view is lent by the fact that the bone ash percentage of chicks receiving Dalda was even lower than that of the bones of the negative control birds, i.e. those receiving the vitamin D-free basal ration only.

CHAPTER IV

CHEMICAL CONSTANTS OF VEGETABLE OILS, VANASPATIS AND LARD

By N. D. KEHAR, T. S. KRISHNAN, S. N. RAY, B. C. JOSHI, and B. C. RAISARKAR

IN different parts of India, a number of vegetable oils like mustard, *til*, etc. are consumed by the people. Often they constitute the only source of fat in the poor man's dietary. Recently, however, some of the refined vegetable oils, but more commonly hydrogenated vegetable oils (*vanaspatis*), have started increasingly to replace *ghee* and the ordinary crude vegetable oils in the dietaries. Since the *vanaspatis* resemble *ghee* in appearance and as, unfortunately, there is no law as yet in India to colour the hydrogenated product in a distinctive way, there has been large scale adulteration of *ghee* with this. In many cases, lard is also used for adulterating *ghee*. This is not only an offence, as the public is defrauded, but there may also be grave risks from the public health standpoint, since some workers feel that consumption of *vanaspati* may have deleterious effects. The food analyst is up against the problem of distinguishing pure *ghee* from such adulterated stuff. This problem becomes more complicated by the fact that very little is known about the chemical constants of the different brands of *vanaspati* and refined vegetable oils. So far as is known to us the only data available for chemical constants of *vanaspatis* are those given by Ram *et al.*, [1943] and Rao and Anantakrishnan [1945]. Data regarding the chemical constants of refined oils are also very meagre. This chapter, therefore, deals with the study of some of the common chemical constants of *vanaspatis* as well as vegetable oils both raw and refined and one sample of lard. The effect of heating on chemical constants has also been studied.

The following six common vegetable oils, ten brands of *vanaspatis* and lard, were employed in this study :

I. Vegetable oils :

- (1) Groundnut oil, (2) cotton-seed oil, (3) coconut oil, (4) *mahua* oil,
- (5) sesame oil and (6) mustard oil.

II. Vanaspatis :

- | | |
|------------------------|--|
| 1. Dalda, M. P. 36° | } Supplied by Hindusthan Vanaspati Manufacturing Co., Bombay |
| 2. Dalda, M. P. 39° | |
| 3. Dalda | } Hydrogenated groundnut oil, purchased from open market |
| 4. First quality No. 1 | |
| 5. Swastika | |
| 6. Kotogem | } Hydrogenated cotton-seed oil, purchased from open market |
| 7. Cotex | |
| 8. Binaula | |
| 9. Temple | } Hydrogenated sesame oil, purchased from open market |
| 10. Rajhans | |

III. Lard

Purchased from open market.

All the crude oils, except cotton-seed oil, were prepared by pressing their seeds under our own supervision, to get the pure samples of the materials. Cotton-seed oil, however, was obtained from the manufacturers as it could not be pressed locally. Both the crude and refined samples of each of the above oils were studied separately.

The refining of the oils was carried out as given in Chapter III.

The following chemical constants were examined :

- (a) Saponification value, (b) iodine number, (c) Reichert-Meissl values, (d) Reichert-Polenske value and (e) Kirschner value.

Further, the acid value of the oils and the solidifying and melting points of the *vanaspatis* were also determined.

Standard methods of analysis, as approved by the A.O.A.C. [1940], were followed. For iodine value, Rosenmund and Kunheim's pyridine sulphate dibromide method, as modified by Yasuda [1931] was used. Only one sample for each oil or *vanaspati* and 37 samples of *ghee* were analysed.

RESULTS AND DISCUSSION

The chemical constants of the oils and fats studied are given in Table VIII. For ready reference the range of variation in the constant of cow *ghee*, as found in our studies (Chapter II) is also included.

The chemical constants of the different oils were generally within the usual specified limits, though there was a tendency, in many cases, for slightly lower saponification and iodine values. This was most marked in the case of the iodine value of cotton-seed oil. Crude vegetable oils generally have a higher acid value than the other fats. Of the lot, *mahua* oil had the highest acid value. One sample of this oil obtained from the manufacturers had as high a value as 21.7. Such values for this oil have also been obtained by Bolton [1928].

Refining of the oils led to a considerable lowering of the acid value but the other constants were not materially altered.

The variations in the values of the constants between the different *vanaspatis* were not at all wide. This is what should be expected in the progressive and partial hydrogenation of vegetable oils used in their manufacture. [Hilditch, 1941.]

As compared with the cow *ghee*, the crude oils generally had a higher acid value. The saponification values of the oils were lower and the iodine values higher than those of the *ghee*, except in the case of coconut oil, where the position was reverse. Cow *ghee* had also a higher volatile acid content than the oils except that coconut oil had a higher R. P. value.

Vanaspatis showed a complete absence of volatile acids. Their saponification values were lower and iodine values much higher than those for *ghee*. When compared against the parent oils, hydrogenation did not seem to materially affect the saponification values, while the iodine values were lowered by about 25-30 per cent. In Lard, values for the different constants were more or less similar to that of the *vanaspatis*.

TABLE VIII
Chemical constants of cow ghee, vegetable oils, vanaspatis and lard

Oil or fat	Melting point °C.	Solidify- ing point °C.	Acid value	Saponification value	Iodine value	Reichert- Meissel value	Reichert- Polenske value	Kirchhafer value
<i>Cow ghee</i>	0.5	214.2-232.8 (223.6)**	27.4-39.8 (34.4)	16.01-34.76 (24.24)	1.1-2.6 (1.77)	12.77-30.0 (19.28)
Groundnut oil, raw	3.2	189.8	86.4	0.44	0.20	0.0
Groundnut oil, refined	0.2	188.8	87.3	0.33	0.20	0.0
Mustard oil, raw	0.6	197.8	90.7	0.11	0.05	0.0
Mustard oil, refined	0.1	167.9	91.1	0.06	0.05	0.0
Sesame oil, raw	1.5	185.1	83.1	0.33	0.15	0.0
Sesame oil, refined	0.2	185.6	82.6	0.29	0.10	0.0
Cotton seed oil, raw	0.5	191.4	95.3	0.23	0.15	0.0
Cotton seed oil, refined	0.2	190.3	95.1	0.17	0.10	0.0
Coconut oil, raw	1.6	247.7	7.9	6.82	15.30	2.0
Coconut oil, refined	0.3	245.8	8.4	6.70	15.70	1.6
<i>Mahua</i> oil, raw	7.6 (21.7)*	197.2	58.3	3.50	1.30	0.5
<i>Mahua</i> oil, refined	3.3	198.2	57.8	3.40	1.20	0.4
'Dakda' I	36	..	0.5	188.4	69.5	0.05	0.0	0.0
'Dakda' II	39	..	0.1	185.7	62.9	0.05	0.0	0.0
'Dakda' III	40-42	27-33	..	180.0	64.6	0.10	0.0	0.0
'First quality No. I'	40-42	25-30	..	189.0	57.4	0.0	0.0	0.0
'Swarika'	40-42	37	..	194.9	57.6	0.2	0.0	0.0
'Kotogram'	41	23	..	190.3	54.4	0.0	0.0	0.0
'Cotex'	41	29	..	189.9	56.4	0.0	0.0	0.0
'Binoola'	37-40	23	..	192.6	59.5	0.0	0.0	0.0
'Temple'	41-43	31	..	190.6	55.7	0.0	0.0	0.0
'Rajhans'	42	23-30	0.5	189.9	57.1	0.0	0.0	0.0
Lard	190.6	60.4	0.28	0.15	0.0

* This value was obtained for a sample of *mahua* oil obtained from the manufacturers

** The figures in brackets give the average for cow ghee

It may be pointed out, however, that from the analyst's point of view, the examination of the chemical constants of a sample of *ghee* may not yield much crucial information regarding its purity. In Chapter II, we have dealt with the wide variability in the chemical constants of the pure *ghee* as influenced by season, environment and the feed of the animal. The range of variations, as found by us, in the constants of cow *ghee*, has been given in Table VIII. Now, supposing we have a *ghee* sample giving a saponification value of 232.8, iodine value of 27.4, Reichert-Meisel value of 34.76 and Polenske value of 2.35, then it will be possible to mix with 100 parts of this *ghee*, 85 parts of a *vanaspati* like Binaula, whose corresponding constants are 192.6, 59.5, 0, and 0 respectively. The resultant mixture will then have a saponification value of 214.3, iodine value of 42.1, Reichert-Meisel value of 18.79 and Polenske value of 1.3, and will thus show constants within the range of pure *ghee*.

In order to test this point further, we analysed six samples of *bazar ghee* which were known to be admixed with *vanaspati*, as evidenced by a positive phytosterol test. The results of these analyses are incorporated in Table IX. It will be seen that only sample No. 6, and possibly also No. 3, could definitely be classed, on the basis of their chemical constants, as being adulterated with *vanaspati*. These two samples had very low saponification, Reichert-Meisel and Polenske values and rather high iodine values. The rest of the samples, except perhaps No. 4, which had a low Polenske value, should pass the test for pure *ghee*, if the chemical constants are taken to be the only criteria. The phytosterol test which can be regarded as a specific one is a time consuming and difficult analytical test, and as such is rarely carried out as a routine measure.

In order to find out whether low saponification, Reichert-Meisel, Polenske and iodine values may develop in *ghee* samples on long storage, samples of *ghee* were analysed for their chemical constants and were then stored for one year. After this period, the samples were again analysed. It was seen that though the specimens had all gone rancid, the saponification and iodine values were hardly affected. The volatile fatty acid concentrations showed some lowering of their values. The data of these findings are given in Table X. It will, therefore, be admitted that the low values of the constants of some of the samples, as given in Table IX, were mainly caused through adulteration and not through storage.

TABLE IX
Chemical constants of bazar ghee samples

Sample	Acid value	Peroxide value	Saponification value	Iodine value	Reichert-Meisel value	Polenske value	Kirschner value
1	5.9	2.9	225.5	30.9	19.5	1.4	12.3
2	1.0	2.3	226.5	27.7	30.8	1.3	24.2
3	5.0	29.7	212.8	36.8	16.9	0.85	14.9
4	1.6	1.3	220.1	28.9	23.7	0.7	20.9
5	5.6	1.7	219.8	33.2	22.2	1.0	19.7
6	4.3	1.4	205.6	49.3	10.9	0.4	9.3

TABLE X

Effect of storage on the chemical constants of pure ghee

Sample	Acid value	Peroxide value	Saponification value	Iodine value	Reichert-Meisel value	Polenske value	Kirschner value
1. (a)	220.8	35.4	26.1	1.1	21.6
(b)	0.8	24.4	220.1	36.6	23.8	0.8	17.15
2. (a)	221.4	35.6	25.2	1.9	21.6
(b)	0.6	23.0	221.1	35.2	20.5	1.4	15.4
3. (a)	224.3	33.4	[31.4	[2.0	26.4
(b)	1.6	13.8	225.3	34.2	23.3	0.9	18.95
(a) Before storage				(b) After storage			

Effect of thermal treatment on the chemical constants and vitamin A potency of fats and oils

The effect of heat treatment on fats was found by Dyme *et al.*, [1941] to be proportional to the surface area exposed and the duration of heating. The temperature to which they are raised would also have a profound influence. The above authors noticed that heating increased the free fatty acids and diminished the iodine value of the fats besides producing aldehydes and ketones, which may be toxic. Roy [1944] observed that thermal treatment of fats and oils caused changes in their absorption coefficient as well as chemical constants. Heating is also likely

to cause loss of vitamin A potency to varying degrees according to the intensity of heating and the duration of the process. Kennelly and Quackenbush [1947] noted that the adverse effects of feeding oxidised-fats could be eliminated by giving vitamins A and E and suggested that the toxic effects were the result of destruction of dietary essentials rather than a direct toxic effect due to the oxidation of the fat. Dyme *et al.*, [1941] have observed that heating in air at 102-105°C. for 24 hours inactivated the vitamin A of cod-liver oil and butter-fat, as was judged by the effect on growth of rats. Karmarker [1944], Ahmed *et al.*, [1946] and De and associates [1946] observed considerable destruction of vitamin A, when *ghee* was heated or used for frying. The last mentioned workers have also noticed severe losses in carotene on heating margarine fortified with carotene.

The effect of heating, on the following chemical constants, of cow *ghee*, *bazar ghee*, crude groundnut oil and *vanaspatis* of melting point 36°C. and 39°C. made from groundnut oil, for 30 minutes at 190°C., was determined :

(a) Acid value, (b) saponification value, (c) iodine value, (d) Reichert-Meisel value, (e) Reichert-Polenske value and (f) Kirschner value.

The losses in carotene and vitamin A suffered by this thermal treatment were also estimated.

The cow *ghee* tested was a fresh sample prepared in the Institute dairy under standard conditions. The *bazar ghee* was a composite sample prepared by mixing material sold as *desi ghee* in the *bazar*, obtained from five different shops situated in different parts of Bareilly city, in order to make it more representative. Groundnut oil was prepared by pressing the seeds under our own supervision. The *vanaspatis* were obtained from the manufacturers. Only one sample of each variety of *ghee*, oil and *vanaspati* was tested.

Vitamin A was determined by separating the unsaponifiable matter by the method of Olson, Hegsted and Peterson [1939]; dissolving it in aldehyde-free pure ethyl alcohol and estimating the vitamin A content by a Hilger vitameter. Carotene was determined by the method of Seshan and Sen [1942].

RESULTS AND DISCUSSION

The chemical constants of the different oils and fats, before and after heating, is given in Table XI. The initial vitamin A and carotene content of *ghee*, *vanaspati* samples and common vegetable oils which had been found to contain some carotene, and the loss in vitamin A potency suffered by them due to thermal treatment, are given in Table XII.

TABLE XI

Chemical constants of oils and fats before and after heating

No.	Oil or fat	Acid value, free fatty acids as oleic acid	Saponifica- tion value	Iodine value	R. M. Value	R. P. Value	Kirschner value
1.	Cow ghee, unheated	0.5	221.1	32.3	31.9	1.85	24.6
2.	Cow ghee, heated	0.2	220.2	32.9	29.3	1.4	23.0
3.	Bazar ghee, unheated	2.1	226.7	30.2	33.8	1.75	26.5
4.	Bazar ghee, heated	1.9	227.6	29.8	32.0	1.4	25.1
5.	Groundnut oil (crude), unheated	3.33	189.5	86.4	0.44	0.2	0.0
6.	Groundnut oil, heated	2.1	188.2	85.6	0.22	0.1	0.0
7.	Vanaspali, M. P. 36°C. unheated	0.5	188.4	69.5	0.05	0.0	0.0
8.	Vanaspali, M. P. 36°C. heated	0.3	188.7	70.0	0.00	0.0	0.0
9.	Vanaspali, M. P. 39°C. unheated	0.1	185.7	62.9	0.05	0.0	0.0
10.	Vanaspali, M. P. 39°C. heated	0.05	184.8	62.3	0.0	0.0	0.0

TABLE XII

Vitamin A and carotene content of fats and oils and their loss on heating

Oil or fat	Initial content in fresh oil or fat of		Loss of vitamin A potency in heat- ing per cent
	Vitamin A/ γ /gm	Carotene/ γ /gm	
Cow ghee	15.5	1.9	43
Bazar ghee	8.8	..	39
Groundnut oil (crude)	..	0.5	16
Cotton-seed oil (")	..	5.4	13
Coconut oil (")
Mahua oil (")	..	3.2	20
Sesame oil (")	..	1.2	13
Mustard oil (")	..	3.6	38
Vanaspati, M.P. 36°
" " 39°

Heating for half an hour at 190°C. did not seem to cause any great change in the chemical constants, but there was a general tendency for the values to be slightly lowered by the heat treatment. Of the different constants, the saponification and iodine values seemed to be the least affected and the acid value most influenced, with the volatile acid occupying an intermediate position.

The increase in the free fatty acids and decrease in iodine value, noticed by Dyme *et al.*, [1941], were not observed here probably because the heating in this test was only of comparatively short duration. The small decreases in saponification and iodine values, as well as the somewhat greater fall in acid value, noted in this experiment, agree with the findings of Roy [1944]. The effect on the chemical constants, due to thermal treatment was more or less similar in all the fats studied.

Heating caused a loss of vitamin A potency to the extent of 13 to 43 per cent in the different fat samples. The destruction of vitamin A potency in the ghee samples was generally much more than in the case of most of the oils. The loss of carotene suffered by the oils on heating was highest in the case of mustard oil and least in cotton-seed oil. The *vanaspatis* did not contain any vitamin A or carotene.

Karmarkar [1944] observed a loss of 75 per cent or more of the vitamin A content of ghee on cooking. Ahmed *et al.*, [1946] noticed that in frying 'purees' 63-69.5 per cent of the vitamin A was destroyed, while in making curries, involving frying from 22-45 minutes, the loss was complete. De and co-workers [1946] also found that both ghee, and margarine fortified with carotene, lost much of their vitamin A activity when used for frying. In this study, heating for half an hour at 190°C. which is similar to that of ordinary cooking or frying, was found to cause somewhat smaller but substantially serious losses of the vitamin A potency of the ghee.

CHAPTER V

DIGESTIBILITY OF FATS AND OILS

By N. D. KEHAR, T. S. KRISHNAN, S. N. RAY, I. KUMAR and A. K. PAL

FAT in the dietary is very important as a source of energy. The availability of this energy to the tissues would depend essentially on the extent to which they are digested and absorbed by the body. A long series of experiments on the digestibility of fats, conducted on humans, have been summarised by Langworthy [1923]. The digestibilities of about two dozen animal, three dozen vegetable and half a dozen hydrogenated fats have been included in this report. It was found that they were generally well utilized, the digestion coefficient being about 95 per cent or more, provided their melting point was not much above the body temperature. No marked differences in digestibility which relate solely to the origin of the fats, were observed. Earlier, Deuel and Holmes [1921, 1922] had reported on the influence of melting point on the digestibility of fats, by carrying out experiments with hydrogenated corn, cotton-seed and peanut oils melting at different temperatures, prepared either by straight hardening or blending. It was found that all these types of hydrogenated oils were well absorbed, the digestibility being about 95 per cent or more when the melting point was 46°C. or below. When the melting point was above 50°C., however, there was a very sharp decline, the absorption of hardened peanut oil with melting point 52.4°C. being only 79 per cent. Under similar conditions there were no differences between the different oils with regard to their digestibility. There was a tendency for the digestibility to decrease with progressive rise in the melting point, the decline being marked only when the melting point was well above body temperature. This has been confirmed by Crockett and Deuel [1947] as well as Augur *et al.*, [1947]. Hoagland and Snider [1940, 1942], however, state that there was no systematic relationship between the melting point and the digestibility of the fats, when the melting point ranged from 39° to 56°C. Nor did they find any consistent relationship between digestibility of the fat and either the linoleic acid or saturated fatty-acids contents. Later, however, they [Hoagland and Snider, 1943a] found that the digestibility of fats was greatly influenced by the amount of the poorly digested stearic acid present in the same. Baldwin [1940] also confirmed that the digestibility coefficient bore a consistent relationship to the stearic acid and arachidic acid content of the series of hydrogenated fats and oils he tested, since these constituted the predominant fatty acids of the faecal lipoids. He did not find any relationship between the melting point, iodine value, or total saturated fatty-acid content and digestibility, in these fats. Further support to the part played by the stearic acid has been given by Crockett and Deuel [1947], whose study of the influence of melting point on digestibility led them to conclude that the increased fat excretion, observed with high melting-point fats, was due to the rats' inability to absorb the large amounts of palmitate and stearate formed by hydrogenation, rather than to a failure of lipolysis. Mattil *et al.*, [1945, 1946] as well as

Norris and Mattil [1946] have also studied this subject and demonstrated that stearic acid was very much unabsorbable, although better utilized when fed as a mixed triglyceride than as a tristearin mixed with triolein. It was suggested that this might be due to inter-esterification of glycerides during digestion. Mattil [1947] after a careful analysis of the published data, concludes that "the primary factor which limits the digestibility of fats is without doubt the amount of solid fatty acids present and the degree of limitation increases with chain length." He also showed that a positive co-relation existed between the digestion of fats found in human adults and those of corresponding fats observed in albino rats.

Most of these experiments on the digestibility of different fats and oils by foreign workers were carried out on a basal ration containing abundant quantities of vitamins. Since dietary surveys in India had shown that the food ingested by the majority of the population, viz. the poorer classes, was sub-adequate in essential nutritive factors particularly vitamin A, it was decided to carry out digestibility tests with different oils and fats to find out whether they showed any variation when added to a basal ration sub-optimum in vitamin A.

Experiments were carried out with adult male rats to find out the digestibility coefficient of the cow *ghee*, buffalo *ghee*, lard, seven brands of *vanaspathis* as well as groundnut, sesame, mustard, cotton-seed, *mahua* and coconut oils. The raw and refined samples of each of the oils were studied separately. Four to six adult male rats, weighing about 300 gm. each, were selected from the stock colony and housed in individual cages, made of 3/16 inch mesh wire netting and of the usual cylindrical pattern. The floor of the cage was about two inches above the ground level and was made of wire grid, with the strands half inch apart, to enable the faeces and urine to pass through, immediately they were voided. Each cage was placed in a galvanised iron tray, in which it fitted loosely. During experimental periods when quantitative faeces collection was made, the tray was lined with filter paper. Fresh water was provided in self-feeding bottles fixed to the top of the cage. The food was given in small glazed earthenware cups, which were cleaned every day. The animals were at first put on the following fat-free ration :

Dextrinised starch	64	} Plus 5 γ of β -carotene plus 10 I.U. of vitamin D per rat per day.
Casein (extracted)	24	
Yeast (extracted)	8	
Salt mixture*	4	
Total		100

* (McCollum *et al.*, 1916)

The rats were maintained on this ration for eight days to completely eliminate the effects of the previous diet. They were fed *ad lib* throughout. All the animals were weighed twice every week during the preliminary period and daily during the experimental period.

At the end of this preliminary period, a digestion experiment was carried out for eight days, in two periods of four days each, with each of these rats. During this period of experiment, the exact food consumption was determined and the

whole of the faeces excreted was quantitatively collected. The daily excretion of faeces was transferred to small conical flasks containing some absolute alcohol, one for each rat, till the four days were over. The second four days collection was also made similarly and stored in separate flasks. The fat content of the faeces was determined in each of the samples and the average endogenous excretion was deduced for each rat.

The fat in the faeces was extracted by a modification of the method of Boyd [1933]. The faeces was ground up thoroughly with pure sand and 3:1 alcohol-ether (made from purified alcohol and ether) and the fat completely extracted by repeated trituration with this solvent, warming on a water bath to help the extraction. The extract was then filtered into a flask, the solvent evaporated off under reduced pressure and dried in a vacuum desiccator. The residue in the flask was next taken up with pure chloroform, filtered into a tared flask, the chloroform evaporated off and the fat estimated by drying to constant weight.

After the collection period of eight days was over, the animals were switched on to the following diet :

Dextrinised starch	59	} Plus 5γ of β-carotene plus 10 I.U. of vitamin D per rat per day.
Casein (extracted)	24	
Yeast (extracted)	8	
Salt mixture	4	
Experimental fat or oil	5	
Total		100

The oils and fats, digestibilities of which were to be determined, were tested one after another. When cow *ghee* formed the dietary fat, the vitamin A intake of the rats was equated to that of the oil or *vanaspati* groups. In these digestibility experiments a preliminary period of four days was allowed which was followed by an experimental period of eight days, divided into two periods of four days each, exactly as on the fat-free diet. The estimation of the food consumption, faeces collection as well as the determination of their fat content was carried out as before.

The digestibility coefficient of the fat was determined after correcting for their endogenous fat excretion, with each individual animal, in both periods and the average of all the eight to twelve figures was taken as the digestibility of the fat or oil.

RESULTS AND DISCUSSION

A consolidated summary of all the digestibility figures of the different fats and oils is given in Table XIII. It would be noticed from the same that all of them were digested practically completely and to about an equal extent. A statistical analysis reveals, however, that the digestibility coefficients of Dalda only, among the *vanaspatis* and raw and refined cotton-seed oils, show figures significantly lower than *ghee* or most of the other fats and oils. The digestibility coefficients of raw coconut oil, refined *til* oil and raw mustard oil were significantly superior to

that of cow *ghee*. However, the differences, i.e. the inferiority or superiority of the *vanaspathis* and oils over *ghee* are all barely significant at 5 per cent level only.

TABLE XIII

Digestibility of fats and oils
(Corrected for endogenous excretion)

Fats or oils	Digestibility coefficient	
		*
1. Cow <i>ghee</i>	97.47	± 0.35
2. Buffalo <i>ghee</i>	98.51	± 0.52
3. Dalda (a)	94.78	± 0.78
4. First quality No. 1	96.37	± 0.45
5. Swastika	96.79	± 0.41
6. Kotogem	97.03	± 0.28
7. Binaula	96.70	± 0.60
8. Temple	98.10	± 0.92
9. Rajhans	96.05	± 0.37
10. Groundnut oil, raw	97.28	± 0.49
11. Groundnut oil, refined	97.60	± 0.36
12. Cotton-seed oil, raw]	94.71	± 0.46
13. Cotton-seed oil, refined	94.55	± 0.77
14. Coconut oil, raw	98.86	± 0.20
15. Coconut oil, refined	97.19	± 0.36
16. <i>Mahua</i> oil, raw	98.54	± 0.28
17. <i>Mahua</i> oil, refined	98.17	± 0.27
18. <i>Til</i> oil, raw	96.19	± 0.65
19. <i>Til</i> oil, refined	98.74	± 0.32
20. Mustard oil, raw	98.75	± 0.18
21. Mustard oil, refined	98.58	± 0.19
22. Lard	98.28	± 0.17
23. Dalda (b)	95.48	± 0.47

* Standard error.

(a) Purchased from open market.

(b) Supplied by manufacturers.

It would be clear from the data given above that there was no material difference of any great practical significance between the digestibilities of cow *ghee*, vegetable oils, *vanaspathis* and lard. In oils also there was no indication of any consistent relationship between refining or hydrogenation and digestibility. These digestibility data are in agreement with the results obtained by foreign workers. Basu and Nath [1946], who carried out digestibility trials with men, obtained values very similar to those observed with the rats in this experiment, for all fats other than hydrogenated groundnut oil. In their case the digestibility of groundnut oil decreased from 99.0 to 89.8 per cent when it was hydrogenated. Hence, they surmised that hydrogenation led to the lowering of the digestibility of the oil to some extent. A similar though much less lowering was seen in this series of experiments when the figures for raw or refined groundnut oil were compared with those of Dalda. As against the above findings Misra and Patwardhan [1948] could not confirm the same, as they found that hydrogenation had no effect on the digestibility of groundnut oil when the melting point of the hardened product ranged from 29.5°C. to 49°C. Even the presence of 9 and 18 per cent of iso-oleic acid did not seem to have any effect on the digestibility of the oil, according to them. Nhavi and Patwardhan [1946] also noticed that hydrogenation had little or no influence on the rate of absorption of the fat. Dutt [1945], however, stated that the utilization of the fatty acids of hydrogenated fat was about 3 per cent less than that of the butter-fat. Basu and Nath [1946] did not find any material differences in digestibility between butter-fat and the natural vegetable oils.

Deuel, Cheng and Morehouse [1948] noticed that crude rape-seed oil gave a comparatively low digestibility of 77 per cent, which was raised to 82 per cent on refining. They remarked that this oil had the lowest digestibility of any fat, liquid at ordinary temperature, which had been investigated in rats. However, in a later paper from the same laboratory, it was found that rape-seed oil had a digestibility coefficient of 99 per cent, when human beings were used as subjects [Deuel *et al.*, 1949]. This value is practically identical with that found by us in rats. Basu and Nath [1946] obtained a value of 94.1 per cent as the digestibility of mustard oil, which is very similar to 98.8 per cent found in this experiment. Refining made no change in the above figure in the present study. Basu and Nath carried out their experiments on men but their figures tally well with the present results obtained on rats. The digestibility values obtained by Deuel *et al.*, (*loc. cit.*), though also carried out on rats, were, however, much lower. In this connection the observation of Bhalerao *et al.*, [1947], that though vegetable oils generally gave an absorption rate not significantly different from that of butter-fat, mustard oil gave a much lower value, may be of help. But Basu and Nath [1946a] who studied the absorption rates of different oils and fats, found that though olive oil and butter-fat showed better rates of absorption at the end of two hours, mustard showed the maximum rate after four hours, and after six hours all the oils behaved nearly alike.

In view of this conflict of findings here, it would be difficult to explain the difference without further study. It is possible, however, that the origin and history of the crude rape-seed oil used by Deuel *et al.*, (*loc. cit.*) had something to do with its poor digestibility, because on refining it showed an appreciable improvement which had not been noticed in this study, where the effect of refining had not been found

to show any consistent or significant effect on the digestibility of six different crude oils.

It would, therefore, appear from the above data, that all the fats tested were digested nearly completely and to about an equal extent, showing no great marked difference in this respect between cow and buffalo *ghee*, vegetable oils, *vanaspatis* and lard. Neither refining nor hydrogenation, as studied in this experiment, seemed to have any significant influence on the digestibility of the oil.

CHAPTER VI

Part I

GROWTH PROMOTING VALUE OF FATS AND OILS WHEN INCORPORATED IN A SYNTHETIC DIET

By N. D. KEHAR, T. S. KRISHNAN, S. N. RAY, I. KUMAR, B. C. JOSHI, A. K. PAL,
K. SAHAI and R. CHANDA.

IN the previous chapter, it has been shown that as compared with *ghee* the vegetable oils and their hydrogenated products (*vanaspatis*), generally have more or less similar digestibility. A study of their chemical constants and composition indicates that the only important difference between *ghee* and other fats is the lack of vitamin A in any appreciable quantity in the latter. In this chapter, an attempt has been made to study whether *vanaspatis* and vegetable oils, when incorporated in a synthetic diet have the same effect as *ghee* on the growth of young rats, taking care to equalise the vitamin A content of the different fat rations. This has been done as there is a wide-spread belief in this country that *vanaspatis* are injurious to health. Even among research workers, as seen in Chapter I, there is considerable difference of opinion on the comparative nutritive value of animal and vegetable fats, particularly of butter-fat (*ghee*) as compared to the common vegetable oils, both in the natural and hydrogenated form.

In one important respect, however, our experiments differ from the foreign studies. Practically in all the investigations referred to in Chapter I, the diets were adequate in all the essential nutritive factors like proteins, minerals, vitamins, etc. However, dietary surveys in this country have shown that the poorer classes, who form the bulk of the population, generally subsist on a sub-adequate diet and unless this fact is taken into consideration in planning the experiments, the results will have little practical value. Further, experiments in this laboratory and elsewhere have shown that the bad effects of a deficient diet may not often be apparent in the first generation but might manifest themselves more clearly in the second and subsequent generations. After taking into consideration the above factors, a synthetic diet sub-optimum in vitamin A was used as the basal ration and the study was extended over three generations of rats, which were used as experimental animals, except in Experiment II, where the investigation was carried out over one generation only.

Experiment I. Ghee versus vanaspatis

Weanling rats, similar in weight and all aged four weeks, were removed from our stock colony and were divided into ten groups. Each group contained four males and six female rats. The fats cow *ghee*, buffalo *ghee*, three brands of hydrogenated groundnut oil, viz. 'Dalda', 'First quality No. 1', 'Swastika', three brands of hydrogenated cotton-seed oil, viz. 'Kotogem', 'Cotex' and 'Binaula', and two brands of hydrogenated sesame oil, viz. 'Temple' and 'Rajhans' were examined. The hydrogenated fats were purchased from the open market in

April 1944 and again in April 1945, whereas, the *ghee* samples were supplied by the Director, Dairy Research Institute, Bangalore, in three lots; the first lot being supplied in April 1944, the second in November 1944 and the third in September 1945. The basal ration, hereafter called 'Diet A', used during the first six weeks of experiments was composed of cane sugar—62 parts, casein (alcohol and ether extracted)—24 parts, mineral mixture [McCollum, *et al.*, 1916]—4 parts, and yeast (petroleum ether extracted)—5 parts. *Ghee* or *vanaspatis* were added to this diet at the rate of 5 per cent. In addition, 5 micrograms of carotene and 1 microgram of calciferol were given to *vanaspati*-fed rats and smaller amount of carotene supplement to rats of *Ghee* groups, so that the quantity of vitamin A received by them was of the same order (i.e. 8-9 I. U. daily) as that received by the *vanaspati*-fed animals.

In a preliminary experiment, the growth obtained on such a diet was rather poor and was traced later to the poor quality of yeast at our disposal. The diet was, therefore, slightly modified by administering 10 ml. of skim milk per rat per day when the growth became satisfactory though not as good as that of rats in our stock colony. As our stock colony animals receive fresh greens and liver regularly, it is possible that the poorer growth rate of rats on the modified Diet A was caused by a lack of sufficient vitamins and perhaps other factors as well. The importance of the use of this sub-optimum diet will be discussed later.

From the seventh week onwards, i.e. from ten weeks of age until they were discarded, the rats were shifted to a cereal diet (Diet B) having a composition of maize—66 parts, whole wheat flour—22 parts, crude casein—9 parts, yeast—2 parts, NaCl—0.5 part and CaCO_3 —0.5 part. Ninety-five parts of this diet were mixed with 5 parts of *ghee* or *vanaspati*, each group receiving the same fat it was getting during the previous period. In addition, each rat in all the groups received daily 10 ml. of skim milk and 5 g. of fresh greens, usually lucerne or berseem.

The change over to Diet B was used for two purposes. Firstly; our main object in undertaking this work was to investigate whether the inclusion of *vanaspatis* daily in natural diet continuously over three generations would have any bad effect. The Diet B represents a good vegetarian diet, containing abundant quantities of vitamins and minerals. The first six weeks of the synthetic diet regime was intended to give some quantitative idea about the comparative nutritive values of the different *vanaspatis* with regard to each other as well as to *ghee*. Secondly, as already mentioned, the Diet A was a sub-optimal feed containing barely sufficient vitamin A and probably slightly deficient in certain B-vitamins. The switch over to an abundant diet helped us to examine whether the supply of excess of vitamins and of possibly other factors was helpful in dietaries containing hydrogenated fats.

As soon as the animals were four months of age the females of each group were mated with the males of the same group, i.e. receiving the same fat. The reproductive and lactating capabilities of the different groups of rats will be dealt in a later chapter.

From the litters thus born in each group, the heaviest member of each litter of the same group was selected (six males and six females being thus selected) and kept on Diet A plus the respective fats their parents were receiving. The experimental procedure followed was the same as before, and in the third generation offsprings obtained were also treated exactly as in the first generation. In this way

the continuous effect of feeding each fat through three complete generations was observed. The average gains in weight during experiment of the rats in the different groups and different generations are shown in Table XIV.

It will be noticed that the growth rate of the rats on the Diet A and *vanaspatis* (i.e. during the first six weeks after weaning) was slower than those on the same diet plus cow or buffalo *ghee*. The effect was more pronounced in the male rats and the rates were significantly lower in the second and third generations. A statistical analysis carried out on the weekly growth rates of the rats fed on the different fats (Table XV) reveals that in the first generation the fats do not differ significantly between themselves in respect of promotion of growth. In the second generation, however, the male and female rats of the cow and buffalo *ghee* groups were found to exhibit growth rates significantly greater than those obtained for all the *vanaspatis*. Among the *vanaspatis*, the rats getting 'Kotogem' showed a significantly lower growth rate than most of the other hydrogenated fats. In the third generation, the rats of the *vanaspati* groups showed slightly better growth rates than those of the corresponding groups in the second generation, whereas the *ghee*-fed rats exhibited similar rates in both the generations. In spite of this, the growth of the male rats in most of *vanaspati* groups was significantly lower than that of the *ghee* groups. The only exceptions were the male rats receiving 'Cotex' and 'Temple' brands of *vanaspatis*, the difference between whose growth rates and those of the cow or buffalo *ghee* groups were not statistically significant. The female rats of this generation, however, grew equally well, irrespective of receiving *ghee* or any of the *vanaspatis*. The significance of these differences in the third generation as compared to the results of the second generation, was perhaps due to the use of fresh and better type of yeast, the previous stock used in the first and second generations having been used up. However, the analysis on the comparative growth rates of male and female rats taken together shows that the 'Rajhans', 'Dalda', 'Binaula' and 'First quality' groups of rats grew at significantly lower rates than the rats of the *ghee* group.

When the results of the male and female rats of all the three generations were pooled together, it was observed that the cow and buffalo *ghee* groups did not differ significantly, whereas all the *vanaspati* groups were found to yield growth rates significantly lower. Among the *vanaspatis*, 'Kotogem' gave the lowest growth rate, which was significantly lower than that given by a number of other *vanaspatis*. These statistical results are given in a precise form in Table XV.

It may be observed from Table XVI that, as soon as the rats were put on Diet B (adequate diet), the *vanaspati*-fed rats made very rapid gains, and their weights at the end of ten weeks were very nearly the same as of those receiving *ghee*. This shows that the *vanaspatis* by themselves are probably not harmful, but that their nutritive effects are dependent to a great extent on the nature of the basal ration used. That the growth inhibiting property of *vanaspatis* is absent in an adequate diet was further brought out in another experiment. Rats of the second generation from the various *ghee* and *vanaspati* groups were put directly after weaning on Diet B plus *ghee* or *vanaspati* at 10 per cent level; the rats in the various groups receiving the same fats their parents were getting. Two male and two female rats were used in each group. Five grams of greens and 10 ml. of skim milk were also given to each animal daily. The growth rates of the various groups are given in Table XVII.

TABLE XIV

Growth of rats on Diet A for six weeks: Average gain in weight in six weeks (in gm.)

Fats	Males			Females		
	1st generation	2nd generation	3rd generation	1st generation	2nd generation	3rd generation
Cow ghee	92.9±4.2	59.3±4.1	65.5±2.6	72.0±3.3	56.2±1.8	54.7±2.0
Buffalo ghee	90.0±6.6	62.8±3.3	63.4±2.0	69.8±3.6	48.5±1.9	53.8±4.4
Dalda	80.8±3.1	36.3±2.3	51.9±5.2	65.3±2.0	47.2±2.2	52.9±2.8
1st Quality	82.9±9.7	40.3±1.8	49.2±2.9	69.0±2.4	43.2±2.5	45.8±4.7
Swastika	82.0±1.9	37.2±2.7	51.3±2.9	66.3±3.9	35.2±1.9	55.9±4.0
Kotogem	75.5±7.5	33.9±3.4	50.0±3.8	64.3±2.9	31.3±1.6	50.7±3.1
Cotex	86.4±4.1	40.3±1.5	57.9±2.5	67.7±2.8	38.8±2.7	49.1±2.9
Binaula	87.8±6.9	36.0±2.4	50.0±3.7	66.6±2.8	38.8±3.8	49.8±3.0
Temple	77.8±3.5	46.8±3.6	55.5±5.8	70.2±2.5	38.0±3.7	58.5±3.9
Rajhans	99.6±8.7	46.5±2.2	46.3±3.8	66.9±1.9	32.9±2.7	57.9±2.5

N.B. Standard error is given for all the means.

TABLE XV

Average growth rate of animals in Experiment I

(in gm. per week)

Fat	Generation I	Generation II	Fat	Generation III	Fat	Pooled for all generations
Cow ghee	13-20	9-66	Cow ghee	10-22	Cow ghee	10-90
Buffalo ghee	13-00	9-71	Buffalo ghee	10-10	Buffalo ghee	10-82
Temple	12-18	7-36	Swastika	9-73	Rajhans	9-32
Dalda	11-87	7-08	Temple	9-56	Temple	9-31
First quality	12-39	6-99	Cotex	9-05	Cotex	9-22
Cotex	12-46	6-68	Kotogem	9-03	Swastika	9-13
Rajhans	13-09	6-57	Rajhans	8-93	Dalda	9-09
Binaula	12-44	6-38	Dalda	8-78	First quality	9-03
Swastika	11-94	6-17	Binaula	8-45	Binaula	8-90
Kotogem	11-17	5-57	First quality	8-27	Kotogem	8-44

NOTE. The averages under the same vertical line do not differ among them at 5 per cent. level of significance.

In this as well as in the other statistical Tables, the growth rates have been computed from the regression equation of the weekly body weights on time.

TABLE XVI

Growth of rats in 4 weeks on Diet B : Average gain in weight (in gm.)

Fat	Males			Females		
	1st generation	2nd generation	3rd generation	1st generation	2nd generation	3rd generation
Cow ghee	52.4±6.4	64.7±1.2	38.3±2.1	33.2±2.0	44.9±2.2	31.4±2.8
Buffalo ghee	43.9±6.1	87.7±4.5	50.7±2.7	31.3±4.0	46.7±2.0	40.8±4.2
Dalda	63.5±2.8	26.6±2.7	65.4±6.3	37.1±0.7	51.8±2.2	34.8±2.2
1st quality	60.9±4.3	97.6±1.9	41.3±3.0	31.0±3.6	50.9±2.2	33.0±3.3
Swastika	39.1±6.2	95.7±1.8	59.8±2.5	37.6±3.1	51.1±1.1	33.7±2.6
Kotogem	62.5±4.0	98.9±3.8	58.5±2.1	31.6±1.6	63.4±1.3	41.5±4.2
Cotex	59.9±3.9	94.9±1.6	78.3±3.3	32.1±2.9	57.1±2.5	59.4±2.6
Binaula	56.8±4.0	94.4±5.1	51.2±3.3	32.0±2.6	53.3±3.3	39.5±1.6
Temple	59.6±2.9	97.4±3.3	58.8±4.7	34.6±2.6	58.3±3.1	39.5±4.8
Rajhans	47.6±6.2	96.1±4.3	60.8±2.3	31.9±1.6	60.9±3.8	43.3±1.8

It will be seen that, in spite of the higher level of fat given, six of the *vanaspali* groups of animals showed as good growth rates as the *ghee*-fed animals, whereas, as may be seen from Table I, their litter mates, on the restricted diet plus *vanaspali*, showed very poor growth rate. The comparative growth rates of the different groups are tabulated below in a numerically descending order :

S. No.	Fat	Average growth rate in gms./week	
1.	Buffalo <i>ghee</i>	20.14	
2.	Dalda	20.08	
3.	First quality	19.79	
4.	Cow <i>ghee</i>	19.24	
5.	'Cotex'	18.37	S. E. of mean = ± 0.62 gm.
6.	'Rajhans'	17.36	
7.	'Binaula'	16.99	
8.	'Temple'	16.95	
9.	'Swastika'	16.37	
10.	'Kotogem'	15.98	

The averages covered by a line do not differ among themselves at 5 per cent level of significance.

TABLE XVII

Growth rate of rats of second generations placed after weaning on Diet B plus 10 per cent fat.

Name of fat	Average increase in weight in 6 weeks (in gm.)
Cow <i>ghee</i>	113.3 \pm 7.6
Buffalo <i>ghee</i>	121.0 \pm 11.2
'Dalda'	117.9 \pm 11.1
'1st quality No. 1'	115.7 \pm 11.2
'Swastika'	98.0 \pm 5.8
'Kotogem'	94.6 \pm 6.3
'Cotex'	106.3 \pm 11.0
'Binaula'	101.3 \pm 10.6
'Temple'	102.1 \pm 10.5
'Rajhans'	99.4 \pm 7.6

These results, together with the data obtained in the investigation discussed before as well as those carried out afterwards, show that the poor growth rate of the rats receiving *vanaspatis* always improved whenever they were shifted from a sub-optimum to a liberal diet. After four to five weeks on the liberal diet, their final weights were generally nearly equal to the *ghee*-fed rats. Thus, it would be seen that hydrogenated vegetable oils, when added to a diet, sub-optimum particularly in Vitamin A and possibly also in other factors, retarded the growth rate of young animals in the second and subsequent generations.

The animals of all the groups maintained good health in general. Though extensive alopecia was seen especially in the second generation in all the *vanaspatis* groups, the animals of the *ghee* groups were not affected. The alopecia disappeared soon after the rats were put on Diet B.

Experiment II. Heated ghee versus heated vanaspatis.

As *ghees* and *vanaspatis* are generally used as frying media and the heated fats form a large percentage of the total fat ingested, it was decided to find out also the comparative nutritive values of heated *ghees* and *vanaspatis*. A perusal of the available literature showed that Beck and Peacock [1941] found that digestibility of the fats was progressively lowered as they were heated for increasing lengths of time and the rate varied considerably from fat to fat.

Young rats, on weaning, were kept on Diet B supplemented with 10 c.c. of skim milk and 5 gm. greens per rat per day. The rats, both male and female, were obtained from the third generation of the *ghee* and *vanaspatis*-fed parents and the

TABLE XVIII

Average increase in body weight (in gm.) in six weeks of rats receiving various heated fats

Name of heated fats	Males	Females
Cow <i>ghee</i>	115.5 ± 5.4	86.2 ± 2.0
Buffalo <i>ghee</i>	114.0 ± 3.6	73.3 ± 2.3
'Dalda'	114.0 ± 2.8	82.5 ± 1.0
'1st quality'	102.7 ± 2.0	85.0 ± 3.1
'Swastika'	96.8 ± 1.9	74.0 ± 4.3
'Kotogem'	109.5 ± 5.7	87.8 ± 2.1
'Cotex'	100.2 ± 2.0	74.0 ± 2.1
'Binaula'	80.0 ± 4.0	63.2 ± 3.4
'Temple'	92.8 ± 1.7	78.3 ± 0.6
'Rajhans'	97.0 ± 5.5	81.8 ± 3.9

groups were so arranged that the offsprings from the cow *ghee* group received 'heated cow *ghee*', those from the 'Dalda' groups received 'heated Dalda', and so on. Three males and three females were kept in each group. The fats were heated to 200°C. and were kept at this temperature for half an hour. After cooling, they were mixed to the extent of 5 per cent with diet B. As in the other experiments, the food was supplied *ad lib*. The growth record for six weeks, on such a diet, is given in Table XVIII.

It will be seen that even with a liberal diet, the male animals of all the *vanaspathi* groups, with the exception of those in the 'heated Dalda' group, showed comparatively lower gain in weight than the animals of the heated cow or buffalo *ghee* groups. Statistical analysis of the results were carried out and the results are given below:

MALE		FEMALE	
Fat	Growth rate in grams per week	Fat	Growth rate in grams per week
Kotogem	17.93	Kotogem	15.30
Buffalo <i>ghee</i>	17.84	First quality	15.17
Cow <i>ghee</i>	17.70	Cow <i>ghee</i>	15.06
First quality	17.47	Dalda	14.07
Kotex	16.25	Rajhans	13.86
Dalda	16.07	Temple	13.60
Swastika	15.90	Swastika	12.95
Rajhans	15.28	Kotex	12.65
Temple	15.02	Buffalo <i>ghee</i>	12.62
Binaula	11.47	Binaula	10.20

NOTE.—Averages under the same vertical line do not differ among themselves at 5 per cent level of significance

It will be seen that the male rats of the heated 'Swastika', 'Rajhans', 'Temple' and 'Binaula' groups were significantly lighter in weight than the corresponding animals of either heated cow or buffalo *ghee* groups, whereas, for the females no such difference was observed. The buffalo *ghee* group females, however, showed comparatively much poorer growth. It appears, therefore, that heating *vanaspatis* seemed to exercise a depressing effect on the growth to a certain extent because from Table XVI and subsequent statistical analysis it was seen that the rats getting Diet B plus unheated *vanaspatis*, even at ten per cent level, except those of 'Swastika' and 'Kotogem' groups, did not show any slackening of growth as compared with the animals on similar liberal diet plus cow or buffalo *ghee*.

Experiment III. Ghee versus vegetable oils and other fats

This experiment was undertaken as vegetable oils form the general fat component of the common man's dietary, and there is dearth of accurate information on the nutritive value of the common vegetable oils as compared to *ghee*. Further, this study was also intended to throw light on whether the lower growth rates seen in the animals receiving *vanaspatis*, under certain dietary regimes, was caused by hydrogenation of the oils or the defect was inherent in the parent oils. Lard was also included in the study as it is in common use. All the raw and refined oils mentioned in Chapter V were used for this study. For experimental convenience, the oils and fats were grouped into two series, A and B. Series A consisted of raw and refined samples of groundnut (first generation only), cotton-seed, coconut and *mahua* oils, lard, 'Dalda' and cow *ghee*, while Series B comprised raw and refined groundnut, *til*, and mustard oils, 'Dalda' and cow *ghee*. 'Dalda' and cow *ghee* were included in both Series as reference fats.

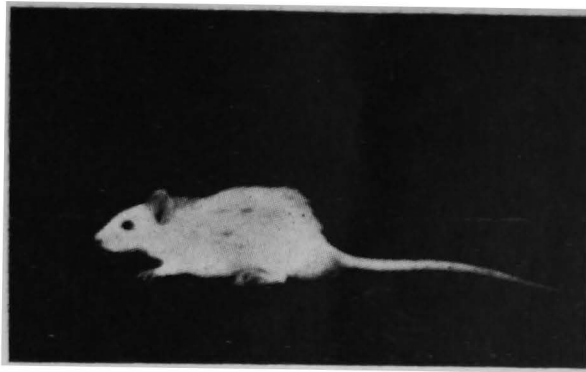
The experimental procedure was very similar to that followed in Experiment I except that (a) the basal ration had a slightly different make up, viz. dextrinised starch—59, casein (extracted)—24, yeast (extracted)—8, salt mixture—4, experimental oil or fat—5 parts, (b) the experimental feeding extended over eight weeks instead of six weeks and (c) in place of a cereal ration from the seventh week, the basal ration was liberalised.

This liberalisation of the diet as mentioned in (c) above was necessary for the following reasons:

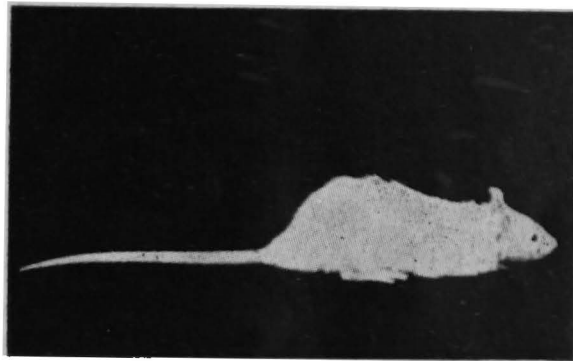
It was originally planned to observe the effect of feeding the above experimental diet over a period of 12 weeks, and then mate them to get the young ones for the next generation tests. The first generation experiments with Series A were carried out in accordance with the above plan. However, when the rats were mated after twelve weeks of feeding on the basal diet, they failed to reproduce.

In order to get the progeny for the second generation studies, those animals which failed to conceive were each given a supplement of 20 ml. skim milk, 5 gm. greens and 0.5 mg. alpha-tocopherol, daily. After four weeks on this regime they were re-mated. This proved successful and so the feeding schedule of the subsequent experiments was slightly altered. The foregoing basal diet was given for the first eight weeks after weaning, and the weight increase recorded in this period was

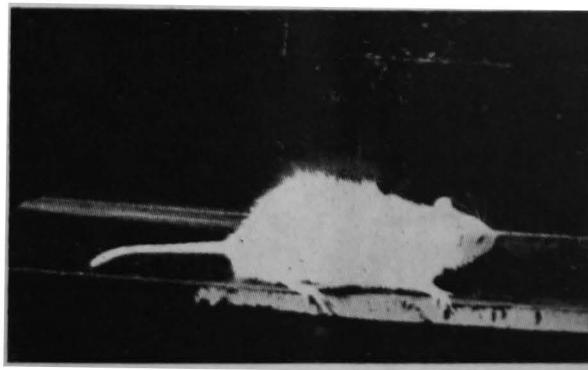
PLATE, I



A

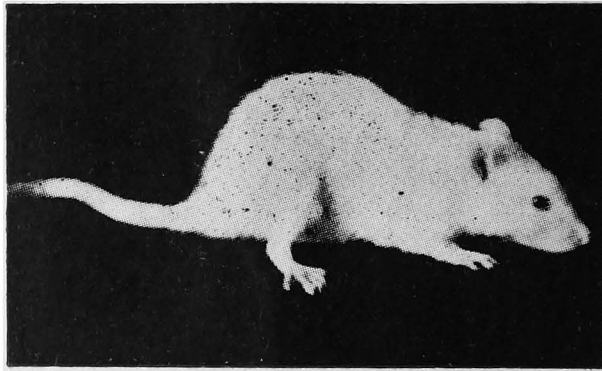


B

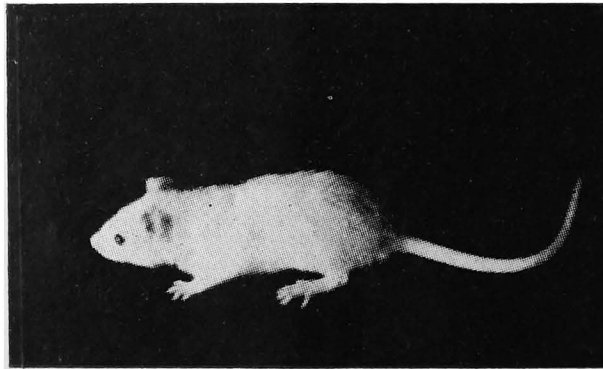


C

In the first generation of the synthetic basal ration series no difference in growth is observed between (A) *ghee* (B) *vanaspati* and (C) oil groups (p. 47)



A



B



C

In the second and subsequent generations of the synthetic basal ration series, rats on (A) cow *ghee* show better growth than those on (B) *vanaspati* and (C) oils (p. 47)

taken to represent the growth promoting value of the fat. From the beginning of the ninth week, the basal ration mentioned above was supplemented with 20 ml. skim milk, 5 gm. greens and 0.5 mg. alpha-tocopherol, per rat per day in all the groups. After four weeks on this liberalised ration, i.e., when they had completed twelve weeks of feeding and were four months of age, the rats were mated as usual within each of the groups to get the next generation offsprings. All the animals that had been mated were kept on this supplemented diet till the young ones were weaned at four weeks of age, i.e., for the whole of the gestation and lactation periods also. For the second generation tests, a representative group of twelve young ones (six males and six females) was selected from the weanlings of each of the fat groups, the young ones nearest the average weight in each of the litters being selected as far as possible. They were then switched on to the basal ration containing the same oil or fat their parents were getting. The feeding, weighing, etc., were carried out in exactly the same manner as in the first generation. The whole procedure was continued and repeated till the fourth generation* feeding tests, lasting for eight weeks, were completed.

This change in feeding procedure, of giving the supplemented diet from the beginning of the ninth week, although primarily meant to obtain litters for the subsequent generation experiments, also helped to study the effect of liberal feeding on the comparative growth-promoting value of the different fats. The growth-promoting values of the fats have been considered both on the basal ration fed for the first eight weeks, as well as on the supplemented one, ingested during the next four weeks. The results are given in Table XIX.

It would be seen from this Table that there was no significant difference between the growth rates of the groups of animals getting the different fats in the first generation (Plate I). In the second and subsequent generations, however, cow *ghee* was significantly superior to most of the other fats and oils (Plate II). It may not be out of place to mention here that we could not extend the observations in all the fat groups to the fourth generation, as in the groups like refined cotton-seed and lard, the majority of the animals died and it was not possible to get the subsequent generation. The stock of *til* oil and crude cotton-seed oil having been exhausted these groups too could not be carried forward to the fourth generation. Among the oils, crude *mahua* group fared better than the others in the second generation. However, in the fourth generation, even these lost ground and resembled the other oils in being significantly inferior to cow *ghee*. When compared against 'Dalda,' the parent oil did not give a significantly different growth effect in any generation. The females generally showed less differences among the different groups than the corresponding males.

The results observed with refined oils were rather interesting. All the refined oils gave somewhat better growth than the corresponding crude oils in the first generation. But in the second and subsequent generations the animals receiving

* Owing to an unfortunate accident the third generation experiments were spoilt and had to be rejected. The study was, therefore, extended to the fourth generation.

TABLE XIX
Increase in weight (in gm.) of rats on synthetic diet—5 per cent fats or oils
(Average increase in eight weeks)

No.	Fat or oil	Series	MALES			FEMALES		
			1st generation	2nd generation	4th generation	1st generation	2nd generation	4th generation
1	Cow ghee	A	93.0 ± 5.77	109.8 ± 2.26	114.5 ± 3.35	86.1 ± 3.62	91.2 ± 2.30	87.3 ± 2.70
2	Cow ghee	B	108.6 ± 3.87	122.4 ± 7.06	..	94.4 ± 3.82	93.4 ± 2.26	..
3	Groundnut oil raw	A	87.6 ± 8.11	82.7 ± 4.51
4	Ground nut oil	B	117.7 ± 4.41	93.3 ± 6.28	96.1 ± 6.40	86.3 ± 3.58	75.7 ± 5.00	70.7 ± 2.91
5	Ground nut oil refined	A	104.1 ± 6.20	95.4 ± 3.75
6	Groundnut oil refined	B	119.7 ± 7.08	99.3 ± 4.15	91.4 ± 2.96	95.8 ± 3.43	76.7 ± 3.39	73.2 ± 3.45
7	Cotton-seed oil raw	A	97.7 ± 8.17	77.7 ± 11.44	..	93.1 ± 6.40	77.1 ± 7.07	..
8	Cotton-seed oil refined	A	98.1 ± 1.81	42.8 ± 4.75	..	83.8 ± 3.51	46.0	..
9	Coconut oil raw	A	101.8 ± 2.76	89.5 ± 5.51	86.1 ± 4.73	87.7 ± 2.37	80.3 ± 5.54	70.4 ± 2.85
10	Coconut oil refined	A	102.3 ± 6.11	54.2 ± 3.73	79.7 ± 8.21	92.3 ± 1.31	61.3 ± 5.69	66.1 ± 2.76
11	Mahua oil raw	A	88.1 ± 9.14	108.0 ± 2.94	98.8 ± 2.02	88.3 ± 2.96	81.3 ± 6.89	68.2 ± 1.15
12	Mahua oil refined	A	104.1 ± 7.44	75.4 ± 6.65	81.8 ± 7.82	83.2 ± 4.47	70.5 ± 5.16	62.3 ± 5.67
13	Til oil raw	B	120.0 ± 4.84	98.0 ± 4.71	..	99.6 ± 5.90	75.5 ± 3.10	..
14	Til oil refined	B	120.8 ± 6.23	93.9 ± 8.66	..	95.5 ± 2.55	80.2 ± 4.92	..
15	Mustard oil raw	B	103.5 ± 8.48	109.0 ± 3.24	92.3 ± 2.97	86.3 ± 5.18	85.8 ± 5.08	67.5 ± 1.35
16	Mustard oil refined	B	124.6 ± 14.18	85.6 ± 6.15	73.5 ± 7.42	99.0 ± 4.33	63.4 ± 5.88	61.6 ± 1.62
17	Lard	A	99.8 ± 2.25	52.7 ± 1.76	..	94.2 ± 3.03	45.0	..
18	'Dalda'	A	90.8 ± 5.35	76.8 ± 3.69	78.7 ± 5.85	83.9 ± 4.82	75.7 ± 2.56	64.2 ± 1.16
19	'Dalda''	B	107.2 ± 6.72	93.8 ± 8.35	..	91.2 ± 3.89	78.7 ± 4.15	..

refined oils generally fared worse than those getting the corresponding raw oils. However, groundnut oil was an exception as the refined product gave a slightly better growth effect in all the generations. The statistical analysis of the growth rates of the males and females pooled together, is given below in a tabular form.

SERIES A

Weekly growth rate (in gm)

Serial No.	Fat	1st generation	2nd generation
1	Cow ghee	10-74	12-86
2	Mahua oil raw	9-93	13-22
3	Coconut oil raw	11-14	11-10
4	Cotton-seed oil raw	11-31	10-12
5	'Dalda'	10-23	9-87
6	Mahua oil refined	11-14	9-27
7	Coconut oil refined	11-53	7-58
8	Lard	11-02	7-18
9	Cotton-seed oil refined	10-78	4-21

SERIES B

Weekly growth rate (in gm)

Serial No.	Fat	1st generation	Fat	2nd generation
1	Mustard oil refined	14-01	1. Cow ghee	13-05
2	Til oil raw	13-89	2. Mustard oil raw	11-66
3	Til oil refined	13-63	3. Til oil refined	10-61
4	Groundnut oil refined	13-54	4. 'Dalda'	10-50
5	Cow ghee	12-63	5. Groundnut oil refined	10-46
6	Groundnut oil raw	12-61	6. Til oil raw	10-40
7	'Dalda'	12-47	7. Groundnut oil raw	9-96
8	Mustard oil raw	11-54	8. Mustard oil refined	8-60

Weekly growth rate (in gm)

Serial No.	Fat	Generation IV
1	Gow ghee	12.65
2	Mahua oil raw	11.09
3	Groundnut oil refined	10.55
4	Mustard oil raw	10.47
5	Coconut oil raw	10.38
6	Groundnut oil raw	10.15
7	Coconut oil refined	9.55
8	Mahua oil refined	9.45
9	'Dalda'	9.39
10	Mustard oil refined	8.43

NOTE.—The averages covered by a vertical line do not differ among themselves at 5 per cent level of significance

After completion of the eight-week feeding on the basal synthetic diet alone three male rats out of each of the fat groups of the second generation, were sacrificed for other studies. The remaining animals were given the liberal supplement from the ninth week, and the weight gains during the four succeeding weeks recorded. In the first generation, Series A animals were not given such liberal feeding during the ninth to twelfth week, for reasons already explained, whereas the Series B animals of the same generation received the supplement. As the fourth generation studies were stopped at the end of the eighth week of feeding, no data for liberalised feeding of these animals were available. The results of the liberalised feeding are given in Table XX.

It would be seen that there was a decided increase in the growth rate due to the supplementation of the ration. This indicates the inadequacy of the basal ration, as has already been stressed in the introductory part of this Chapter. However, when the different groups were compared in the first generation, liberalised feeding produced more or less the same weight gain, irrespective of the nature of the dietary fat. In the second generation, however, as a rule cow ghee animals showed the lowest weight gains during this period as compared with the other fat groups. In many cases the weight gain among males during the four weeks (ninth to twelfth) of supplemented feeding was more than that recorded during the first eight weeks of active growth on the basal ration. Among females, however, the growth effect during the liberal feeding period was observed to be only about half of that made by the males during the same period.

Statistical analysis did not reveal any significantly better growth in most of the fat groups during this period. This may be due to the smaller number of animals

TABLE XX

Mean increase in weight (in gm.) of rats in 4 weeks of liberal feeding

(EXPERIMENT III)

Series A (2nd generation)

Oil or fat	Males	Females
Cow ghee	61.4 ± 3.55	28.5 ± 3.33
Cotton-seed oil, raw	78.3 ± 5.03	31.3 ± 3.03
Cotton-seed oil, refined	83.8 ± 11.75	..
Coconut oil, raw	59.0 ± 7.29	30.8 ± 2.50
Coconut oil, refined	59.4 ± 3.20	32.0 ± 2.77
Mahua oil, raw	65.7 ± 2.06	34.1 ± 1.83
Mahua oil, refined	71.1 ± 4.23	37.2 ± 3.98
Lard	84.5 ± 4.19	..
'Dalda'	71.3 ± 3.57	31.2 ± 2.00

SERIES B

Oil or fat	MALES		FEMALES	
	1st generation	2nd generation	1st generation	2nd generation
Cow ghee	56.3 ± 1.54	52.3 ± 5.49	28.9 ± 3.83	33.9 ± 1.79
Groundnut oil, raw	55.8 ± 3.85	88.0 ± 3.61	28.9 ± 2.19	38.2 ± 2.72
Groundnut oil, refined	49.8 ± 2.48	76.5 ± 4.04	31.4 ± 2.08	37.4 ± 4.10
Til oil, raw	57.8 ± 2.06	70.5 ± 8.13	30.4 ± 3.54	36.6 ± 2.43
Til oil, refined	55.2 ± 2.93	83.2 ± 5.18	35.1 ± 3.95	37.3 ± 2.72
Mustard oil, raw	55.5 ± 8.55	66.3 ± 4.33	38.2 ± 2.50	33.4 ± 3.51
Mustard oil, refined	53.9 ± 5.73	71.5 ± 2.18	27.9 ± 1.65	41.8 ± 3.71
'Dalda'	53.7 ± 3.64	78.8 ± 4.57	28.6 ± 1.49	40.0 ± 1.92

used during the ninth to twelfth week, as three male animals from each group were sacrificed for other experiments. However, animals fed on refined cotton-seed oil, lard, 'Dalda' and crude groundnut oil, showed significantly higher growth rate during the liberalised-feeding period in the second generation than the corresponding

ghee-fed ones. This would seem to indicate an attempt on the part of those fat groups which had made rather poor growth on the basal ration, to gain weight at an accelerated rate when the ration was liberalised, to make up for the stunting during the basal ration period. In other words, they tried to equalise in body weight with the cow-*ghee* animals.

Thus, while on a sub-optimum diet the cow-*ghee* animals fared better than the other fat groups, under liberalised feeding there was no marked difference between their growth effects (Plate III). It might be recalled that similar observations were obtained in Experiment I also.

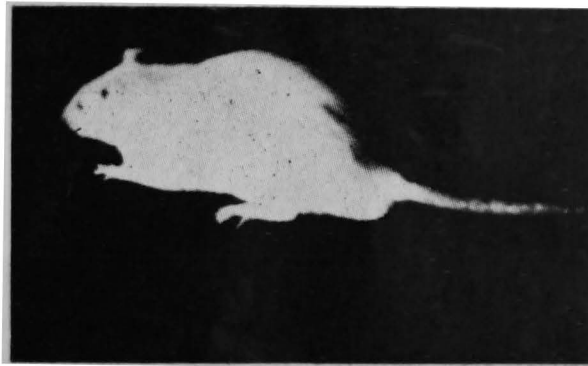
Health of animals

The animals of the first generation, in both the Series A and B getting different fats maintained fairly good health throughout. Only one animal in each of the groundnut oil (refined) group of Series A and mustard oil (raw) group of Series B died of unspecified reasons. Their mortality could not be ascribed to any deficiency disease. In the second generation, animals of all the groups in the Series A, except those getting cow *ghee*, exhibited symptoms of ill health and deficiency diseases, at some stage or the other. Those fed refined cotton-seed oil and lard were the worst affected. They developed a rough coat, showed alopecia and became weak and unthrifty, losing weight steadily after about the fifth week of experimental feeding on the basal ration (Plate IV). At about the same time, they also began to exhibit signs of dryness and inflammation of the eyes. In many of the animals this developed into total closing of the eyes and blindness. Several of them also exhibited a dragging gait, ending in paralysis of the hind quarters and legs and ultimately in death. Out of the twelve rats in each group at the commencement of the feeding test with the basal ration, only two (one male and one female) of the refined cotton-seed oil group and four (three male and one female) of the lard group survived at the end of eight weeks. The rats of the cotton-seed oil (refined) group also showed cannibalistic tendencies, as one of the rats of this group was eaten away by the others. The animals of the refined coconut oil group also showed clinical symptoms. These were, however, very much milder than the above. In this group, though there was severe inflammation of the eyes, they were not closed. Whereas, in the former two groups the rats showed loss of weight, in this case there was only great reduction in the rate of their growth after about the fifth week till the end of the eighth week. The severity of manifestation of alopecia, roughness of the coat, as well as the unthrifty appearance, however, were not greatly different from those seen in the less affected animals of the lard and cotton-seed oil (refined) groups. In the coconut-oil (refined) group no symptoms of paralysis were observed nor did any of the rats die. Other groups of this Series showed alopecia and roughness of the coat which were somewhat less severe than was observed in those fed on refined coconut oil. Animals getting raw *mahua* oil were the least affected among this Series. There was one death in this group but nothing special was noticed in the post-mortem examination of the same. Cow *ghee* fed rats did not show any noticeable clinical symptoms, except a slight alopecia, which cleared up soon after. When the supplementary feeding of milk and greens was commenced from the beginning of the ninth week, there was a remarkable improvement in the health and general condition of all the animals.

PLATE, III



A



B

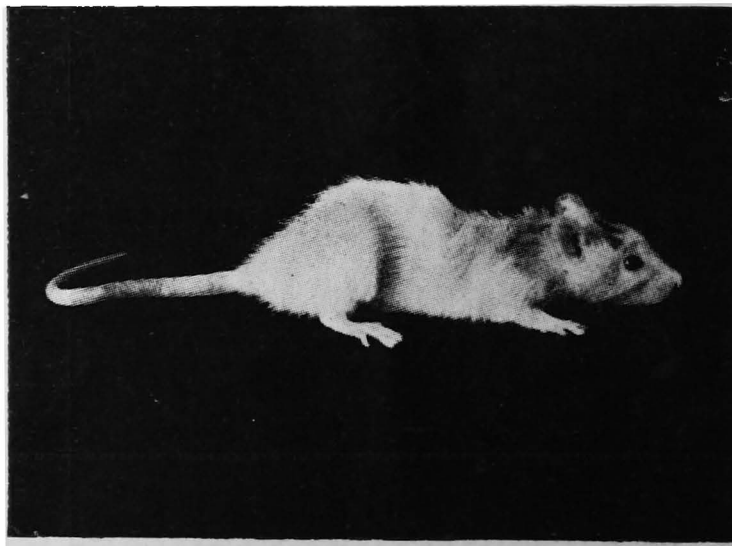


C

On liberal diet, no difference in growth was observed between (A) *ghos* (B) *varanasi* and (C) oil-fed rats. (p. 49)



A



B

Normal coat in (A) cow *ghee* group and (B) alopecia in *vanaspati* and oil groups (p. 49)

They put on weight rapidly and the deficiency symptoms of the eyes, fur, etc. also disappeared quickly. In about three to four weeks they appeared normal and more or less resembled the cow-ghee fed animals in general appearance.

Animals of Series B of this generation did not show any such abnormalities. Except for a slight alopecia in few of the rats, they appeared fairly normal throughout the eight weeks of the basal ration. Here also, the liberal diet improved their condition and appearance as well as their weight very considerably. There was no material difference in behaviour of the different fat groups in this Series, from their health and appearance point of view. There were no deaths in any of these groups.

Fourth Generation : The rats of this generation maintained fairly good health throughout the eight weeks of growth after weaning when they were fed the basal ration sub-adequate in vitamin A and containing the different experimental fats. Except for a mild manifestation of alopecia in some of the oil-fed groups, which was more particularly noticeable in the refined coconut-oil group, no other marked clinical symptoms were observed. The animals of some of the vegetable oils and 'Dalda' groups stopped gaining weight by the end of the seventh week of feeding. Of these, 'Dalda', coconut oil and mustard oil groups were more affected than the rest. Cow-ghee fed animals were practically free from any of these symptoms. Since the experiment was stopped at the end of the eighth week, further behaviour of the animals could not be pursued. There were no deaths in any of the groups.

The marked mortality and other pathological symptoms seen in the second generation of Series A animals were probably due to the fact that the parents of these animals were on a sub-optimum diet for 18 weeks as against only eight weeks for the parents of second generation animals of the Series B. It may also be recalled that in experiment I no mortality or any prominent pathological symptoms were seen in any group in any generation. This was due, possibly, to the fact that the animals were kept on the sub-optimum diet for six weeks only.

Discussion

On consideration of all the above data it would appear that under the above experimental conditions when only a sub-optimal basal ration was ingested, the growth promoting value of different vegetable oils, raw, refined or hydrogenated, as also lard, was generally significantly lower than that of cow-ghee. The difference, however, seemed to manifest itself only on prolonged ingestion of the fats, i.e. in the second and subsequent generations. It would also appear that there were no marked consistent differences between either the various *vanaspatis* or the different oils in this respect. Hydrogenation did not appear to affect, materially the growth promoting value of the oil. Refining, however, seemed to lower the growth promoting value of the oils on prolonged usage. The refined cotton-seed oil gave the worst results. Groundnut oil, however, differed from others, in that it gave a slightly better growth on refining, in all the three generations of the experiments. All the differences between the various fats and oils, more or less disappeared when they were fed with a liberal diet.

These results are in general agreement with the findings of Datta [1945] and Basu and Nath [1946]. Whereas Datta (*loc. cit.*) found mustard oil to be much poorer than hydrogenated fat, in this experiment the raw mustard oil was found to be

somewhat better than 'Dalda' while the refined one was found to be slightly poorer. The difference between cow-*ghee* and other oils and fats, in their growth promoting value, seems mainly to be the result of deficiencies in some essential nutritive factor or factors like vitamin A and B-complex which were given only in sub-adequate amounts in the different basal rations. The animals on oils, *vanaspatis* and lard, either did not perhaps utilize the ingested vitamins with the same efficiency as those getting cow-*ghee*, or the requirement of these accessory factors was higher when the oils and *vanaspatis* replaced cow-*ghee* as the dietary fat. This was indicated by the lower vitamin A reserves found in the liver of rats getting the oils and 'Dalda' as compared to those fed cow-*ghee*, even when vitamin A ingestion in the various groups were equated (Chapter XII). A higher requirement of thiamin and riboflavin, when oils and *vanaspatis* are sources of fat in the ration, has also been described later (Chapter XIII). Further support for this view is provided by the fact that all these differences in growth practically disappeared when a liberal diet was ingested. Under the latter dietary regime, the possibly increased requirements of some essential factors when fats other than *ghee* were ingested, would seem to have been met and, therefore, perhaps all these fats proved more or less of equal value to *ghee*. The fact that females showed less differences than the corresponding males in the growth promoting value of the different fats and oils under the restricted vitamin A ingestion, may be due to their requirements of vitamins being less than that of the males, as has been shown by Coward *et al.*, [1931], Bult and Sorgdrager [1938 ; 1940], Coward [1942], Brenner *et. al.*, [1942], Popper and Brenner [1942], Heftman [1947] and Esh and Sutton [1948].

The slightly superior behaviour of raw *mahua* and mustard oils, as compared with other oils, which was observed on the basal ration in Experiment III, might probably be due to their containing some carotene, as was seen in Chapter IV. Since the carotene ingestion of all these animals was on the border line of sufficiency, any additional supply, though small, would tend to have a beneficial effect. The reason why raw cotton-seed oil, which also contained about an equal quantity if not slightly more, of carotene did not equal raw *mahua* and mustard oils in the second generation, might conceivably be due to the presence in the cotton-seed oil of gossypol, which is known to enhance the vitamin A requirement. The refined oils fared generally worse than the corresponding raw ones in the second and fourth generations, probably because of the lower absorption of carotene, due to its greater destruction in the gut, consequent on the removal, during the refining process, not only of the carotene but also of the stabilizing factors in the natural oil like phospholipids, low molecular weight phenolic bodies, free fatty acids and perhaps other factors which, acting together are said to confer, according to Hickman [1943] a stability 10-100 times more than is associated with pure tocopherol and oil.

There is also another possible reason for the superiority of cow-*ghee*. Whereas the animals of all the groups other than that of cow-*ghee* got their entire dietary vitamin A as carotene, the cow-*ghee* fed rats ingested their vitamin A mainly as preformed vitamin A, since cow-*ghee* which was their only source of vitamin A contained the same mainly as vitamin A. As it is known that the biological efficiency of vitamin A is higher than that of carotene [Grey *et al.*, 1940; Treichler *et al.*, 1942], when equal amounts of both are ingested, the cow-*ghee* animals might have benefitted thereby. However, this difference in biological efficiency between vitamin A

and carotene may not likely be much at the low level of carotene ingestion taking place on the basal diet. Though these differences in vitamin A ingestion and availability were existing to the same extent in the first generation also, no differences were found between the growth promoting value of cow-*ghee* and the different fats in this generation. This might probably be because the rats used for this test were taken from stock and consequently would be having large reserves of vitamin A in the liver. Moore [1940] has demonstrated that, with high vitamin A intake, the liver of a young rat can store enough vitamin A to last the animal the rest of its life. Consequently, deficiencies arising in the first generation of oil or *vanaspati*-fed rats, from insufficient ingestion or decreased availability of vitamin A, might have been made up for by drawing on their liver reserves so that no differences in growth rate from cow-*ghee* group were noticeable. This behaviour would also mean additional support for the presumption that differences in the growth promoting value of the fats were caused by variations in factors like vitamin A ingestion and requirement.

These results, viz. cow-*ghee* being superior to other vegetable oils, might seem to be conflicting with the observations of Deuel and co-workers [1943; 1944; 1945a; 1950] who found no difference in the growth promoting values of butter-fat and other vegetable oils, margarines, etc. even when fed over several generations. There is, however, no contradiction because their experiments were conducted under entirely different conditions. Whereas the above authors fed an adequate ration, if not super-adequate in vitamin A and other essential nutritional factors (their animals were receiving at least 40 I. U. of vitamin A per head per day), the experiments discussed here were carried out with a diet containing only a border-line sufficiency of vitamin A (8 I. U.) and possibly of B-complex as well. That the present results are not only not conflicting with the findings of Deuel *et al.* [*loc cit.*] but actually confirm them is shown by the fact that the superiority of cow-*ghee* over other vegetable fats disappeared when the experimental animals were switched on to a liberal diet, which made good the dietary deficiencies particularly of vitamin A. Our work only stresses the fact that, in the comparative estimation of the nutritive value of fats, proper definition of the basal ration used should be put forth, as the defects of a sub-optimum ration are likely to be aggravated in the presence of oils or *vanaspati*. This conclusion assumes a significant meaning when the average poor Indian diets are considered. Such diets are usually low in vitamins and minerals and the addition of certain oils and *vanaspati* may, therefore, bring about adverse symptoms, unless precautions are taken to fortify these vegetable dietaries with sufficient fat soluble vitamins and other similar essential nutritional factors.

PART II

THE COMPARATIVE GROWTH PROMOTING VALUE OF FATS AND OILS WHEN INCORPORATED IN INDIAN DIETARIES

IN part I of this chapter, it was shown that when *ghee* was incorporated in a synthetic basal ration containing sub-optimum quantity of vitamin A and perhaps of other factors as well, animals showed better growth, especially in the second and subsequent generations than rats fed the same basal ration plus other oils or *vanaspatis*, even when the vitamin A intake in the different groups was equated. When, however, the diets were liberalised by addition of greens and skim milk, the difference between *ghee* and the other fats disappeared.

In this series of experiments, studies have been made on the effect of incorporating different fats in some of the common Indian dietaries. As is well-known, many of the Indian dietaries are deficient in several essential nutritive factors like protein, calcium, vitamins, etc. but there is little precise information about the effects of adding different fats and oils to such dietaries. Mason *et al.*, [1945] had observed adverse effects when butter-fat was added to a poor South Indian diet but they did not show whether other fats would also produce similar effects.

Experiment IV

For this experiment, the diet used by a poor Bengali was chosen. Since the work of McCarrison at Coonoor had labelled this diet as one of the poorest in the country, it was expected that any adverse effects on the health and well-being of the experimental animals due to the addition of different fats to it would be manifested quickly and prominently. The composition of the Bengali diet employed for this work was that used by MacDonald and Bose [1942] which itself was based on the earlier work of McCarrison. It consisted of rice—25 parts, *arhar* (*cajanus indicus*)—0.4 part, leafy vegetables—0.2 part, potatoes—3.5 parts, brinjals (*solanum melongena*)—1.75 parts, *torai* (*Luffa acutangula*)—1.75 parts, mustard (*Brassica juncea*) oil—0.3 part, salt—0.1 part and fish—0.7 part. The fats studied comprised cow *ghee* and six brands of *vanaspati*, viz. 'Dalda', 'Kotogem', 'First quality No. I', 'Binaula', 'Rajhans' and 'Temple'.

These fats were fed at 5 per cent level along with the basal diet as in earlier experiments. For each of the fat groups similar lots of twelve rats (six males and six females) were taken from the progeny of the parents receiving the same fat plus diet B(Chapter VI) for four months before the birth of the litters. The weekly growth records were kept from weaning onwards. In the preparation of the diet fish alone was cooked by boiling in water while the vegetables were minced and mixed together

in the raw state. Powdered raw rice was added and all the ingredients were thoroughly mixed—95 parts of this diet being mixed with 5 parts of the fat. The weight gains recorded in twelve-week feeding are given in Table XXI.

TABLE XXI

Average increase in weight in gm. in 12 weeks, of rats getting Bengali diet plus different fats at 5 per cent level (Experiment IV)

Fats	Males	Females
Cow <i>ghee</i>	37.6 ± 1.5	39.2 ± 3.8
'Dalda'	36.4 ± 1.6	35.8 ± 2.5
'Kotogem'	24.9 ± 3.4	22.0 ± 3.6
'First quality No. I'	20.5 ± 2.5	29.0 ± 2.9
'Binaula'	26.1 ± 5.5	23.1 ± 4.2
'Rajhans'	25.0 ± 3.9	21.5 ± 4.6
'Temple'	23.8 ± 4.1	24.8 ± 3.8

It would be noted from the data given in Table XXI that there was very poor growth in all the groups. This is not surprising at all in view of the multiple deficiencies of the diet. However, it will be seen that with the exception of the 'Dalda'-fed animals, all other *vanaspati*-ingesting rats made significantly poorer gains in comparison with those getting cow *ghee*. There was practically no difference between the cow *ghee* and the 'Dalda' so far as growth effect was concerned. This superiority of 'Dalda' over other *vanaspatis* was also seen in Experiment II (Chapter VI), where the comparative nutritive values of heated fats were examined. In view of the multiple deficiencies present in the basal ration and also because many of the animals developed pathological symptoms, the results of statistical analysis of the growth rates of the different groups in this experiment may not have much practical significance.

Though the comparative growth promoting abilities of the different groups have been given for a period of twelve weeks only, the experiment was further continued to examine the effect of long period feeding on the health of the animals, as well as to study the capability of the animals of the different groups for reproduction and lactation. The data on reproduction and lactation are discussed in the next Chapter. The observations, during 25 weeks of feeding regarding the general condition of the animals, are given below.

In all the *vanaspati* groups, some animals, after third week, showed alopecia varying in intensity from mild to severe. Only two animals of the cow-*ghee* group showed very slight alopecia which disappeared in one case within a week. In most of the other affected animals, the time of disappearance was about three weeks though the symptom recurred again in all the *vanaspati* groups.

PLATE, V



A



B

Rats fed on poor Bengali diet with certain *canaspatia* for a prolonged period exhibit (A) xerophthalmia and (B) paralysis (p. 58)

After eight weeks of feeding, some of the animals of the *vanaspati* group except 'Dalda' and 'Rajhans', started showing symptoms of paralysis (Plate VB). Hind legs were mostly affected. In the 'Kotogem' group, the symptoms were severe and two animals died. In most of the other cases, the manifestation disappeared in one to four weeks. None of the cow-*ghee* animals developed this affliction.

From the 13th week onwards, the extrusion of the genital organ of one or more animals of each group, including the cow-*ghee* group, was observed. This condition did not disappear with the progress of feeding.

From the 14th week onward, quite a large percentage of animals in all the *vanaspati* groups started showing symptoms similar to those seen in vitamin A deficiency, viz. inflammation of eyes, drying of the cornea, exudation of pus from eyes and in severe cases xerophthalmia (Plate VA). All the affected animals died within about eight weeks after the development of these symptoms. None of the cow-*ghee* animals showed these symptoms.

The number of animals of each sex thus affected in the various fat groups is given below :

Number of male and female rats out of 12 affected in various fat groups

Fats	Males	Females	Total
Cow <i>ghee</i>	0	0	0
'Dalda'	1	1	2
'Kotogem'	2	2	4
'First Quality No. 1'	2	1	3
'Binaula'	1	1	2
'Rajhans'	3	4	7
'Temple'	1	1	2

This is not at all surprising when it is remembered that the cow-*ghee* animals were each receiving about 6-8 I.U. of vitamin A per day whereas those of the *vanaspati* groups could get only about 1 I.U. per rat per day.

In some of the rats, a diarrhoea-like condition was also noted, but it ceased within a day or two. Four animals of the 'Kotogem' group died from retention of urine. In two of these, the formation of urinary calculi was detected on post mortem examination. At the end of 25 weeks of experiment all the animals of the cow-*ghee* group, except one which died of pneumonia in the 23rd week, were alive. The corresponding number of the survivals in the *vanaspati* groups was as follows: 'Dalda' -7, 'Kotogem' -5, 'First quality' -1, 'Binaula' -5, 'Rajhans' -4 and 'Temple', 8. The 'Rajhans' and 'Temple' groups could be maintained for only 22 and 21 weeks respectively as the stock of the fats became exhausted. It will thus be seen that the mortality in the *vanaspati* groups ranged from 42 to 92 per cent as compared to 8 per cent in case of the cow-*ghee* group.

Experiment V

After the above experiment was completed, it was decided to carry out a similar series of investigations to compare the effects of addition of *ghee*, vegetable oils or hydrogenated vegetable oils to a Bengali diet. A slightly different basal ration was used which is more accurate and representative of present conditions, as revealed by a consideration of all the published data on dietary surveys in India as well as from the information obtained from surveys carried out by this Institute. It may be recalled that the previous basal ration was a modified McCarrison diet as used by MacDonald and Bose [1942]. In order to avoid, as far as possible, associated effects of other fats, all free fats and oils which were part of the above dietary, as recorded in the surveys, were excluded and only the experimental fats were given.

As most of the hydrogenated fats (*vanaspatis*) sold in the markets, are prepared from groundnut oil, this oil, in the natural crude form, was employed in this study. As there is some doubt about the inferior nutritive value of *vanaspatis* having a melting point higher than that of the body temperature, two hydrogenated products prepared from groundnut oil and having different melting points, one below body temperature and another above it, were tested. The following fats were studied :

1. Crude groundnut oil
2. *Vansapati* of m.p. 36° C. made from groundnut oil
3. *Vanaspatis* of m.p. 39° C. made from groundnut oil
4. *Bazar ghee*
5. Cow *ghee*

Pure cow *ghee* prepared in the Institute dairy under standard conditions, was used as the control. The *bazar ghee* was a composite sample prepared from material purchased from five different shops from different localities in Bareilly.

The composition of the diet was rice—65.5 per cent, *masoor dal* (*Lens esculenta*)—2.0 per cent, *moong dal* (*Phaseolus radiatus*)—0.5 per cent, onion—2 per cent, fish—2 per cent, potatoes—11 per cent, leafy vegetables—1.2 per cent, brinjals (*solanum melongena*)—4.5 per cent, *torai* (*Luffa acutangula*)—4.5 per cent, egg—1 per cent, meat—0.5 per cent, common salt—0.3 per cent, and experimental fat or oil—5 per cent.

The protein, lime and phosphoric acid contents of the diet were 9.8 per cent, 0.02 per cent and 0.45 per cent, respectively. The vitamin A potency of the ration was 75.8 I. U. per 100 gm.

Since the diet is low in vitamins and as the provision of adequate vitamins in a diet is of very great importance, this aspect was also taken up for investigation. The influence of the fortification of the diet with vitamins was determined by running simultaneously an exactly similar duplicate series of trials in which the experimental animals were given an adequate supplement of the vitamins. Since the rat does not require vitamin C this was omitted and only vitamin A, D and B-complex, were given. The schedule of the vitamin supplements given daily to each animal was ; vitamin A concentrate—60 I. U., calciferol—10 I. U., thiamin—60 γ, riboflavin—50 γ, pyridoxin—50 γ, Ca-pantothenate—100 γ and nicotinic acid—1 mg.

As the oils and fats are generally used by people in India for frying purposes, and as little is consumed in the raw state, they were all first heated to 190° C., for 30 minutes and cooled, before feeding to the rats. This heat treatment was carried out to simulate, as far as possible, general consumption practice.

The requisite amounts of the vegetables as well as the cooked meat and fish were weighed out and reduced to a fine consistency by passing them through a meat mincer. They were then incorporated into the proper amount of the powdered cereal and pulse mixture. The exact quantities of the other components like eggs, milk, etc., were also added to this and the whole thoroughly mixed to a uniform texture. To each 95 parts of this basal mixture of Bengali diet, five parts of one of the experimental fats which had been heated and cooled, was added and again thoroughly mixed to get a homogenous product. This was then transferred to tightly stoppered bottles and kept in a refrigerator.

The usual experimental technique given earlier, using twelve animals in each group (equal number of males and females), was followed here also. The rats which were to get the vitamin supplements were dosed individually with vitamin A and D while the vitamin B-complex was added to their food. To ensure the ingestion of the full dosage of B vitamin by each rat, 20 per cent more of the scheduled daily allowance was added to their food daily.

All the experimental rats were weighed individually at the commencement and twice every week thereafter till the completion of twelve weeks of feeding. The weight gain during this period was taken as the growth promoting index of the different fats.

TABLE XXII

Growth of rats (in gm.) in 12 weeks when fed Bengali Diet
(Experiment V)

Fats or oils	Males		Females	
	Without vitamins	With vitamins	Without vitamins	With vitamins
Cow ghee	49.1 ± 4.30	92.9 ± 7.50	41.2 ± 5.43	78.3 ± 4.34
Bazar ghee	49.7 ± 6.32	87.9 ± 13.29	39.1 ± 5.35	80.6 ± 8.20
Groundnut oil.	41.9 ± 5.33	68.2 ± 11.71	34.6 ± 3.32	76.3 ± 6.37
Vanaspati, m.p. 36°C.	49.8 ± 7.33	90.6 ± 16.16	38.5 ± 3.62	88.8 ± 3.81
Vanaspati, m.p. 39°C.	40.0 ± 4.47	99.7 ± 1.98	38.3 ± 4.77	81.4 ± 5.54

From the growth results given in Table XXII it would be seen that on the unsupplemented basal diet the weight gains in all the fat groups were very poor and nearly the same as those seen in the last experiment. This is probably due to

the fact that though the present diet was slightly richer than the previous one, yet it was still grossly deficient in calcium, protein and other essential ingredients and was also highly unbalanced. It will also be noted that with such a poor basal ration, no significant difference between cow *ghee* and the other fats could be demonstrated.

That this diet was also seriously deficient in vitamins was seen from the fact that supplementation with vitamins induced practically 100 per cent more weight gain in all the groups than on the basal ration alone. In the vitamin supplemented groups also there was no significant difference between the growth promoting values of the fats. Even this increased growth rate was much lower than that of normal rate, as seen in our stock rats, again pointing to the multiple deficient nature of the basal ration.

The rats in the unsupplemented series all looked stunted, and had rough coats. Alopecia occurred in practically all the fat groups. Supplementation with vitamins did not modify the manifestation of the above symptoms to any material extent except that since the growth was better, the animals were somewhat bigger in size. Animals getting the pure cow *ghee* fared somewhat better than the rest.

A tendereness of the body was observed in practically all the animals in the different groups in both the unsupplemented and vitamin supplemented series. One animal of the *vanaspati*-39° group showed paralysis of the hind legs after 16 weeks on the basal ration. The animals in this series were kept under observation for 18 weeks only as against 25 weeks in the previous experiment (Experiment IV). Even then, the mortality figures for the different groups were as follows :

Unsupplemented

Cow *ghee*—nil, *bazar ghee*—1, groundnut—1, *vanaspati* 36°—2, *vanaspati* 39°—2

Vitamin supplemented

Cow *ghee*—2, *bazar ghee*—3, groundnut oil—4, *vanaspati* 36°—3, *vanaspati* 39°—4. It will thus be seen that the mortality rate was higher in the *vanaspati*-fed rats as compared to that of the pure cow *ghee*-fed animals. A curious finding was that the number of deaths were much higher in the vitamin supplemented rations. That all the fat groups had shown increases in mortality on the vitamin supplemented diet seems to lend support to the presumption that these deaths might not be connected mainly with the nature of the ingested fat but to other causes which are not clear at present. Besides congestion of lungs, nothing very special was noticed on postmortem examination of the animals which died.

It may be recalled that in the previous experiment (Experiment IV), animals of most of the *vanaspati* groups showed multiple deficiency symptoms and higher mortality, whereas cow *ghee*-fed animals were more or less free from these afflictions. In Experiment V, the animals fared much better and the mortality was much less, especially in the unsupplemented groups. This might have been due to several reasons. Firstly, the basal diet used in the latter experiment was of higher nutritive value, for example, the rats of the unsupplemented groups had each been receiving daily on an average 3—4 I. U. of vitamin A from the basal ration, as compared with

1 I. U. in the previous experiment. This explains why eye symptoms, seen in the *vanaspati*-fed rats of the earlier experiment, were absent even in the *vanaspati*-fed animals of the latter experiment. Secondly, the experiments in the earlier test were carried on for a much longer duration (25 weeks) than the present one (18 weeks). It may also be stated here that most of the deaths in the previous experiment occurred after the 20th week. Thirdly, the *vanaspati*s used in the present series correspond to 'Dalda' of the earlier test, on which, manifestation of clinical symptoms and incidence of mortality was the least as compared with other brands of *vanaspati*s used.

Taking all these facts into consideration, the behaviour of the *vanaspati*-fed rats in this test can be said to follow more or less the same trend as was observed in the former study.

Experiment VI.

Since the previous two experiments had been with the poorest type of diet prevalent in the country, it was thought that, as a comparison, a similar study should be carried out with a better type of Indian dietary. The North Indian diet was, therefore, chosen as it had been shown by McCarrison *et al.*, to be one of the best Indian dietaries. The percentage composition of the diet used was as follows:

Wheat—44.4, *bajra* (*Pennisetum typhoideum*)—9.0, maize (*zea mays*)—5.5, *jowar* (*Sorghum vulgare*)—3.5, arhar (*cajanus indicus*)—10.0, leafy vegetables—2.0, potatoes—4.2, brinjals (*Solanum melongana*)—1.0, *torai* (*Luffa acutangula*)—1.0, whole milk—3.0, separated milk—9.0, *gur* (jaggery)—1.3, fish—0.1, meat—0.5, eggs—0.2, common salt—0.3, and experimental fats or oils—5.0. The protein, lime and phosphoric acid contents were 14.2, 0.13 and 0.79 per cent respectively. The vitamin A potency was approximately 150 I. U. per 100 gm. of the ration. In this case also the effect of supplementing the basal ration with extra vitamins was studied in an exactly similar manner as in the last experiment. The composition of the vitamin supplement given was the same as in the Experiment V. The fats studied were also the same as those used in the previous experiment. The growth of the animals in 12 weeks feeding is given in Table XXIII.

TABLE XXIII

Growth of rats (in gm.) in 12 weeks when fed North Indian Diet
(Experiment VI)

Fats and oils	Males		Females	
	Without vitamins	With vitamins	Without vitamins	With vitamins
Cow ghee	108.0 ± 5.86	133.6 ± 4.36	76.3 ± 2.34	109.0 ± 4.36
Bazar ghee	96.8 ± 4.94	139.6 ± 7.61	71.3 ± 3.45	96.1 ± 4.39
Groundnut oil	81.7 ± 6.22	131.8 ± 4.27	72.2 ± 3.91	99.5 ± 3.16
<i>Vanaspati</i> , m.p. 36°C.	79.9 ± 4.07	135.4 ± 6.07	75.0 ± 0.57	102.2 ± 3.60
<i>Vanaspati</i> , m.p. 39°C.	86.3 ± 6.01	130.8 ± 2.19	72.8 ± 3.09	99.1 ± 2.78

It would be clear from the above Table that the rats on even the basal ration made much better growth than those on the basal Bengali diet and about the same as that of the vitamin supplemented Bengali diet. This would probably be due to the fact that the North Indian diet was much better, since its content of calcium, protein, etc., was higher than in the Bengali diet. Whereas in the Bengali diet there was no significant difference between the growth promoting values of *ghee*, vegetable oil and *vanaspatis*, on this diet the growth of the male rats getting the oil and *vanaspatis* was significantly poorer than that of the rats receiving either the pure or *bazar ghee*. Between the oil and *vanaspatis* or between the two *vanaspatis*, however, there was no significant difference. This finding is similar to the other experiments described in Experiment III. The female rats showed much less variation in growth rate from group to group, so that there was no significant difference in the growth of female rats getting oils or *vanaspatis* as compared to that of similar *ghee*-fed rats. Similar behaviour of the female rats was seen in Experiment I and III as well, and is in agreement with the results found by foreign workers as cited in Chapter VI (Part 1). On the addition of vitamins, there was increased growth here also, though the proportionate rise was only about 50 per cent over that of the unsupplemented ration. The final body weights in the vitamin supplemented samples were more or less of a normal order. On supplementation with vitamins, however, the differences between the fats disappeared.

In other words, when an adequate supply of vitamins is made to the North Indian diet, *ghee*, vegetable oils and *vanaspatis* had similar growth promoting values. This effect is more or less similar to that observed in Experiments I and III, where on liberalisation of the diet, the differences between *ghee* and the other fats disappeared. The probable reasons for this influence of vitamins would be examined in greater detail in the later chapters. Except alopecia, no other pathological symptoms were seen in any animal in any of the groups. Even with regard to the incidence, extent and degree of manifestation of alopecia, there seemed to be no material difference between group and group, whether supplemented or unsupplemented with vitamin, except that the rats getting pure cow *ghee* showed comparatively less symptoms. The mortality score was also low. Surprisingly enough, the only mortalities were seen in the *ghee*-groups, one in the pure *ghee* and the other in *bazar-ghee* group. No specific cause of these deaths which could be attributed to fats, was determined. It will thus be seen that the animals of this Series fared much better than those getting the Bengali diet.

DISCUSSION

It would thus be seen that the superiority of cow *ghee* over other fats and oils seems to manifest itself only when the diet is on the border-line of adequacy. If the ration is too poor (as in the Bengali diet) resulting in extreme stunting, or comparatively rich (as in the supplemented ration) enabling more or less normal growth, these differences between the fats fail to show up. Boer *et al.*, [1947b] stated that the superiority of butter-fat over vegetable-fats could be best demonstrated when rice flour formed the carbohydrate of the basal diet. In our Bengali diet experiments this was not observed even though rice formed the staple cereal. This may perhaps be due to the extremely unbalanced and deficient nature of the diet used by us. Though the Bengali diet was very similar to the South Indian diet used by Mason

et al., [1945], the adverse effects noticed by them on the addition of butter-fat, were not observed in these experiments. It would be clear from the growth data obtained in the present series of experiments that the cow-*ghee* diets gave equal, if not better, growth than any of the other fats. In another series of experiments conducted in these laboratories by Kehar and Sahai [1949] only beneficial effects and no adverse effects have been observed by the addition of butter-fat to a poor rice diet similar to that prevalent in South India. On the other hand, in the North Indian dietary, when wheat formed the staple grain, the difference between *ghee* and the fats was manifested. The superiority of cow-*ghee* over the vegetable-fat, observed in the vitamin low North Indian diet and its disappearance on a liberal supply of vitamins, agrees well with the findings of Heftmann [1947]. However, no difference in growth promoting value between the oil and its hydrogenated products could be found in any of the dietaries used, nor was the melting point of the *vanaspati* within the limits studied, found to affect its growth promoting value.

CHAPTER VII

EFFECT OF FATS ON REPRODUCTION AND LACTATION

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It is an accepted fact that fats are essential for proper reproduction and more particularly for efficient lactation. Failure or poor reproduction on fat-free diets has been noticed by Escudero *et al.*, [1939], Quackenbush *et al.*, [1942a] and Deuel and co-workers [1947]. Whereas Galamini [1939], Sure [1941], Maynard and Rasmussen [1942], Deuel *et al.*, [1944c ; 1945a], Euler *et al.*, [1947 ; 1947a], as well as others, found no difference between various fats, Schantz, Elvehjem and Hart [1940], Loosli *et al.*, [1944], Miller [1943] and others considered that fats differed among themselves regarding their efficiency for reproduction and specially so for lactation. Owing to this conflict of opinion a comparative study of different vegetable oils, *vanaspatis*, lard and cow *ghee*, for this purpose, was carried out. Since the requirements for reproduction and lactation are more rigorous and exacting than for growth, it was presumed that any differences between the different fats would be shown up to better advantage in such a study.

The animals in all the experiments given in Chapter VI have been utilized for studying the comparative values for reproduction and lactation of various fats when added to either a synthetic diet or to common Indian dietaries. The results for each experiment are discussed one by one. Except where otherwise mentioned, males and females of each group, i.e. receiving the same fat have always been mated together at four months of age. The number of females giving birth to litters, as well as the number of live litters born, have been used as the index of reproductive ability. The weight and number of young alive at the time of weaning, i.e. at four weeks of age, have been taken to be indices for the lactating power of the mothers.

Experiment I. (Synthetic diet plus ghee or vanaspatis)

The results are given in Table XXIV. As shown in the Table, no consistent difference in reproductive ability in the various groups in the different generations could be found. The litter size varied from group to group and from generation to generation but the differences within each group were so wide that, no general trend could be traced nor any significant differences observed. The average weights of the young at birth, in the different groups, were also very similar and lay between 4.0—5.0 gm. each. Taking the weights of the litters, as well as the percentage of young weaned at 28 days, as the criteria for lactation, no significant difference between the effects of the various fats could be seen. These results have been analysed and it is found that fertility, litter size and lactation capacities do not

appear to be affected differently by different fats in any generation, except that in the second generation 'Dalda'-fed animals appeared to be superior to all other fats including cow *ghee* so far as lactation performance was considered.

These results are a little surprising in the face of the clear cut results seen in the growth records, but it must be remembered that the animals of all the groups had been receiving adequate diets for 18 weeks before mating, as well as during the time of giving birth and rearing the litters and thus were not probably affected by the *vanaspatis*.

TABLE XXIV
Reproduction and lactation of rats in Experiment I

Fat	Per cent giving birth to litters	Average size of litters	Average weight in gm. of youngs at 28 days	Per cent weaned
<i>1st generation</i>				
Cow <i>ghee</i>	100	8.3 ± 0.50	14.1 ± 0.36	69.7 ± 6.37
Buffalo <i>ghee</i>	100	6.3 ± 0.50	15.5 ± 0.44	84.0 ± 5.87
'Dalda'	100	7.5 ± 1.19	15.2 ± 1.44	86.7 ± 4.39
'1st Quality'	100	8.4 ± 0.50	14.6 ± 0.96	78.6 ± 6.25
'Swastika'	100	7.0 ± 0.63	15.2 ± 0.42	82.9 ± 7.45
'Kotogem'	100	7.8 ± 0.50	15.1 ± 0.46	80.7 ± 9.35
'Cotex'	80	8.0 ± 0.58	15.4 ± 0.69	70.8 ± 2.79
'Binaula'	100	7.5 ± 0.87	15.1 ± 0.55	70.0 ± 2.78
'Temple'	100	8.0 ± 0.71	13.9 ± 0.44	81.3 ± 8.39
'Rajhans'	100	8.0 ± 0.55	14.0 ± 0.90	67.5 ± 13.55
<i>2nd generation</i>				
Cow <i>ghee</i>	100	8.2 ± 1.08	30.8 ± 0.58	59.2 ± 20.62
'Buffalo <i>ghee</i>	100	9.0 ± 0.58	32.7 ± 0.56	92.6 ± 3.15
'Dalda'	100	6.7 ± 1.10	38.1 ± 0.82	97.5 ± 1.67
'1st Quality'	100	7.8 ± 1.30	31.3 ± 0.91	97.9 ± 1.52
'Swastika'	100	9.6 ± 0.22	28.7 ± 0.53	95.8 ± 4.44
'Kotogem'	100	9.8 ± 0.80	29.0 ± 0.67	89.7 ± 11.71
'Cotex'	100	8.0 ± 1.21	30.9 ± 0.70	77.1 ± 12.00
'Binaula'	100	7.5 ± 0.55	35.1 ± 0.54	58.9 ± 15.82
'Temple'	100	8.8 ± 0.50	30.1 ± 0.64	79.5 ± 20.00
'Rajhans'	100	9.0 ± 0.44	26.4 ± 0.48	85.2 ± 16.67

TABLE XXIV—*contd.**Reproduction and lactation of rats in Experiment I*

Fat	Per cent giving birth to litters	Average size of litters	Average weight in grams of youngs at 28 days	Per cent weaned
<i>3rd generation</i>				
Cow ghee	100	6.2 ± 0.55	30.5 ± 0.90	86.5 ± 15.01
Buffalo ghee	83.3	5.6 ± 1.32	25.8 ± 1.17	92.9 ± 19.44
'Dalda'	100	5.8 ± 0.80	31.5 ± 1.19	94.3 ± 3.13
'1st Quality'	83.3	5.2 ± 0.59	32.8 ± 1.60	73.1 ± 20.00
'Swastika'	100	5.5 ± 0.75	27.6 ± 0.52	97.0 ± 2.78
'Kotogem'	100	6.5 ± 0.18	26.2 ± 0.52	94.9 ± 3.28
'Cotex'	100	6.8 ± 0.50	29.5 ± 0.56	86.9 ± 7.80
'Binaula'	100	6.8 ± 0.63	29.2 ± 0.70	95.2 ± 4.27
'Rajhana'	100	7.8 ± 0.55	27.1 ± 0.50	95.7 ± 5.54
'Temple'	100	6.0 ± 0.82	31.5 ± 0.84	80.5 ± 2.34

NOTE.—In column 4, the weights of the youngs of the 1st generation at 14 days of age have been given.

Experiment II

Table XXV shows the reproduction and lactation performance of the animals getting heated *ghees* and *vanaspatis* when incorporated into Diet B (Chapter VI). The weight of the young at 14 days of age has been used here as one of the indices of lactating capacity [Maynard and Rasmussen, 1942]. It will be seen from the Table that except for the 'Binaula' group (in which none of the animals cast any litter), there is no difference between fat and fat in respect to fertility and lactating capacity of the animals. The statistical analysis reveals that the fertility of rats does not appear to differ significantly from fat to fat. As regards lactation capacity, 'Temple'-fed rats appear to give results better than any other fats. The comparatively small number of animals used here however robs the statistical data of any great practical significance.

TABLE XXV
Reproduction and lactation records of animals of Experiments II

Name of fat	Per cent giving birth to litters	Average size of litters	Average weight of youngs at 14 days	Per cent weaned
Cow <i>ghee</i>	66.6	6.5 \pm 1.5	10.3 \pm 1.15	84.6 \pm 3.8
Buffalo <i>ghee</i>	100	6.3 \pm 0.57	9.1 \pm 0.16	78.9 \pm 14.70
'Dalda'	66.6	6.5 \pm 0.50	9.3 \pm 0.30	84.6 \pm 7.2
'1st Quality'	100	6.3 \pm 0.99	11.3 \pm 1.54	57.9 \pm 19.5
'Swastika'	100	6.7 \pm 0.74	10.5 \pm 0.86	80.0 \pm 9.0
'Kotogem'	100	6.0 \pm 1.53	11.0 \pm 1.36	61.1 \pm 5.9
'Cotex'	66.6	7.0 \pm 0.00	11.9 \pm 0.75	92.9 \pm 7.2
'Binaula'	0	—	—	—
'Temple'	100	5.3 \pm 0.54	14.1 \pm 1.43	62.5 \pm 8.8
'Rajhans'	100	7.0 \pm 1.00	10.4 \pm 0.30	71.4 \pm 18.8

This finding is again in contrast to the growth rates of the animals, as it may be remembered that significant differences in the growth rates between various groups had been observed. For example, the 'Cotex' fed females grew at a significantly slower rate than the cow-*ghee* fed ones, yet the reproductive capability of the former was equal to, if not slightly better than that of the latter.

Experiment III

It has already been mentioned in Chapter VI, that animals fed solely on the synthetic basal ration as used in this experiment, failed to reproduce, irrespective of the fat used, and that this failure to cast litters was set right by feeding each of the rats a supplement of 20 ml. of skim milk, 5 gm. greens and 0.5 mg. alpha-tocopherol, daily for at least four weeks before mating. The reproduction and lactation performance of the rats getting the different fats and oils, are given in Table XXVI. It might be observed from the same that the reproduction record was satisfactory in all the groups. The average birth weight of the young ones was normal, varying from 4.5 to 5.5 gm. each. The lactation performance of these animals was also satisfactory. The record of the fat groups of Series A was, however, not as good as that of those in Series B both as regards mortality of young as well as of average weaning weight. As mentioned before in Chapter VI, this comparatively poorer performance was perhaps due to the fact that the animals of Series A were on the sub-optimum basal ration for about 18 weeks after weaning, whereas the rats of Series B were given this diet for eight weeks only. Both series were on the liberal ration for an equal period, viz. about 11 weeks. Hence it is possible that both sets of animals were not in the same state of nutritional status at the time of mating and lactation. This view is strengthened by the fact that the animals in the different fat groups, in both the Series, exhibited in the second generation similar reproduction

TABLE XXVI

Reproduction and lactation performance of animals of Experiment III

Fats or oils	Per cent giving birth to litters		Average size of litters		Average weight of youngs at 28 days		Per cent weaned	
	1st generation	2nd generation	1st generation	2nd generation	1st generation	2nd generation	1st generation	2nd generation
<i>Series A</i>								
Cow ghee	100	100	8.0±.68	7.7±.98	23.4±2.19	36.5±2.70	79.2	78.3
Cotton-seed oil, raw	66.6	66.6	5.8±.83	5.8±1.20	30.8±3.49	44.2±3.37	65.2	100
Cotton-seed oil, refined	100	100	6.7±.68	8.0	24.6±2.35	28.0±5.71	82.5	100
Coconut oil, raw	83.3	83.3	7.8±.75	7.8±1.69	23.5±2.60	30.7±4.32	69.2	89.7
Coconut oil, refined	83.3	100	8.6±.75	8.5±2.40	22.3±2.32	30.6±2.43	79.1	86.3
Mahua oil, raw	100	100	8.2±.68	8.4±1.07	21.8±2.09	39.2±2.52	85.7	97.6
Mahua oil, refined	66.6	66.6	7.0±.83	8.3±1.20	23.9±2.71	44.0±2.81	89.3	100
Lard	100	—	7.2±.68	—	25.9±2.56	—	65.1	—
'Dalda'	100	66.6	7.2±.68	7.5±1.20	23.6±2.19	36.7±2.28	88.4	96.7
<i>Series B</i>								
Cow ghee	100	100	9.8±.65	8.5±.78	34.8±2.38	29.9±1.70	93.2	94.1
Groundnut oil, raw	100	83.3	8.2±.65	7.8±.84	39.0±2.55	29.7±1.96	93.9	92.3
Groundnut oil, refined	100	100	8.2±.65	8.0±.78	39.5±2.48	33.0±1.73	100	95.8
Til oil, raw	100	66.6	7.7±.65	8.0±.95	35.4±2.71	30.7±2.12	89.1	96.9
Til oil, refined	100	80	7.8±.65	8.8±.95	41.7±2.64	32.4±2.19	91.5	82.9
Mustard oil, raw	100	100	7.8±.65	7.3±.78	37.6±2.61	31.1±1.82	93.6	95.5
Mustard oil, refined	100	83.3	8.7±.65	5.6±.84	35.2±2.45	32.9±2.36	96.2	89.3
'Dalda'	100	50	9.3±.65	6.7±1.10	40.1±2.61	37.5±2.70	94.6	95.0

and lactation performances. However, the majority of the rats in the raw and refined coconut oil as also the single rat on refined cotton-seed oil groups, in Series A, failed to reproduce when mated at 16 weeks of age, when they had been on the supplemented diet for only four weeks. But when continued on the liberal diet for some weeks more and then remated, they showed satisfactory reproduction and lactation. The rather high mortality of 21.7 per cent observed among the litters of the cow-ghee group in Series A of the second generation, does not give a true picture of the lactation capacity of the female rats of this group because eight of the ten litters, which died, were killed as the result of an accident.

On the whole, therefore, it would be seen that in the different generations there was no difference between the various fats with regard to their effect on reproduction and lactation. This view is strengthened by the statistical analysis of the data which shows that the fats did not differ significantly in any generation so far as fertility, litter size and lactating capacity were concerned.

Experiment IV

Reproduction was more or less a total failure in all the groups of rats receiving the Bengali diet and either *ghee* or *vanaspati*. Only in the cow-ghee group, one rat gave birth to four litters all of which died within a few hours of their birth. The very poor quality of the basal diet is thus apparent.

Experiment V

In this experiment also, it was found that even with an improved Bengali diet reproduction was a complete failure in all the fat groups including cow *ghee* one. The reproduction and lactation performance was little better when the diet was fortified with vitamins. Only one out of five females mated, in each of the cow *ghee* and *bazar-ghee* fed groups, reproduced and gave one and four litters respectively. None of the females in any of the oil or *vanaspati* fed groups gave birth to young. Even the few litters born, in the cow-ghee and *bazar-ghee* groups, died shortly after birth. It would be clear, therefore, that reproduction was very unsatisfactory and that fortification with vitamins made little improvement. Also, all the fats including cow *ghee* fared equally badly in this respect.

Experiment VI

With the North Indian diet, more rats gave birth to litters than was seen in the case of the Bengali diet. On the basal North Indian diet, i.e. without any vitamin supplementation, while none of the groundnut-oil fed rats reproduced, all the mated females on *vanaspati* 36°C. cast litters. Out of the five rats mated in each of the cow *ghee*, *bazar ghee* and *vanaspati* 39°C groups, only 1, 2 and 3 animals respectively, gave birth to young ones. The litter size was very low, ranging from an average of 1.5 to 3.2. The total number of litters cast by all the rats of the *vanaspati* 36°C group, which showed 100 per cent reproduction, was only 16. The single rat in the cow *ghee*-group, which reproduced, gave birth only to two litters. The new borns were under-sized and puny, weighing only 3.5 to 4.0 gm. each, on an average. That lactation was a complete failure was shown by the fact that not a single one of the litters in any of the groups survived even for one week. All of these died within two to three days of birth or earlier. Supplementation of the North Indian diet

with vitamins did not seem to have brought about any substantial change in the behaviour of the rats regarding reproduction or lactation. On this supplemented diet too, none of the females getting the groundnut oil gave any litters. Whereas on the unsupplemented North Indian diet all the females of the *vanaspati* 36°C group reproduced, in the group of vitamin supplemented diet none of them gave birth to any young ones. The cow-*ghee* group showed no change because on both the supplemented and unsupplemented diets, only one rat of the whole lot cast litters. In the *bazar ghee* and *vanaspati* 39°C groups, whereas on the unsupplemented diet, two and three respectively of the five in each gave litters, on the supplemented diet four and one respectively out of five in each, reproduced. The litter size too was practically unaltered, varying from an average of one to four in the different groups. The birth weight of the young was slightly better in some groups, the average weight ranging from 3.5 to 4.5 gm. each. The mortality rate and lactation performance were exactly the same as on the unsupplemented diet in which all the young ones died either just after, or within two to three days of birth.

DISCUSSION

It will thus be seen that with basal rations which permitted reproduction and fairly satisfactory lactation, there was no significant difference between fat and fat. These results are in agreement with the observations of Galamini [1939], Sure [1941], Maynard and Rasmussen [1942], Deuel *et al.*, [1946c, 1945a] and Euler *et al.*, [1947, 1947a]. The inability of various fats and oils, including butter-fat, to promote satisfactory lactation on an inadequate diet, noticed by Sure [1941a] has been confirmed by the present results of both the sub-optimum synthetic ration as well as of the Indian dietaries. Boelter and Greenberg [1943] had observed that on a diet very low in calcium, successful mating was difficult. Cerecedo and Vinson [1944] had pointed to the importance of the level of protein in the diet for this purpose. As the Indian dietaries were sub-adequate in both these important proximate principles, it is not surprising that rats on such poor dietaries failed to reproduce or rear litters. Though supplementation with vitamins slightly improved the reproduction capacity, the females failed entirely to suckle their litters. It might, therefore, be surmised that the deficiencies of the dietary in calcium, protein and other factors were so overwhelming that successful reproduction and lactation were impossible.

These findings, therefore, indicate that on a poor human dietary, characterised by multiple deficiencies or on a sub-adequate synthetic diet as used in the above studies, rats fail to reproduce, irrespective of the nature of dietary fat, including cow *ghee*. When the synthetic diet was liberalised by the addition of skim milk, greens and vitamin E, reproduction and lactation was satisfactory and no significant difference in these functions was seen in animals getting *ghee* or other fats like vegetable oils raw, refined or hydrogenated.

CHAPTER VIII

BLOOD PICTURE OF RATS INGESTING VARIOUS FATS AND OILS

BY N. D. KEHAR, T. S. KRISHNAN, D. N. MULLICK, D. N. MUNRO and B. DUTT

IN rats deprived of riboflavin and in those with restricted food intake, Shukers and Day [1943] noticed that blood counts showed a low level of leucocytes, an increase in the per cent of neutrophiles and a decrease in lymphocytes, which were suggestive of inanition. Ershoff and Adams [1946] observed that the addition of folic acid corrected the reduction in leucocytes and suggested that rats on a restricted food intake were either unable to synthesize or utilize the folic acid present, or that they required more of this substance than animals on an unrestricted diet. Vitamin A deficiency was found by Abbott *et al.*, [1939] to lead to a decrease in total leucocytes. Hassan, Ibrahim and Khanna [1948] also noticed that leucocytes, both collectively and differentially, responded to the level of vitamin A in the blood. These authors, however, state that with increase in vitamin A, the total number tends to decrease, lymphocytes and monocytes to increase, and neutrophiles and eosinophiles to decrease. Brenner and Roberts [1943] had also observed a relationship between vitamin A and the general defence mechanism of the body. Berry *et al.*, [1945] found that rats on a basal deficient diet, compared with those on an adequate diet, showed a total white cell count of only a third, with a relative increase in granulocytes and decrease in lymphocytes, which confirmed their working hypothesis that resistance to certain bacterial infections may be lowered by an inadequate diet. Callaway and Noojni [1940] noticed that animals on a low-fat diet had a lesser degree of leucocytosis after infection than those on a normal or high fat diet. The differences were due principally to polymorphonuclear cells. No difference in the red cell count or haemoglobin of the blood of children, getting either butter-fat or margarine as their dietary fat, was observed by Leichenger and associates [1948]. The following study was carried out to find out the effect of ingesting different fats and oils along with a standard synthetic diet on the blood picture when the ingestion of vitamin A was restricted to a sub-optimum level.

Experimental

The blood picture of fourth generation rats, which were fed the basal synthetic diet (mentioned in Experiment III, Chapter VI) containing 5 per cent of cow ghee, groundnut, mustard, mahua or coconut oil or 'Dalda', was studied. The experimental procedure, feeding, management of animals and other cognate matters, are given in detail in Chapter VI.

The total count of the red blood corpuscles, white blood corpuscles, haemoglobin content, as well as the differential count of the leucocytes of the blood of the above

TABLE XXVII

Effect of different fats on the blood picture of rats

Fat or oil	R. B. C. millions per cu. mm.	W. B. C. thousands per cu. mm.	Haemoglobin g/100 ml.	Differential count per cent			
				Polymorphs	Lymphocytes	Monocytes	Eosinophiles
<i>Cow ghee</i>	9.0±0.4	11.8±0.9	17.0±0.8	31±6.0	42±9.8	25±5.6	2±0
<i>Groundnut oil, raw</i>	8.4±0.1	10.5±0.6	13.1±0.4	31±4.5	51±5.4	16±3.5	2±1
<i>Groundnut oil, refined</i>	8.5±0.1	12.0±0.8	14.3±0.4	31±8.9	34±17.2	33±10.9	2±1.1
<i>Mustard oil, raw</i>	9.1±0.6	11.2±0.8	14.7±0.4	33±4.0	49±5.0	17±1.2	1±0.5
<i>Mustard oil, refined</i>	9.4±0.3	11.0±0.5	14.0±1.2	32±1.5	57±3.6	11±3.5	—
<i>Makua oil, raw</i>	9.7±0.5	12.5±0.8	13.8±0.1	30±4.3	47±1.4	22±3.9	1±0.9
<i>Makua oil, refined</i>	9.0±0.0	11.6±0.7	12.5±0.3	36±3.1	53±1.6	10±2.9	1±0.6
<i>Coconut oil, raw</i>	9.1±0.6	12.0±0.3	14.5±0.1	23±5.2	52±4.0	24±0.9	1±0.5
<i>Coconut oil, refined</i>	8.6±0.6	11.5±0.5	13.4±0.1	21±3.7	56±7.9	21±8.9	2±0.4
<i>'Daldā'</i>	8.2±0.3	12.0±1.0	14.6±0.2	25±6.5	61±5.5	12±2.2	2±1.7
<i>Stock diet</i>	9.1±0.2	13.3±0.9	17.5±1.0	31±2.1	43±3.7	23±5.2	3±0.3

rats, were carried out when they had just completed eight weeks of experimental feeding, after weaning. All the above determinations were made according to the procedure outlined by Napier and Das Gupta [1945]. Three male rats from each of the oil and fat groups as well as an equal number of similar rats from the stock colony, were examined for the above constituents.

The data relating to the total and differential counts of the cells as well as the haemoglobin content of the blood of the rats getting different oils, cow *ghee* or *vanas-pati*, besides those on the stock diet, are given in Table XXVII. It might be observed from the same that the average total R. B. C. count was nearly the same in the stock as well as experimental rats on the different fats. The average total W.B.C. count, which ranged from 10.5×10^3 to 12.5×10^3 , was also about the same in all the experimental rats including cow-*ghee* fed ones, but slightly lower than that observed in the stock animals, viz. 13.3×10^3 . The differential counts, too, were roughly the same in all groups including cow-*ghee* fed and stock rats. There was, however, a slight tendency for the average percentage of polymorphonuclear cells to be slightly lower in the coconut oil and 'Dalda' groups, as compared with the others. The most pronounced difference, however, was in the haemoglobin content of the blood. While the average figures for stock rats and the experimental cow-*ghee* fed ones were practically the same, all other groups gave somewhat lower figures. Among the latter, the average figures ranged from 12.5 to 14.7 gm., which were more or less within the normal values for such animals, while those obtained with stock rats and the cow-*ghee* fed animals in this experiment, viz. 17.5 and 17.0 gm. respectively, were somewhat higher than what are usually considered normal values.

DISCUSSION

Leichenger *et al.*, [1948] did not find any difference in the red cell count and the haemoglobin content of the blood of children getting butter-fat or margarine as their dietary fat. In the present experiment too, no differences in the red cell counts were found in the blood of rats getting different fats, though there was a tendency for the haemoglobin content of the cow-*ghee* fed animals to be somewhat higher than those of the other fat groups. However, the latter figures were approximately equal to that given by Geyer and associates [1947a] as normal. The total W. B. C. count found by the latter workers in their rats was somewhat higher than that obtained in this experiment, but their minimum value of 13.5×10^3 agreed fairly well with that obtained on our stock rats, viz. 13.3×10^3 . This lowering in the total W. B. C. count has been associated with vitamin A deficiency by Abbott and co-workers [1939]. Brenner and Roberts [1943] had also found some relationship between vitamin A deficiency and the general defence mechanism of the body. Since all the experimental animals were on a diet supplying only sub-optimum amounts of vitamin A, this tendency for a slight lowering of the total W. B. C. may be an indication of incipient vitamin A deficiency. The low vitamin A reserves found in the livers of these animals (Chapter XI) would be a further support for this presumption. Though the extent of decrease in the average total W. B. C. count observed by Berry *et al.*, [1945] was not seen here, yet the values were slightly lower than those of the stock rats. With regard to the differential count, there seemed to be no material difference between the different experimental or stock animals. However, in the coconut oil and 'Dalda'-fed rats there was a

tendency for the polymorphs to be somewhat lower than the others. When this is coupled with the slightly lower total W. B. C. counts as well, it would seem that they indicate a condition which might render them more susceptible to acute bacterial infection than others. This is in line with the hypothesis of Berry and associates [*loc. cit.*] that resistance to certain bacterial infections may be lowered by inadequate diets. As has been shown elsewhere (Chapter XII), the vitamin A reserves in the liver of the animals of these two groups were also about the lowest among all the groups. However, since the number of observations were small and as there were considerable individual variations, more work would seem essential before any definite conclusions regarding the comparative effects of the different fats on the blood picture could be drawn.

CHAPTER IX

THE EFFECT OF DIFFERENT FATS AND OILS ON PROTEIN METABOLISM

By N. D. KEHAR, T. S. KRISHNAN and R. CHANDA

THOUGH a considerable amount of work has been done on the influence of different dietary fats on the absorption and utilization of vitamins, minerals, etc., very little seems to have been done regarding their effect on protein metabolism. Maignon [1933-34] observed that fats had a favourable effect on the utilization of protein for synthetic purposes and on nitrogen metabolism. Forbes *et al.*, [1946, 1946, a, b, c] have studied the effect of the level of dietary fat on protein and energy metabolism. As, however, no work seems to have been carried out regarding the relative effects of different fats on protein absorption and metabolism work, a comparison of different vegetable oils as well as lard and *vanaspathi* with cow *ghee* is reported here.

Metabolism experiments were carried out with adult rats to compare the comparative effect of groundnut, cotton-seed, coconut, *mahua*, *til* and mustard oils, three brands of hydrogenated groundnut oil and two brands each of hydrogenated cotton-seed and sesame oils, as well as lard and cow *ghee*, to compare their effect on protein metabolism, as measured by the digestibility coefficient and biological value of the dietary protein. Both raw and refined samples of each of the oils were studied. The synthetic diet employed in the growth experiments, reported in Chapter VI was used.

Six adult male rats from the Institute stock colony about 300 gm. in weight each, were selected and housed in individual cages with raised wire screen bottoms. They were then put on the following fat-free diet :

Dextrinised starch	64	plus 5 γ of β -carotene
Casein (extracted)	24	plus 10 I.U. of vitamin D
Yeast (extracted)	8	per head per day
Salt mixture	4	
	<hr/>	
	100	
	<hr/>	

The animals were fed *ad lib* and had constant access to fresh water from a self-feeding bottle. After a preliminary period of ten days, a metabolism experiment lasting three days, was carried out, during which period the exact food consumption was determined and the total excretion of urine and faeces was collected quantitatively and analysed.

Analyses of the food, faeces and urine were carried out and the actual daily ingestion and excretion of nitrogen determined for each animal. The data for this group of animals is given in Table XXVIII under fat-free diet.

TABLE XXVIII

Effect of different fats and oils on the true digestibility coefficient and biological value of protein

Groups of rats indicated by nature of fat	True digestibility coefficient of protein	Biological value
Fat-free	67.2±0.8	45.0±0.8
Cow ghee	91.1±0.5	73.1±3.4
Groundnut oil, raw	90.7±0.6	54.0±2.3
Groundnut oil, refined	92.3±1.0	59.7±1.6
'Dalda' (m.p. 36°C.)	93.1±0.4	58.6±3.0
'Dalda' (m.p. 40-42°C.)	90.2±1.1	58.2±4.2
'First quality'	84.0±0.4	52.4±1.8
Til oil raw	89.9±0.4	59.3±2.4
Til oil refined	92.2±0.2	56.3±1.1
'Temple'	88.1±0.6	51.8±2.5
'Rajhans'	89.3±0.7	57.1±3.3
Cotton-seed oil, raw	90.0±0.7	50.5±3.5
Cotton-seed oil, refined	86.1±2.7	55.5±2.2
'Binaula'	88.8±0.7	60.7±1.8
'Kotogem'	89.2±1.2	54.7±2.4
Coconut oil, raw	93.1±0.4	57.0±2.7
Coconut oil, refined	90.8±0.3	55.6±2.4
Mahua oil, raw	93.5±0.3	52.8±1.6
Mahua oil, refined	92.1±0.3	54.8±2.2
Mustard oil, raw	92.6±0.8	55.5±2.4
Mustard oil, refined	94.0±0.7	59.0±2.1
Lard	91.8±0.4	53.9±1.6

Simultaneously, a group of 126 adult male rats, each weighing about 300 gm. was selected from the Institute stock colony and divided into 21 similar groups of six each. They were then put on the following diet, sub-adequate in vitamin A :

Dextrinised starch)	59	} Plus 5 γ of β -carotene plus 10 I.U. of vitamin D, per head per day
Casein (extracted)	24	
Yeast (extracted)	8	
Salt mixture	4	
Experimental oil or fat	5	
	<hr/> 100 <hr/>	

Each of the groups was given the above diet containing one of the different oils and fats. When cow *ghee* formed the dietary fat, the dosage of vitamin A was equated. The experiment with each fat or oil consisted of a preliminary period of four days, followed by an experimental period of three days during which (latter period) the exact food consumption was determined and the total excretion of faeces and urine collected quantitatively and measured, as before. To determine the exact intake and outgo of N, the analysis of the food, faeces and urine of each of the experimental rats was also carried out as already described.

The excretions of metabolic faecal N and the endogenous urinary N were determined in a similar separate group of adult male rats which were fed the following N-free ration :

Dextrinised starch	81
Sucrose	10
Cow <i>ghee</i>	5
Salt mixture	4
	<hr/>
TOTAL	100 <hr/>

Each animal received daily 8 I.U. of vitamin A, 1 γ calciferol and 1 c.c. of 20 per cent marmite solution.

The determination of the exact nitrogen intake and excretion, as well as the analysis of the food, faeces and urine, were carried out for each of the different animals. From these, the average daily excretion of metabolic faecal nitrogen, expressed as gm. N per 100 gm. of dry faeces, as advocated by Kehar and Mukherjee [1949], and endogenous urinary nitrogen expressed as mg. N per 100 gm. body weight, were found to be 1.38 and 15.7 respectively. These figures were used to determine the true digestibility coefficient as well as the biological value of dietary protein, in each of the fat-diets. The biological value was estimated by the method of Mitchell [1923-24] as modified by Mukherjee and Kehar (unpublished results).

The results of the experiments are given in Table XXVIII. From the same, it may be seen that the digestibility as well as the biological value of the protein was raised by the addition of fat to the diet. Replacement of 5 per cent of the starch of the above fat-free diet by an equal weight of cow *ghee*, improved the digestibility of the protein by 36 per cent and increased its biological value by 62 per cent. Replacement of cow *ghee* by an equal weight of any of the vegetable oils, *vanaspati* or lard did not seem to materially affect in general the improvement in digestibility 'of the protein'. Statistical analysis reveals that 'First Quality', 'Temple' and 'Binaula' fed rats showed significantly lower whereas 'Dalda' (m.p. 36°C.) fed rats gave significantly higher digestibility of protein, as compared to the cow-*ghee* fed rats. The rise in the biological value as compared to that on the fat-free diet was much less, being only about 12 to 35 per cent. Hence, when the vitamin A ingestion was restricted to a sub-optimum level, cow *ghee* seemed to be significantly superior to other oils and fats, as it enabled a more efficient utilization of the dietary protein as judged by its higher biological value. This beneficial effect of fat on protein metabolism, observed here, is in agreement with the observations of Maignon [1933-34] and Kehar and Chanda [1948]. The superiority of cow *ghee* over other fats, enabling a better utilization of protein, may be due to the ability of cow *ghee* to make better use of the sub-optimum ingestion of vitamin A as has been demonstrated further by the higher storage of vitamin A in the liver by animals getting cow *ghee* (Chapter 11). The effect of low dietary provision of vitamin A in decreasing protein utilization has been observed by Reitzman *et al.*, [1945] on calves. There were no marked differences between oils and fats other than cow *ghee*, in their effect on protein metabolism. Neither refining nor hydrogenation seemed to have a significant effect on the parent oil, with regard to its effect on the digestibility and utilization of the dietary protein.

The poorer growth of the oils or *vanaspati* fed rats observed in the second and subsequent generations when given a basal ration sub-adequate in vitamin A (Chapter IV) may perhaps be due, at least partly, to this lower efficiency of protein utilization on such a diet.

Effect of supplementation with B-vitamins

Hartwell [1922 ; 1928], Reader and Drummond [1926] as well as Hassan and Drummond [1927] have shown that an adequate provision of B-vitamins is essential for the proper utilization of dietary protein. Boutwell *et al.*, [1945] also indicated that the requirements of B-vitamins were enhanced when corn oil was fed in place of butter-fat in the diet. In order to study the effect of supplementation with B-vitamins on protein metabolism, the group of rats receiving raw groundnut oil and 'Dalda' (M.P. 36° C.) were used. After the test with the above fats on the basal diet was over, they were given the following amounts of B-vitamins and the experiments repeated in an exactly similar manner.

Thiamine	30γ	} per rat per day
Riboflavin	30γ	
Pyridoxin	30γ	
Calcium pantothenate	100γ	
and		
Nicotinic acid	1 mg.	

The results were as following :

Fat fed	Digestibility of protein per cent		Biological value per cent	
	Without B-vitamins	With B-vitamins	Without B-vitamins	With B-vitamins
Groundnut oil, raw	90.7 \pm 0.6	91.6 \pm 1.1	54.0 \pm 2.3	60.8 \pm 1.1
Groundnut oil, hydrogenated (‘Dalda’ M.P. 36°C.)	93.1 \pm 0.4	93.7 \pm 0.5	58.6 \pm 3.0	66.5 \pm 0.8

It would be clear from the above that addition of extra B-vitamins, while not affecting the digestibility of the dietary protein, had improved its biological value by about 13 per cent and 14 per cent respectively in the case of the oil and *vanaspatt* respectively. Even these improved values are, however, lower than that observed with cow-*ghee* as the dietary fat.

CHAPTER X

INFLUENCE OF FATS ON ABSORPTION OF CALCIUM AND PHOSPHORUS

By N. D. KEHAR, T. S. KRISHNAN and R. CHANDA

It is generally admitted that dietary fat has a beneficial influence on the absorption of calcium. Though the antirachitic properties of fat in the diet have been noticed by several workers, [Jones, 1940, Booth *et al.*, 1942, French, 1942, Dutt, 1945, Datta, 1948 and others], there is no complete unanimity regarding the comparative value of different fats for this purpose. Whereas Booth *et al.*, [1942], Euler and associates [1941, 1942] and others did not notice any difference between butter-fat, margarines, vegetable oils, lard, etc. in this respect, other investigators like Hagnell-Zethraeus and collaborators [1942], French and Elliott [1943], Dutt [1945], Basu and Nath [1946 b] and Westerlund [1947-48] have observed differences between them, generally finding butter-fat superior to the others. Consequently, the following study was carried out to compare the effect of ingesting cow *ghee*, vegetable oils, *vanaspatis* and lard, as dietary fats on the calcium and phosphorus assimilation in the rat.

To determine their effect on calcium and phosphorus retention, metabolism experiments were carried out with cow *ghee*, six different vegetable oils generally used for human consumption and/or for the manufacture of *vanaspatis*, seven brands of *vanaspati* made by the hydrogenation of three different oils and lard as dietary fat. Both raw and refined samples of each of the oils were studied. The experimental technique employed, the diet used, the fats and oils tested, and other cognate factors were exactly the same as described earlier in Chapter IX.

The fat-free diet (Chapter IX) served as the negative control while the basal diet plus cow *ghee* formed the positive control.

Another experiment to determine the percentage of ash in the bones of growing animals fed different fats, was carried out as follows :

Female stock rats were kept in a dark room and mated. The young ones were weaned at 28 days of age and divided into seven similar groups of six rats each. These were given the same basal ration as fed to the rats in the metabolism tests, the fats tested were cow *ghee*, two *vanaspatis* made by hydrogenation of groundnut oil ('Dalda' M.P. 40°-42°C) and ('First Quality No. 1'), two *vanaspatis* made by hydrogenation of sesame oil ('Temple' M.P. 41°-45°C), and ('Rajhans' M.P. 42°C.) and two *vanaspatis* made by hydrogenation of cotton-seed oil ('Kotogem' M.P. 41°C. and 'Binaula' M.P. 37°-40°C). The amounts of vitamins A and D fed also remained exactly the same as in the growth experiments, viz. 8 I.U. of vitamin A and 30 I.U. of vitamin D per rat per day. This ration was fed to the weanling rats for four weeks. The technique of feeding, housing, management, etc. were according to the standard

procedure described earlier. During the entire period of the experiment the animals were kept in the dark room. At the end of four weeks the rats were sacrificed and the fat-free femurs were analysed for ash, calcium and phosphorus.

RESULTS

The calcium and phosphorus retention results of the different groups are given in Table XXIX. It would be noticed that in the absence of fat in the diet, the absorption of calcium and phosphorus is seriously affected, the average figures being 5 per cent and 7.5 per cent respectively. With addition of cow *ghee* to the extent of 5 per cent of the diet, the retention of calcium rose to 45 per cent and of phosphorus to 57 per cent. When the different oils or fats were substituted for cow *ghee*, weight for weight, the percentage absorption of calcium ranged from 22.6 to 40.7.

TABLE XXIX

Effect of different fats and oils on calcium and phosphorus metabolism

Fats or oils	Percentage absorption of calcium	Percentage absorption of phosphorus
Fat-free	-5.27 ± 1.47	7.54 ± 2.03
Cow <i>ghee</i>	44.62 ± 1.42	56.75 ± 1.72
Groundnut oil, raw	39.63 ± 2.20	47.78 ± 2.29
Groundnut oil, refined	40.66 ± 0.94	46.77 ± 0.98
'Dalda' <i>vanaspathi</i> (m.p. 36°C.)	38.65 ± 1.42	44.35 ± 2.53
'Dalda' <i>vanaspathi</i> (m.p. 40°-42°C.)	36.17 ± 2.15	43.57 ± 1.87
'First quality' (m.p. 40°-42°C.)	22.62 ± 1.39	41.80 ± 1.76
Sesame oil, raw	38.73 ± 1.02	43.82 ± 0.76
Sesame oil, refined	38.27 ± 1.31	36.10 ± 2.83
'Temple' (m.p. 41°-43°C.)	34.68 ± 1.22	28.43 ± 2.01
'Rajhans' (m.p. 42°C.)	25.40 ± 1.29	29.90 ± 3.27
Cotton-seed oil, raw	33.07 ± 3.17	40.88 ± 2.51
Cotton-seed oil, refined	37.20 ± 1.35	47.17 ± 1.51
'Binaula' (m.p. 37°-40°C.)	34.18 ± 1.68	37.45 ± 1.99
'Kotogem' (m.p. 41°C.)	29.35 ± 2.31	34.97 ± 2.20
Coconut oil, raw	34.1 ± 1.52	42.9 ± 1.81
Coconut oil, refined	33.9 ± 1.21	39.9 ± 1.79
<i>Mahua</i> oil, raw	29.9 ± 1.18	38.0 ± 1.78
<i>Mahua</i> oil, refined	32.1 ± 1.65	35.0 ± 1.09
Mustard oil, raw	32.7 ± 1.18	38.3 ± 1.44
Mustard oil, refined	33.8 ± 2.41	36.4 ± 1.47
Lard	30.8 ± 1.47	37.2 ± 1.51

The substitution of different vegetable oils for *ghee* in the diet caused a reduction in the calcium absorption by about 9 to 33 per cent, while the replacement of *ghee* by the *vanaspathis* brought about a lowering of about 13 to 47 per cent in the same. It would, therefore, seem that hydrogenated products were less efficient in promoting calcium absorption than the vegetable oils. A careful scrutiny of the relevant figures for the parent oils and their hydrogenated products reveals that all *vanaspathis*, even when made from the same oil, do not behave alike, for whereas, 'Dalda' M.P. 36° C and 'Binaula' showed more or less similar retention of calcium as the parent oil, 'Dalda' M.P. 40 -42°C, 'Kotogem' and 'Temple' showed a reduction of about

10 per cent from the same. 'First Quality No. 1' and 'Rajhans', however, recorded much lower calcium absorption than the corresponding parent oils, the decrease being about 47 per cent and 34 per cent respectively. When lard replaced the *ghee*, the calcium value was reduced by about 31 per cent.

In the case of phosphorus too, the absorption figures were lower when the vegetable oils, lard or *vanaspathi* replaced the cow *ghee*, as was observed with calcium. The range of the figures of absorption with different fats in the diet was also roughly of the same magnitude for both the minerals. With the oils in the diet, the phosphorus absorption figures were about 16 to 38 per cent lower than that recorded when the cow *ghee* formed the dietary fat. The lard-fed animals gave an absorption value about 35 per cent less than the cow *ghee* group. The substitution of the *vanaspathi* for the *ghee* or parent oils in the dietary led to a decrease in retention of phosphorus which ranged from about 22 to 50 per cent and 7 to 35 per cent respectively. The behaviour of the different hydrogenated products from the same oil was more uniform in this case than was seen with calcium. The maximum decrease was seen in the hydrogenated sesame oils and the least with hydrogenated groundnut oils. Between the different vegetable oils (other than groundnut oil), there was generally no significant difference with regard to phosphorus absorption. Raw groundnut oil was slightly better than the rest. Lard was somewhat less efficient than 'Dalda'.

The absorption of phosphorus was somewhat better than that of calcium in nearly all the fats tested. In the case of refined *mahua*, *til* and mustard oil, the difference between the calcium and phosphorus absorption figures was less than that observed with other fats and oils. There did not seem to be any consistently marked difference between the raw and refined oils in their capacity to promote calcium and phosphorus absorption. Hydrogenation appeared to affect significantly the efficiency of the parent oil to promote calcium and phosphorus absorption though the extent of the loss of efficiency varied with the nature of the hydrogenated product. The statistical analysis of the data reveals that refined groundnut oils yield results comparable to cow *ghee* for the absorption of calcium but cow *ghee* appears to give better results than any other fat for the absorption of phosphorus.

The bone ash percentage was somewhat higher in *ghee*-fed rats than in the *vanaspathi*-fed ones but the calcium and phosphorus contents as well as the calcium phosphorus ratio of the bones were practically unaffected by the nature of the dietary fat (Table XXX).

DISCUSSION

The antirachitic property of fat as evidenced by improved calcium assimilation, noticed by Knudson and Floody [1940], Jones [1940] and others has been substantiated by these experiments. Whereas Booth *et al.*, [1942] as well as Euler and associates [1941; 1942] found no differences between different fats, like butter-fat, vegetable oils, margarines and lard in their effect to promote increased calcium assimilation, the present results show a significant superiority of *ghee* over the others. These results are in agreement with the findings of the work of Hagnell-Zethraeus *et al.*, [1942], French and Elliot [1943], Dutt [1945] and Westerlund [1947-48] as well as Kehar and Chanda [1948]. - Whereas Dutt [1945] found a decrease of 14

per cent in calcium absorption by replacing cow *ghee* with hydrogenated fats, the reduction observed in this experiment was to the extent of 13.47 per cent. Basu and Nath [1946, b] studied the effect of different fats on the calcium and phosphorus metabolism of adult men. They observed that inclusion of each of the fats, except coconut oil resulted in an increase in the calcium balance due to diminished faecal and urinary excretion of calcium. Similar, though not such marked or regular, results were obtained for phosphorus. When coconut oil was added to the basal diet, however, there was a marked increase in the faecal excretion of calcium and the urinary calcium excretion was higher than when the other fats were given and in every subject there was a fall in calcium balance. In the present experiment, addition of 5 per cent of coconut oil to the fat-free ration resulted in a decrease in both the faecal and urinary excretions of calcium thus enabling a greatly increased retention of 34 per cent of the same as compared to 5 per cent of the basal fat-free ration. With regard to the behaviour of coconut oil as compared with other fats, particularly in the matter of urinary excretion of calcium, no marked differences were observed and no increased urinary calcium excretion, over that seen with other fat groups, was noticed. These observations were equally true of phosphorus absorption as well. There is, however, one difference between the experiments of Basu and Nath and the present one, viz. they carried out their studies on men while these tests were made on rats. Further they fed a natural diet which was not fat-free while in the present experiment the diet was a synthetic one, composed of pure fat-free materials and with the vitamin A intake restricted to a sub-adequate level, viz. 8 I.U. of β -carotene. Calcium and phosphorus, however, were provided in adequate quantities and in proper proportion. Enough vitamin D was also fed. It is possible that some or all of the differences between the present results and that reported by others might have been caused by the differences in experimental conditions as discussed above.

TABLE XXX

Calcium and phosphorus contents of bones of animals fed different fats

Groups of rats indicated by the nature of fat	Per cent ash in fat-free bone	Per cent calcium in bone ash	Per cent phosphorus in bone ash	Calcium: phosphorus in bone ash
Cow <i>ghee</i>	63.10 \pm 0.71	38.15 \pm 0.22	18.17 \pm 0.12	2.10 \pm 0.020
'Dalda' <i>vanaspati</i> , m.p. 40-42°C.	57.40 \pm 0.79	37.90 \pm 0.19	18.08 \pm 0.11	2.10 \pm 0.004
'First quality' m.p. 40-42°C.	56.27 \pm 1.01	37.98 \pm 0.15	18.20 \pm 0.11	2.09 \pm 0.004
'Temple' m.p. 41-43°C.	54.60 \pm 1.02	38.07 \pm 0.16	18.05 \pm 0.12	2.11 \pm 0.008
'Rajhans' m.p. 42°C.	54.00 \pm 0.82	38.05 \pm 0.16	18.10 \pm 0.16	2.10 \pm 0.008
'Kotogem' m.p. 41°C.	55.00 \pm 1.07	38.32 \pm 0.11	18.20 \pm 0.12	2.11 \pm 0.008
'Binaula' m.p. 37-40°C.	53.40 \pm 1.02	38.13 \pm 0.19	18.15 \pm 0.15	2.10 \pm 0.008

It would, therefore, appear that under the above experimental conditions, when cow *ghee* formed the sole dietary fat, there was significantly better calcium and phosphorus assimilation as compared with that when an equal weight of any vegetable oil, *vanaspati* or lard was consumed. There were some differences between the vegetable oils; groundnut oil being a little better and *mahua* oil somewhat poorer than the others. In this respect, the animal-fat, lard, occupied no higher rank than some of the poorer oils. Refining did not seem to have any material effect on the ability of the oils to promote calcium and phosphorus absorption. Hydrogenation decreased calcium and phosphorus retention as compared with the parent oils, the extent of variation depending on the nature of the *vanaspati*.

It might be recollected that, in the growth experiments discussed in Chapter VI earlier, animals fed raw *mahua* or mustard oils exhibited a better gain in the weight than those given groundnut oil or 'Dalda' in the second generation and to a somewhat lower extent even in the fourth generation, particularly among males. However, from the results of calcium and phosphorus absorptions observed in this experiment, it would be noticed that groundnut oil and 'Dalda' promoted a relatively higher retention of the above minerals than mustard or *mahua* oils. It would, therefore, seem that the differences in growth promoting value between the various oils and fats, observed in the experiments described in Chapter VI, may not be due to variations in their relative antirachitic values.

Effect of supplementation with B-vitamins

In an exploratory experiment when a group of rats was given a ration, containing *vanaspati*, and supplemented with marmite, the absorption of calcium and phosphorus showed a marked improvement in the marmite supplemented groups. The observation seemed to point to an important relation between B-vitamins and calcium and phosphorus metabolism. Since marmite contains factors other than B-vitamins, the experiment was repeated by supplementing the synthetic ration fed in the metabolism studies detailed earlier in this chapter, with synthetic B-vitamins. The fats studied were raw groundnut oil and 'Dalda' M.P. 36°C. In addition to the ration given in the metabolism experiments, these rats were fed a supplement of 30γ thiamine hydrochloride, 30γ pyridoxin, 50γ riboflavin, 100γ calcium pantothenate and 1 mg. nicotinic acid per head per day. The calcium and phosphorus retentions, as determined in an exactly similar manner as already described are given below :

Fat or oil fed	Calcium per cent		Phosphorus per cent	
	Basal ration only	Basal ration plus B-vitamins	Basal ration only	Basal ration plus B-vitamins
Groundnut oil raw	39.6 ± 2.20	46.6 ± 0.62	47.6 ± 2.29	52.1 ± 0.77
'Dalda' <i>vanaspati</i> , M.P. 36 C.	38.7 ± 1.42	45.0 ± 2.01	44.4 ± 2.53	51.0 ± 0.75

It would be clear from the above data that calcium balance was raised by 18 and 16 per cent and phosphorus retention by 9 and 15 per cent when the oil and *vanaspathi* respectively were supplemented with additional B-vitamins. The increased retention equalled that obtained with the *ghee* in the case of calcium and approached it in the case of phosphorus.

CHAPTER XI

ABSORPTION OF CAROTENE AND LIVER STORAGE OF VITAMIN A

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SINCE differences between the growth promoting values of *ghee*, vegetable oils, *vanaspathi* and lard were observed in the experiments reported in Chapters V and VI, and as the dietary allowance of vitamin A was sub-adequate in many of the tests, it was decided to find out the comparative efficiencies of the different fats to promote the storage of the ingested carotene and vitamin A in the liver as vitamin A. Though growth response is often used as a general index of the efficiency of vitamin A utilization, yet, as growth is influenced by several factors besides the level of vitamin A ingestion, it is not as reliable an index of vitamin A and carotene utilization as storage of vitamin A in the liver which had been used for this purpose ever since the classical work of Moore (1929) demonstrating the conversion and storage of carotene as vitamin A in the liver. The animals of the Experiments I, III, V and VI were utilized for studying this aspect in the different dietaries. The results are given below. The determination of the liver vitamin A was carried out by the method of Davies [1933] except that the unsaponifiable matter was dissolved in aldehyde-free pure ethyl alcohol and the vitamin A measured by a Hilger vitamer.

Experiment I

After the young ones in the various generations were weaned, their parents were sacrificed and the livers were examined for vitamin A content. As the total vitamin A content per gram of liver was found to increase with the age of the animals and as animals of various ages were examined, the average rate of deposition of the vitamin, i.e. the total vitamin A content divided by the age of the animals, was used instead. The results are given in Table XXXI. It will be seen that in spite of the fact that all the groups were receiving plenty of carotene in the form of green fodder (five grams daily for the last three to four months of their lives), the livers of the *vanaspathi*-fed rats contained much less vitamin A than those of the *ghee* ingesting ones. The amount of vitamin A and carotene fed to *ghee*-fed rats through the *ghee* was very small as compared with the total amount of carotene fed as greens. Thus the total carotene ingested daily through greens was about 250-300 I.U., while the maximum amount of vitamin A and carotene supplied through *ghee* never exceeded above 10 I.U. per head per day. In spite of this fact, the liver storage of vitamin A by most of the rats of the *vanaspathi* group was significantly lower than that of the cow *ghee*-fed animals. It is reasonable to believe, therefore, that the presence of *vanaspathi* in the diet, interfered with the absorption or utilization of dietary carotene. Statistical analysis of the data confirms that cow *ghee* appears to

be best in respect of vitamin A concentration in liver. Next come buffalo *ghee* and 'Rajhans'. The other *vanaspatis* appear to be more or less of the same order and inferior to the above fats.

TABLE XXXI

Storage of vitamin A in liver of rats receiving various fats (Experiment I)

Name of fat	Concentration of vitamin A in γ /gm. liver	Rate of storage of vitamin A
Cow <i>ghee</i>	102.71 \pm 20.74	12.13 \pm 1.85
Buffalo <i>ghee</i>	66.78 \pm 8.59	8.34 \pm 0.29
'Dalda'	34.80 \pm 6.91	4.33 \pm 0.78
'1st Quality'	40.08 \pm 6.30	4.99 \pm 0.86
'Swastika'	44.22 \pm 4.74	5.78 \pm 0.61
'Kotogem'	39.98 \pm 5.01	5.12 \pm 0.63
'Cotex'	39.93 \pm 7.03	5.71 \pm 0.97
'Binaula'	46.39 \pm 11.31	5.71 \pm 1.00
'Temple'	46.50 \pm 12.60	5.52 \pm 1.39
'Rajhana'	56.99 \pm 10.94	7.07 \pm 0.95

Note: Rate of storage = Concentration of Vitamin A in γ /gm. liver / Age of animal in months.

Experiment III

Synthetic Diet experiment

The vitamin A content of the livers of the rats of the second and fourth generations, fed cow *ghee*, vegetable oils (both raw and refined), or *vanaspati*, are given in Table XXXII. The hepatic vitamin A concentration in these animals was estimated when they had just completed eight weeks' experimental feeding on the basal diet in which the vitamin A ingestion was only 8 I.U. per rat per day, i.e. before they were put on to the liberal diet containing greens. In both these experiments the storage of vitamin A in the liver was highest in rats getting cow *ghee* and least in those receiving 'Dalda' (hydrogenated groundnut oil), while the other oils occupied intermediate positions. It might also be observed that there was a general tendency to store less vitamin A in the liver, in the animals ingesting the refined oils as compared to those getting the corresponding raw ones. The vitamin A content of the liver observed in the fourth generation animals, was consistently lower than that of the corresponding ones of the second generation in each and every one of the fat and oil groups, including cow *ghee*.

TABLE XXXII

Effect of different fats on the vitamin A content of liver (Experiment III)
(γ /gm. liver)

Fats	Second generation	Fourth generation
Cow ghee	7.3 ± 0.67	4.3 ± 0.20
Groundnut oil, raw	4.8 ± 0.36	3.5 ± 0.32
Groundnut oil, refined	4.5 ± 0.23	3.1 ± 0.10
Cotton-seed oil, raw	4.3 ± 0.19	—
Coconut oil, raw	4.7 ± 0.42	3.2 ± 0.39
Coconut oil, refined	4.1 ± 0.26	3.0 ± 0.15
Mahua oil, raw	4.8 ± 0.12	3.7 ± 0.31
Mahua oil, refined	4.7 ± 0.26	3.4 ± 0.25
Til oil, raw	5.5 ± 0.59	—
Til oil, refined	4.8 ± 0.12	—
Mustard oil, raw	6.4 ± 0.39	4.0 ± 0.13
Mustard oil, refined	6.3 ± 0.24	3.9 ± 0.22
'Dalda'	3.9 ± 0.12	2.8 ± 0.22

Experiments V and VI

Vitamin A storage in liver with Bengali and North Indian diets

The concentration of vitamin A in the liver of the animals fed on the basal and vitamin supplemented Indian diets, when different fats were ingested, is given in Table XXXIII. From the figures in this Table it may be observed that the liver reserve of vitamin A found in the different fat groups of the two diet Series were approximately of the same order and the figures for the concentration of vitamin A found, were also about equal in the corresponding experiments of the two Series. Since the Bengali diet was poorer in vitamin A potency than the North Indian diet, it is natural to expect the animals on the latter dietary to show larger storage than the former. The explanation for this apparent discrepancy may probably be found in the fact that the Bengali diet rats were smaller than the corresponding North Indian diet groups at the end of the experiment and as such their livers also were proportionately smaller in size. As is but natural, the rats getting the vitamin supplements showed a larger reserve than those not receiving the additional allowance. In both the dietary Series, the maximum concentration of vitamin A in liver was observed in the case of rats getting cow ghee as the dietary fat, both in the vitamin supplemented and unsupplemented regimes, and comparatively, the least content was seen in the livers of rats fed on the two *vanaspathis*. The groundnut oil group showed a slightly

higher concentration of vitamin A in the liver than the *vanaspati* group, on both dietaries, whether supplemented with vitamins or not.

It will thus be seen that in all the experiments the *ghee*-fed animals showed a better storage of vitamin A in the liver than rats ingesting oils or *vanaspati*; whereas between the oils and *vanaspati*, the animals receiving the latter stored comparatively less vitamin A than the corresponding ones on the parent oil. On analysis of the data, of Experiment III (Table XXXII) it was found that cow-*ghee* feeding was associated with the maximum storage of vitamin A. It does not, however, differ significantly from raw and refined mustard oils but appears to be superior to the remaining oils excepting raw *mahua* oil in the fourth generation.

The amount of vitamin A found in the liver in the Experiment III, was rather low as compared with the animals of Experiments I, V and VI, probably because, while the former animals were given only 8 I.U. of vitamin A as carotene, the others received correspondingly much higher quantities of vitamin A or carotene. Lewis *et al.*, [1942] did not find any vitamin A in the liver when the ingestion was 10 I.U. or less per day, while Myburgh [1943] observed that an intake of 8.4 I.U. daily, allowed small stores to accumulate in the liver. Callison and Knowles [1945] also noticed vitamin reserves of 4.6—15.9 I.U./gm. liver when 80 I.U./Kg., was fed daily. Caldwell and associates [1945] as well as Campbell *et al.*, [1945] found that rats receiving 3 I.U./gm. air dry food, stored negligible amounts of the vitamin in their liver. The liver reserves obtained in this experiment were more or less in general agreement with the above findings. The quantum of storage noticed in the above experiments seems to be higher than that recorded by Lewis *et al.*, or Sherman and associates (*loc. cit.*).

TABLE XXXIII

Vitamin A content (in γ /gm.) of liver of rats fed Indian dietaries plus different fats and oils (Experiments V and VI)

Fat or oil	Bengali diet		North Indian diet	
	Without vitamin	With vitamin	Without vitamin	With vitamin
Cow <i>ghee</i>	23.2 \pm 1.83	49.9 \pm 4.10	23.0 \pm 1.22	53.3 \pm 1.76
Bazar <i>ghee</i>	15.1 \pm 1.44	32.3 \pm 3.50	18.1 \pm 1.48	31.5 \pm 1.75
Groundnut oil	11.3 \pm 1.02	24.4 \pm 2.89	11.7 \pm 1.35	25.1 \pm 1.45
<i>Vanaspati</i> m.p. 36°C.	8.7 \pm 0.94	15.7 \pm 1.25	9.9 \pm 0.99	16.5 \pm 1.37
<i>Vanaspati</i> m.p. 39°C.	9.9 \pm 0.70	15.8 \pm 2.31	10.6 \pm 0.80	17.4 \pm 0.84

The higher storage of vitamin A in the liver, shown by rats ingesting *ghee*, as compared with the other oil-fed animals getting an equivalent amount of vitamin A as carotene, may be due to their getting their vitamin A mostly as preformed

vitamin A whereas others got it as carotene, since Gray *et al.*, [1940] as well as Treichler and associates [1942] have shown that greater storage in liver takes place from vitamin A than from an equivalent amount of ingested carotene. However, it has been found in Experiments I, V and VI, that when rats were given even plenty of carotene or vitamin A, on the same rations, the *ghee*-fed animals invariably stored a higher percentage of the ingested vitamin in the liver than the others. Hence, the form in which the vitamin is ingested, whether as carotene or vitamin A, may not be the only or even the main reason for this difference in liver storage. Similarly, in the groundnut oil and Dalda or *vanaspati*-fed animals of the synthetic and human diet series, even when both fat groups ingested equal amounts of carotene or vitamin A, the oil-fed rats recorded a higher vitamin A content in the liver than the corresponding rats ingesting *vanaspati*.

Lease *et al.*, [1939] found approximately equal stores of vitamin A in the liver of rats when equal quantities of vitamin A dissolved in different fats were administered. They found no difference between butter-fat, vegetable oil or hydrogenated fat, in this respect. Brown and Bloor [1945] found higher stores of vitamin A in the liver of rats when the dietary fat was rich in unsaturated fatty acids. In these experiments, cow *ghee*, which had a lower degree of unsaturation than groundnut oil or *vanaspati* showed a much larger capacity to promote storage of vitamin A in the liver than the latter fats. The difference in tocopherol content of the fats, which has been given as the explanation of the difference by Guggenheim [1944] and Rao [1945; 1945a], also does not seem to be very satisfactory in this case, because, though the tocopherol content of groundnut oil is presumably higher than that of cow *ghee*, the hepatic storage of vitamin A was higher in animals receiving the *ghee* than in those getting the oil on the same regime. The difference between the various oils themselves, as also between groundnut oil and 'Dalda', observed in Experiments III, V and VI, does not seem to be completely explained by the above. It would, therefore, seem that there are other factors also controlling the efficiency of utilization of, or regulating the requirements of ingested carotene and vitamin A when different fats and oils are ingested in the diet.

The utilization of equal amounts of carotene dissolved in different fats and oils, as revealed by growth response of rats, has been found to be nearly similar by Coward [1936] as well as Lease *et al.*, [1939]. Abramson and Brunius [1942] also found the biological potency of β -carotene dissolved in arachis oil and in margarine to be similar, as measured by weight gains of vitamin A depleted rats. Sherman [1941] as well as Brown and Bloor [1945] have found the stability of β -carotene solutions to be greater in oils of soyabean, sun-flower, mustard, etc. than in comparatively less saturated fats like lard, refined tallow, coconut oil, etc. suggesting that the degree of unsaturation had some influence on the same. Guggenheim [1944] and Rao [1945; 1945a], however, attributed the above difference between the oils and fats to their variation in content of alpha-tocopherol.

In view of the above, carotene absorption tests were carried out and the amounts of carotene digested and stored in the liver as vitamin A, in the presence of different dietary fats, were measured.

Experiment A

Preparation of rats for carotene absorption study

Stock females, immediately on giving birth to litters, were kept on the following stock diet :

Wheat <i>atta</i>	95 parts	} plus whole milk 20 ml. per rat per day
Bran	3 „	
Common salt	1 part	
Calcium carbonate	1 „	

The daily supplement of greens was excluded. From the tenth day onwards the whole milk was replaced by skim milk. The young animals were weaned on the twentyfirst day or as soon as they attained a weight of 30 grams. They were then separated from their mothers and kept on the following vitamin A and carotene deficient diet.

Starch	64 parts
Casein (extracted)	24 „
Yeast (extracted)	8 „
Salt mixture	4 „

After two weeks on this diet the animals were ready for the experiment.

Details of experimental procedure

Faeces were collected from each individual rat on vitamin A and carotene deficient diet, supplied *ad lib.* over a period of seven days. The daily collection was transferred to coloured bottles covered with black paper and stored in a refrigerator. The endogenous excretion of carotene was determined, on these collections, for each individual rat.

The rats were then divided into several groups. One group, serving as control, was getting vitamin-A deficient ration plus cow *ghee*, while the others were receiving *vanaspatis* instead of *ghee*, at a level of 5 per cent, mixed up with the same ration. This preliminary feeding continued for four days.

After four days, each rat in each group was given extra carotene at the rate of 100 I.U. (=60 γ) per kilogramme body weight, per day, in addition to their experimental fat ration, as stated above.

The carotene was given dissolved in either *ghee* or the different *vanaspatis* as laid out in the plan. Twenty four-hourly collection of faeces was started from the very day the dosing of carotene was introduced and continued over a period of nine days in the same way as stated above. Carotene determined in the collected faeces corrected for endogenous excretion, determined as above, gave the actual excretion in the faeces. The digestibility of carotene in the presence of each of the fats was then worked out.

All the carotene determinations were made by colorimetric method [Sen and Seshan, 1942].

On the last day of collection, the rats were sacrificed, their livers removed, weighed and sampled and the vitamin-A content estimated. This served as an index of carotene utilization. The estimations of blue units were made by Lovibond Tintometer.

The results obtained with some of the hydrogenated oils ('Dalda', 'First Quality', 'Swastika', 'Binaula'), are given in Table XXXIV. The data show that the substitution of hydrogenated oil for cow *ghee* in the diet depressed absorption of carotene. These differences were subjected to statistical analysis and found highly significant.

TABLE XXXIV

Influence of ghee and vanaspatis on the carotene absorption and storage of vitamin A in liver (Experiment A)

Fats	Per cent of absorption of carotene (after correction for endogenous secretion)	Vitamin A (γ) in the whole liver
Cow <i>ghee</i>	86.63 ± 0.84	9.35 ± 0.22
'Dalda'	73.91 ± 0.30	Trace
'First Quality'	72.00 ± 3.02	Trace
'Swastika'	72.50 ± 0.65	Trace
'Binaula'	79.46 ± 0.59	Trace

The livers of rats receiving cow *ghee* showed 6.63 to 7.14 blue units per gm. of liver, when vitamin A was estimated by Lovibond Tintometer. The livers of all other groups showed only traces which could not be measured.

Experiment B

As practically no vitamin A was found to have been stored by the rats ingesting the different *vanaspatis* in the experiment described above, another experiment with a much higher level of carotene ingestion was carried out. In this, in addition to *ghee* and hydrogenated fat, all the oils mentioned in Chapter IV as well as lard, were studied. The experimental procedure used was exactly similar to that followed in Experiment A above, except that the amount of carotene fed to the depleted rats was raised three-fold. When the cow *ghee* formed the experimental fat of the diet, the dosage of carotene was adjusted by allowing for the vitamin A and carotene present in the fat of the diet eaten, so that the total intake was at the same rate as in the case of the other fat groups. The small amounts of carotene present in some of the oils was allowed for in the same manner.

It would be seen from the data given in Table XXXV, that the average coefficient of digestibility of carotene varied from 77.3 per cent in the raw cotton-seed oil group to 99.1 per cent in the refined *til* oil group. It was observed that the digestibility of carotene was lower in the presence of the raw oils than when their corresponding refined samples were fed. The difference was highest in the case of cotton-seed oil and least with *til* oil. The digestibility figure in the cow-*ghee* group

was similar to those given by the animals getting the refined oils. Lard also resembled cow *ghee* in this respect. 'Dalda' and raw groundnut oil groups gave more or less similar values for the digestibility of carotene.

TABLE XXXV

Digestibility of carotene and its recovery in liver as vitamin A when different fats and oils are ingested (Experiment B)

Oil or fat fed	Total amount of carotene ingested (I.U.)	Digestibility coefficient per cent	Vitamin A in whole liver (I.U.)	Recovery in liver per cent
Cow <i>ghee</i>	254.7	95.5±0.98	178.2	70.0±4.11
Groundnut oil, raw	218.6	83.3±1.34	85.8	39.2±2.20
Groundnut oil, refined	213.2	95.0±1.50	75.0	35.2±4.40
Cotton-seed oil, raw	249.0	77.3±0.96	72.3	29.0±1.93
Cotton-seed oil, refined	228.2	96.5±0.15	64.6	28.3±1.32
Coconut oil, raw	161.6	88.3±1.16	67.0	40.7±7.99
Coconut oil, refined	156.8	95.4±1.07	45.1	28.7±3.33
<i>Mahua</i> oil, raw	161.6	86.2±2.62	70.3	43.5±3.45
<i>Mahua</i> oil, refined	179.5	97.0±0.80	59.9	33.4±1.99
<i>Til</i> oil, raw	155.4	97.7±0.55	74.2	47.7±4.46
<i>Til</i> oil, refined	167.1	99.1±0.50	60.4	36.2±2.70
Mustard oil, raw	173.7	88.9±0.96	109.4	63.0±2.29
Mustard oil, refined	182.1	93.9±1.29	97.5	53.6±4.89
Lard	178.3	95.2±0.29	69.3	38.9±3.68
'Dalda'	169.7	82.1±1.15	45.3	26.7±0.90

The quantities of vitamin A, stored in the liver of these experimental rats, showed differences between the different fats in their ability to promote the storage of the ingested carotene as vitamin A in the liver. The cow *ghee* fed animals showed a considerably higher reserve of vitamin A in their liver than those of any of the other groups even though all of them ingested carotene at an equal rate. The recovery of the ingested carotene as vitamin A in the liver, when the different oils and fats were fed, would be a better basis for comparing the efficiencies of the various fats and oils for promoting the absorption and utilization of the ingested carotene. The data relating to the same show that cow *ghee* was the most efficient dietary fat for this purpose. Cow *ghee* proved to be significantly better than all other fats.

'Dalda' was found to be significantly poorer than groundnut oil with regard to its efficiency for promoting the utilization of the ingested carotene for storage in the liver as vitamin A. The efficiency of 'Dalda' was less than 40 per cent of that of cow *ghee* for promoting liver storage of vitamin A from ingested carotene or vitamin A and was only about two-third as efficient as groundnut oil from which it was prepared. Lard was more or less similar to groundnut oil in this respect.

The statistical analysis suggests, that raw and refined *til* oil promote the maximum digestibility of carotene and are perhaps as good as refined *mahua*, cotton-seed and cocoanut oils in this respect. Refined *til* oil appears to be better than cow *ghee*. An analysis of the data on the storage of vitamin A has yielded that cow *ghee* helps such storage most and is better than any other oil. Raw and refined mustard oils came next in order and appear to be better than other oils.

One interesting observation was the lack of any consistent relationship between the growth promoting value and liver storage of vitamin A. It was demonstrated in previous chapters that cow *ghee* gave a significantly better growth effect in the second and subsequent generations than oils and other fats. It has been shown here that the liver reserve of vitamin A in cow-*ghee* fed animals was higher than that of the oil and *vanaspati*-fed ones. This would seem to indicate a direct and close relationship between the two. However, this relationship was only generally true and not always strictly proportional, as there was no consistently regular correlation between growth effect and the liver content of vitamin A in the same group. As an example, it might be pointed out that in Experiments I and III, the 'Dalda'-fed rats which showed the lowest liver vitamin A content did not consistently record the least weight gain in all the tests. Similarly, though the refined mustard oil group showed lower growth than many of the other oil groups, it consistently gave higher liver reserves of vitamin A than all other oil groups except mustard raw. In Experiments V and VI also the same tendency was exhibited. Whereas cow *ghee* and *bazar ghee*-fed animals consistently showed considerably higher hepatic vitamin A storage than the corresponding oil and *vanaspati*-fed groups, under all comparable dietary regimes, the growth recorded on the different fats including the *ghees* was not significantly different except on the basal North Indian diet. This lack of consistent correlation between growth effect and hepatic vitamin A storage might probably be due to the fact that, whereas vitamin A storage in the liver is directly dependent upon the level of ingestion of carotene or vitamin A, growth is the result of several factors some of which may have nothing at all to do with the dietary vitamin A level such as quality and amount of protein, minerals, other vitamins, etc. of the food.

The faecal excretion of carotene, observed in these experiments, was also of about the same order as that reported by Wagner and associates [1941], Guggenheim [1942] and Ramasarma and Hakim [1942]. In the case of refined *til* oil, however, the excretion observed here was very low, i.e. less than one per cent of that ingested. Since it is a well established fact that storage of vitamin A in the liver is directly dependent upon the amount of vitamin A or carotene ingested, a close correlation between the digestibility of carotene and its recovery as vitamin A in the liver might be expected. A scrutiny of the relevant figures, however, fails to show any

such relationship. For example, whereas raw groundnut oil and 'Dalda' groups showed more or less similar digestibilities of carotene, the hepatic recovery of the ingested carotene in the latter group was only about two-third of that of the former. The reasons for this lack of correlation between carotene digestibility and vitamin A storage in liver is probably due, at least to a considerable extent, to the destruction of carotene and vitamin A taking place in the gastro-intestinal tract, as has been pointed out by Hickman [1943]. He states, "the quantity of vitamin and provitamin A recovered (from the faeces), subtracted from the quantity ingested, has hitherto been assumed to represent the quantity absorbed. Since tocopherol increases the quantity excreted as well as the biological potency of that retained, it is evident that a third figure, the quantity destroyed in the intestine, must be added to balance the equation. Until further work has evaluated this factor, figures for the intestinal absorption of vitamin A and carotene will be untrustworthy. The possibility of intestinal biogenesis further complicates the intake—discard—utilization balance of all vitamins." The greater apparent digestibility of carotene along with lower liver storage of vitamin A, seen in the refined oils, may also be due to the greater gastro-intestinal destruction consequent on the greatly lowered stability of the refined oils brought about by the removal, during the refining process, of "phospholipids, low molecular weight phenolic bodies, free fatty acids and perhaps other factors" accompanying tocopherol in natural oils "which, acting together, are said to confer a stability from 10—100 times more than we associate with pure tocopherol and oil" [Hickman, 1943]. It would, therefore, seem that carotene digestibility figures, by themselves, are, perhaps, only of complementary value. The percentage of the ingested carotene found to be stored in the liver as vitamin A in this experiment, was somewhat higher than that obtained by Kemmerer, Fraps and DeMottier [1947] or Lemley *et al.*, [1947].

The results of these series of experiments are different from those of Coward [1936] and Lease *et al.*, [1939], who found no difference in the biological potency of carotene administered in different oils or fats. Euler and Euler [1945] and Brown and Bloor [1945] had found that the degree of unsaturation of the dietary fat plays an important role on the utilization of ingested carotene. Our results, however, show, that though cow *ghee* is much less unsaturated than most of the other fats and oils, yet animals getting the former fat stored much greater vitamin A in their liver. Similarly, the tocopherol content of the fats also does not seem to have any consistent relationship to the hepatic storage of vitamin A, though Guggenheim [1944] and Rao [1945, 1945a] have stressed that the biological response of carotene, dissolved in different vegetable oils, is more or less the same when the tocopherol contents of the oils are equated.

To sum up, it would seem from the data presented above that, under the conditions studied, cow *ghee* as dietary fat, was more efficient in promoting the absorption and utilization of ingested vitamin A or carotene, as judged by the recovery of the same as vitamin A in the liver, than any of the vegetable oils, either raw, refined or hydrogenated, which have been tested. The animal fat, lard, seemed to be no better than most of the vegetable oils and definitely poorer than cow *ghee*. Refining of the oils led to a decrease in efficiency to promote storage of vitamin A in the liver. Hydrogenation also seemed to lower the ability of the parent oil to promote liver storage of vitamin A from ingested carotene.

CHAPTER XII

EFFECT OF FATS AND OILS ON THE REQUIREMENTS OF THE B-VITAMINS

By N. D. KEHAR, T. S. KRISHNAN and B. C. JOSHI

THE B-vitamins play a very important part in fat metabolism. The close inter-relationship of pyridoxin, pantothenic acid and unsaturated fatty acids in rat nutrition [Quackenbush *et al.*, 1939; 1942*b, c*; Richardson *et al.*, 1941]; the sparing action of dietary fat on thiamine [Evans and Lepkovsky, 1929, Salmon and associates, 1930, 1931, 1937; Guerrant and Dutcher, 1934; Whipple and Church, 1936; MacDonald and McHenry, 1940; Banerji and associates, 1940, 1941, 1942; Wainio, 1942], pantothenic acid [Unna *et al.*, 1941] and biotin [Nielson and Elvehjem, 1942] needs of the rat, and the enhanced riboflavin requirements with increased fat ingestion [Mannering *et al.*, 1941, 1944], are some of the examples indicating the effect of B-vitamins on fat metabolism. Though it is known that fats have a thiamine sparing action, there is very little knowledge of the comparative value of different fats and oils for this purpose. Arnold and associates [Arnold and Elvehjem, 1939; Stirr *et al.*, 1939] demonstrated no marked difference in the thiamine sparing action of olive oil, autoclaved peanut or cotton-seed oil and autoclaved lard or butter-fat.

However, since it was observed in the foregoing chapters that there were differences in the growth promoting value, vitamin A storage in liver, Ca and P absorption, etc. between cow *ghee* and different vegetable oils and other fats, it was considered desirable to find out whether there was any difference in the requirements of these fats and oils for thiamine and riboflavin.

Effect of different fats and oils on thiamine requirement

The procedure employed was an adaptation of the improved rat growth method of assay of vitamin B₁, of Kline, Hall and Morgan [1941], incorporating cow *ghee*, raw and refined groundnut oil or 'Dalda' (hydrogenated groundnut oil), in the basal diet. These were selected in order to study the effect of refining and hydrogenation on vegetable oils as also to find out the difference, if any, from cow *ghee*. The cow *ghee* was separated from casein or any other extraneous matter by repeated melting and filtering through filter paper.

Weanling rats from the stock colony, 24 to 26 days old and 40 to 50 gm. in weight, were distributed evenly into 16 groups of eight rats each (four males plus four females). These 16 groups were sub-divided into four series of four groups each. One series formed the negative control while the other three were tested at three levels of pure thiamine ingestion, viz. 3, 5 and 8γ per rat per day. Each of the four groups in all the series was given one of the four experimental fats. Thus

each of the fats was tested at three levels of thiamine intake, besides the negative control. Cow *ghee* group formed the positive control in each series. The distribution of the young rats was made in such a way that litter mates were allotted to the four groups of each series on the same level of thiamine intake, one on each of the four fats. All the rats were housed in individual cages, with raised wire grid bottoms, the strands of which were $\frac{1}{2}$ inch apart, as described in Chapter III. After grouping them in this manner they were put on the following B₁-free ration, to deplete them of their body reserves of thiamine.

Sucrose	67
Casein (alcohol extracted)	24
Salt mixture	4
Experimental oil or fat	5
	<hr/>
	100
	<hr/>

In addition, the following were given to each of the rats daily :

Vitamin A	60 I.U.
Vitamin D	10 I.U.
Vitamin E	0.5 mg. alphotocopherol
<i>Vitamin B :</i>	
Riboflavin	30 γ
Pyridoxin	50 γ
Ca-d-pantothenate	100 γ
Nicotinic acid	1 mg.
Choline chloride	20 mg.
Vitamin B ₁ -free liver extract	1 ml.

The liver extract, free from vitamin B₁, was prepared by sulfite treatments according to the method of Kline, Hall and Morgan [1941] and was kept in the refrigerator. The casein used was made vitamin B₁-free by repeated alcohol extractions. The vitamins A, D and E were dissolved in propylene glycol and the strength of the solution was so adjusted that two drops contained the daily allowance of all the above three vitamins for each animal. The two drops were placed straight into the mouth of the animals. The members of the B-complex were dissolved in 25 per cent alcohol and the strength adjusted so that 0.5 ml. of the solution contained the daily allowance of each rat. Each animal was given 0.5 ml. of the above B-complex solution and 1 ml. of the B₁-free liver extract mixed with a little of its food in a small cup, every day. It was licked up completely in a few minutes. Enough fresh food was weighed out daily for each of the animals so that there was about two gm. residue the next day. Fresh water was given in self-feeding bottles. All the rats were weighed individually at the commencement, twice a week during the first week and daily thereafter, till they ceased to show any increase in weight. The cessation of growth was taken as the end of the depletion period. This usually took about 10 to 14 days.

After the body reserves of thiamine had been depleted in the above manner, the rats were given graded doses of pure vitamin B₁ as an aqueous solution of thiamine hydrochloride. The requisite quantity of solution was fed along with the B-complex and the liver extract, in the manner mentioned above. The fourth group was continued on the basal B₁-free diet to serve as the negative control. All the rats were weighed on the day the dosing of thiamine started and twice every week thereafter, till three weeks from the date of B₁ administration were completed. The increase in weight of each rat in the different groups and at all levels of B₁ ingestion were recorded and the average growth of each rat on the different fats during these three weeks at each level of thiamine ingestion was deduced. These were statistically examined to find out whether there were any significant differences between the fats, as revealed by the growth effect. The results are given in Table XXXVI.

TABLE XXXVI

Average weight increase of B₁-depleted rats for different fats and oils and getting varying doses of thiamine (Average weight gain in 3 weeks in gm.).

Fat or oil fed		Dosage of pure thiamine hydrochloride given γ/day/rat			
		0	2	5	8
Cow-ghee	(1)	Dead	9.0 ± 5.71	26.1 ± 8.63	32.6 ± 8.86
	(2)	"	6.3 ± 2.82	24.4 ± 5.69	32.6 ± 8.93
	(3)	"	7.6 ± 3.00	25.3 ± 8.14	33.3 ± 5.83
Groundnut oil, raw	(1)	"	-11.9 ± 8.81	21.1 ± 10.45	37.0 ± 4.67
	(2)	"	-13.9 ± 2.33	11.5 ± 11.02	41.2 ± 4.45
	(3)	"	-12.9 ± 4.02	16.3 ± 7.26	39.1 ± 8.27
Groundnut oil, refined	(1)	"	-12.0 ± 1.06	9.3 ± 0.85	20.0 ± 15.77
	(2)	"	-17.0 ± 5.03	11.4 ± 9.04	28.6 ± 13.53
	(3)	"	-14.5 ± 2.56	10.3 ± 4.23	24.3 ± 9.76
'Almonds'	(1)	"	-6.4 ± 8.69	7.8 ± 9.95	26.1 ± 7.76
	(2)	"	-2.4 ± 8.52	4.9 ± 5.76	22.6 ± 7.87
	(3)	"	-4.4 ± 5.68	6.3 ± 5.60	24.4 ± 5.16

(1), (2) and (3) represent males only, females only and total animals respectively

It was found that there were wide individual variations in the response to thiamine administration, among all the fat groups, at each of the levels of B₁ intake studied. The variations were more or less of the same order in all the groups but there was a slight general tendency for more uniform growth in the cow-ghee group

as compared with the other fat groups. The animals of the negative control group started dying by about the end of the second week after depletion and were all dead by the end of the third week. None of the animals, on any of the fat groups, survived three weeks after depletion, on a B₁-free diet.

It would also be seen from the above Table that at all levels of thiamine ingestion cow-ghee animals generally showed greater average weight gains than those of the other groups. The only exception was the raw groundnut oil group at 8 γ level. The differences in growth shown by the different fat groups, at the 8 γ level, were not significant statistically. Since most of the animals at the 3 γ level had lost weight, they would not be suitable for a proper statistical comparison. At the 5 γ level, cow-ghee was significantly better than both refined groundnut oil and 'Dalda'. At this latter level of 5 γ thiamine ingestion, there was no significant difference in growth between rats fed raw or refined groundnut oil or 'Dalda.'

Effect of different fats and oils on riboflavin requirement

The method employed was an adaptation of that recommended by Day and Darby [1947]. The same fats, viz. cow-ghee, groundnut oil raw and refined as well as 'Dalda' (hydrogenated groundnut oil) used in the case of thiamine, were studied. The selection of the rats, their grouping, housing, management, feeding, and other cognate matters were also exactly similar. Here too, 16 groups of eight weanling rats each (four males and four females) were taken and divided into four series of four groups each. Each of the four series was given a different dose of riboflavin while each of the groups in the different series was fed one of the four test fats.

All the young rats were first depleted of their body reserves of riboflavin by putting them on a diet similar to the previous one except that in the vitamin supplement, riboflavin was dropped out and 30 γ thiamine per rat per day was substituted and sucrose in the thiamine deficient ration was replaced by dextrinsed starch.

The rats were on this ration till growth practically ceased, i.e. not more than one to two gm. in a week. This preliminary depletion period took three to four weeks. After the body reserves of riboflavin had been thus depleted, they were given graded doses of pure riboflavin. The rats continued to be weighed twice every week even after riboflavin administration commenced and followed till all the groups had completed four weeks on the riboflavin supplemented regime. The negative control was also carried on for the same period. The weight changes of the individual rats in each of the groups, in all the series, were recorded and the average weight gains over the four weeks period of riboflavin administration, worked out. The growth effects recorded by the different fat groups at each of the levels of riboflavin ingestion, were statistically examined. The results are presented in Table XXXVII.

The young rats, when put on the riboflavin-free ration, grew fairly well for the first two weeks. Their growth then showed a considerable slowing down and in the fourth week became practically stationary. When continued further on the same ration, as in the negative control groups, they did not show any further material gain in weight. Male rats getting cow-ghee remained more or less steady while the other groups, receiving the oils or *vanaspati*, started losing weight steadily. The maximum loss

TABLE XXXVII

Average weight increase of riboflavin-depleted rats fed different fats and oils and given varying doses of riboflavin (Average weight gain in 4 weeks in grams)

Fat or oil fed		Dosage of pure riboflavin given γ /rat/day			
		0	2	4	8
Cow-ghee	(1)	-0.9 ± 1.53	22.0 ± 1.55	39.9 ± 4.52	64.8 ± 4.36
	(2)	-9.8 ± 4.61	18.0 ± 7.63	42.0 ± 3.57	52.6 ± 6.47
	(3)	-5.3 ± 2.80	20.0 ± 3.68	40.9 ± 2.70	58.7 ± 4.28
Groundnut oil, raw	(1)	-9.0 ± 3.01	13.1 ± 5.58	34.1 ± 7.28	57.6 ± 2.93
	(2)	-8.6 ± 1.57	14.6 ± 3.46	25.4 ± 5.80	41.9 ± 8.05
	(3)	-8.8 ± 1.57	13.9 ± 3.05	29.8 ± 4.60	49.8 ± 4.97
Groundnut oil, refined	(1)	-7.0 ± 6.21	11.3 ± 4.26	32.6 ± 3.38	64.4 ± 8.03
	(2)	-11.4 ± 2.56	13.9 ± 4.37	21.1 ± 1.97	45.5 ± 7.03
	(3)	-9.2 ± 3.22	12.6 ± 2.85	26.9 ± 2.83	54.9 ± 5.70
'Dalda'	(1)	-13.6 ± 3.26	11.1 ± 3.27	31.3 ± 5.26	49.3 ± 8.03
	(2)	-13.8 ± 7.50	11.8 ± 2.71	18.9 ± 3.82	48.3 ± 2.07
	(3)	-13.7 ± 3.79	11.4 ± 1.97	25.1 ± 3.81	48.8 ± 3.85

(1), (2) and (3) represent males only, females only and total animals respectively

in weight in the ensuing four weeks was seen in the rats getting 'Dalda' as their dietary fat. When pure riboflavin was given, all the groups responded by showing increase in weight. The higher the dosage of riboflavin the greater was the rate of gain. Even here, it was observed that at all levels of riboflavin ingestion, cow-ghee fed animals showed comparatively larger gains in weight than those getting oils or *vanaspati*. Considerable individual variations in the response to riboflavin depletion or administration were observed in all the fat groups and at all levels. When the increase in weight recorded by all the rats, over the four weeks experimental period of riboflavin administration, was statistically analysed, it was observed that when the ingestion of riboflavin was 4 γ per rat per day, cow-ghee fed animals showed a significantly better growth than those getting refined groundnut oil or 'Dalda'. The difference between the groups receiving cow-ghee and the raw oil was only just above the significance level. Between the oils and the *vanaspati* there was no significant difference. When the growth of all the experimental animals was considered, there was no significant difference at any other level of riboflavin intake nor even on the riboflavin-free diet. However, when the growth of the male rats alone was considered, the cow-ghee fed animals showed significantly better growth than those on 'Dalda' at the 2 γ level of riboflavin feeding

as well as on the riboflavin-free diet. At none of these levels was there any significant difference between the growth of the oil-fed and *vanaspati*-fed rats. At the 8γ level of riboflavin ingestion, ♂ rats getting the different fats did not show any significant differences between the various fats, as judged by the growth of all the rats or males only.

DISCUSSION

It would be seen from the above results that when the riboflavin ingestion is deficient or lacking, there is a tendency for cow-*ghee* fed animals to show better growth than litter mates fed groundnut oil or 'Dalda'.

These results differ from those of Arnold and Elvehjem [1939] or Stirr *et al.*, [1939] who did not find any marked difference in the thiamine sparing action of vegetable oils, lard or butter-fat. This is not surprising as the level of thiamine used by these workers was 15γ per rat daily. The results of this experiment also indicate that no differences in the fats can be observed when the thiamine ingestion is 8γ or more.

The rats of the negative control groups, which were kept on a riboflavin-free diet for four weeks after depletion of their body reserves of the same, did not show any of the characteristic pathological symptoms of dermatoses described by Sullivan and Nicholls [1941]. Excepting some loss in weight, they showed no other acute signs of riboflavin deficiency. As noticed by Jones *et al.*, [1945], these observations were similar to the behaviour of mice deprived of riboflavin. The authors recorded that after cessation of growth, the first and the last death occurred after 34 and 202 days respectively. Their mice showed normal growth for the first three weeks, which was also seen in rats in this experiment. In this study too, when low or marginal amounts of riboflavin were administered (0—4γ per head per day), the cow-*ghee* fed animals showed significantly better growth than the *vanaspati* fed rats. The oil-fed rats behaved like the 'Dalda' fed ones. At higher levels of riboflavin intake, the above difference ceased to be significant. Mannering *et al.*, [1944], who fed rats on an animal fat (lard) or a hydrogenated fat (crisco) at 25 per cent level, however, did not find any difference between these even when the riboflavin intake varied from 0—12 γ per rat per day.

From the above results it would seem that at very low levels of thiamine or riboflavin ingestion, the requirements of these vitamins would seem to be less when cow-*ghee* formed the dietary fat than when oils or *vanaspati* were fed instead. However, at higher levels of thiamine and riboflavin intake, no differences in this respect between the different fats were noticed. Also no difference between raw and refined oils or between the oils and *vanaspati*s was noticed at any level of thiamine or riboflavin intake.

SUMMARY AND CONCLUSIONS

The investigations outlined in this bulletin were taken in hand with the following objects in mind :

1. To make a survey of market *ghee* in order to assess the vitamin A available to the consumers through this medium.
2. To assess the effect of breed, feeding, etc. of animals, on the vitamin A content of *ghee*.
3. To determine the nutritive values of *vanaspatis* (hydrogenated vegetable oils), raw and refined vegetable oils, as well as of lard as compared with that of pure *ghee*.

In order to examine the above points, a number of experiments have been carried out, the results of which have been given in detail in the foregoing chapters. A general summary of the results obtained and the broad conclusions drawn from these, are briefly given below :

(I) *Chemical constants of pure ghee*

The chemical constants of pure *ghee* from different breeds of cattle and buffalo in different parts of India and for different seasons have been determined. The constants varied from breed to breed and from place to place.

The saponification, Reichert-Meissl, Polenske and Kirschner values of *ghee* prepared from the milk of Indian cattle in certain localities and seasons, may fall much below the minimum limits set for butter-fat in the Western countries.

The saponification, Reichert-Meissl and Kirschner values were significantly lowered in summer. The iodine values did not show much seasonal variation.

Ghee samples obtained from North India showed higher saponification and Polenske values, but definitely lower iodine values. The Reichert-Meissl and Kirschner values of the North and South Indian samples were not much different.

Buffalo *ghee* had higher saponification, Reichert-Meissl and Kirschner values and lower iodine values, as compared with cow *ghee*. Polenske values of the two fats were similar.

(II) *Vitamin A and carotene content of fats and oils*

The vitamin A content of *ghee* samples, of different breeds of cattle in different parts of India, varied from 5.35 to 15.0 $\mu\text{g/gm.}$ and that of carotene from trace to 7.5 $\mu\text{g/gm.}$ Buffalo *ghee* had no carotene content and, generally, a lower vitamin A concentration than cow *ghee*. A very low carotene content, even after liberal green feeding, characterised the *ghee* prepared from the milk of the Ongole breed of cattle.

Under similar conditions of feeding, *ghee* from different breeds of cattle had similar vitamin A potency.

The vitamin A content and the carotene values of *ghee* declined during winter and summer. This was due, perhaps, to the lack of green feeding during these seasons.

Ghee as sold in the market had no carotene and a very low vitamin A content.

The presence of carotene in cotton-seed, *mahua*, *til* and groundnut oils, has been observed for the first time. Refining of the oils resulted in the total loss of the carotene content. The samples of *vanaspatis* and lard examined by us contained absolutely no vitamin A or carotene.

The rate of destruction of pure vitamin A added to *vanaspatis* was much faster than in pure *ghee*.

Vanaspatis have been found to contain little or no vitamin D.

(III) *Chemical constants of vegetable oils, vanaspatis and lard*

The important chemical constants of common vegetable oils (both raw and refined), several brands of *vanaspatis* and one sample of lard, have been determined.

The oils generally have higher acid and iodine values and lower saponification and R. M. values than those seen in the cow *ghee*. Coconut and *Mohua* oils differ from other oils in having comparatively higher R. M. and R. P. values. Coconut oil has also a very low iodine value.

Hydrogenation of vegetable oils results in lowering of iodine values and a virtual loss of all volatile acids. The saponification values of *vanaspatis* are nearly the same as that of the parent oils and lower than that of cow *ghee*. The limitations of chemical constants in evaluating the purity of *ghee* samples have been fully discussed, and it has been shown that examination on the basis of the chemical constants only may not yield clear cut results regarding the purity of *ghee*; for example a *ghee* sample may be adulterated to the extent of 70 per cent with *vanaspatis* and yet can pass all the common tests for pure *ghee*.

A short period of heating does not materially affect the chemical constants of *ghee*, oils and *vanaspatis*. There is, however, a substantially serious loss in the vitamin A potency of *ghee* samples. The carotene loss of the oils due to heating was of a lower order.

(IV) *Digestibility of different fats and oils*

When fed at 5 per cent level *ghee*, *vanaspatis* and vegetable oils appear to be digested by rats to more or less the same extent.

(V) *Comparative nutritive values of vanaspatis, vegetable oils, lard and ghee*

This work was carried out in two series. In the first series, the fats were incorporated at a 5 per cent level in a synthetic diet containing sub-optimum quantities of vitamin A and possibly also of vitamin B-complex. In the second series, these fats and oils were incorporated at the same level in various poor Indian dietaries. The indices for comparison were growth, general health, as well as reproduction and lactation in the various groups of animals in both the series. In the first series, the experiments were carried over three or more generations, whereas in the second

series, the experimental period was confined to only one generation but the observations were extended over a longer period. The main conclusions are given below :

In the first series, where a sub-optimum synthetic diet was used, young rats were kept for a period of six to eight weeks on this basal ration. From seventh to ninth week onward a liberal diet was given, as it was found that satisfactory mating and birth of litters was not possible on this basal ration. The period on basal ration only has been used to evaluate the comparative growth promoting value of the different fats. It was found that, whereas in the first generation there was hardly any difference in the growth promoting values of the different fats as compared to cow *ghee* or between themselves, in the second and subsequent generations cow *ghee*-fed animals showed, in general, a much better growth rate than animals receiving *vanaspatis*, vegetable oils (raw or refined) or lard. In most cases the differences between cow *ghee*-fed and other fats and oil-fed animals of the second and subsequent generations have been found to be significant. Between the different oils or *vanaspatis* themselves or between the oils and *vanaspatis*, there was hardly any material difference in any generation.

On transfer to a liberal feeding regime, the oils and *vanaspati*-fed animals, which had shown poor growth rate on the basal ration, made rapid gains in weight and most often equalled in weight the *ghee*-fed rats, within four to five weeks.

As regards the reproduction and lactation abilities, no marked difference has been found between the various groups and in successive generations.

Except in one series of experiments, viz. the second generation of Series A animals of experiment III (Chapter VI), all the animals have kept more or less good health except that alopecia has been very frequent and quite extensive in rats fed either oils or *vanaspatis*. Cow *ghee* animals have shown a milder type of this symptom. Mortality has been quite low except in the series mentioned before. In that series, the parents of the rats of the second generation had been on the sub-optimum basal ration for about 18 weeks. Consequently there had been quite high mortality among animals fed with refined cotton-seed oil and lard. These latter groups of animals also exhibited severe symptoms resembling those seen in vitamin A deficiency. Similar but much milder symptoms had been observed in rats of the series getting coconut oils (raw or refined) and raw cotton-seed oil. All these symptoms disappeared as soon as the animals were put on the liberal diet.

It may thus be seen that, on a sub-optimum ration, i.e. when the basal ration is poor in vitamins and perhaps other essential nutritive factors, *ghee*-fed animals generally fare much better than other animals on the same ration plus oils or *vanaspatis*. This difference between *ghee* and the other fats, however, practically disappears when the diet is liberalised with the addition of vitamins and other essential factors.

As fats are generally used as frying media, the effect of addition of heated fats to an adequate cereal ration was also studied. It was found that even on this liberal diet, the addition of certain heated *vanaspatis* seemed to have a slight depressing effect on the growth of rats. Here again no difference in the reproduction and lactation capacities of the animals of the different groups, either *ghee* or *vanaspati*, could be seen.

In the second series of experiments *ghee*, *vanaspatis* and oils had been added to either a Bengali or a North Indian dietary, used by an average poor man. The Bengali diet used was very poor in calcium, protein and vitamin A content. The North Indian diet was comparatively much richer with regard to these nutrients. On the Bengali dietary regime the growth of rats getting either *ghee* or any of the other fats is very poor and as such hardly any significant difference could be observed between different groups. Even when this diet was fortified by the addition of extra vitamins A, B-complex and D, there was no difference in the growth promoting effect of the various fats, though the total weight gain by the experimental animals was much higher than that on the unsupplemented diet. When rats were kept on the unsupplemented poor Bengali diet for a long period, viz. 25 weeks or more, various pathological symptoms were seen, especially in rats consuming some of the brands of *vanaspatis*. Cow *ghee*-fed animals were found to be comparatively more immune to such symptoms. As the *vanaspatis* contained no vitamin A, and whereas the cow *ghee* is a fairly rich source of vitamin A, it was concluded that most of these deficiency symptoms, seen in the *vanaspati*-fed rats might be due to chronic vitamin A deficiency.

On the other hand, rats on a North Indian dietary, getting cow *ghee*, grew at a much faster rate than their litter mates on the same diet plus either oil or *vanaspatis*. The differences in the growth rate were statistically quite significant. As may be expected, the rats of all the groups in this experiment grew at a much better rate than the rats fed on Bengali diet. However, supplementation of the North Indian diet with extra vitamins also helped the animals to make still better growth showing that even the North Indian dietary can hardly be called an adequate diet. One interesting finding was that, whereas on the unsupplemented North Indian diet cow *ghee*-fed animals were best, in the vitamin supplemented North Indian diet series there was no significant difference between the different groups of animals getting *ghee*, oils or *vanaspatis*. Thus it was seen again that, whereas on a sub-optimum diet cow *ghee* was the best fat, on a liberal diet animals fared equally well whether receiving *ghee* or any other fat. On a very poor dietary, like poor Bengali diet, which is deficient in many essential factors, no such difference between fat and fat could be observed.

Reproduction and lactation in all the groups were more or less a total failure, pointing once more to the unsatisfactory nature of the Indian dietaries under study.

In order to find out the reason for the superiority of cow *ghee* over other fats and oils when incorporated in a sub-optimum ration, a number of experiments were planned to find out the comparative effects of different dietary fats on protein metabolism, calcium and phosphorus absorption, absorption and utilization of carotene as well as requirements of B-vitamins.

(i) Protein metabolism

It had been observed that the addition of fat to a fat-free diet, sub-optimum in regard to vitamin A, improved the digestibility of the dietary protein and also increased its biological value.

Whereas all the fats and oils studied, viz. *ghee*, raw and refined oils and *vanaspatis*, were more or less equally efficient in improving the digestibility of the ingested protein, the cow *ghee* was significantly superior to the others in raising the biological value of the dietary protein. There was no material difference between the other fats with regard to their effect on the biological value of the protein. Neither refining nor hydrogenation seemed to have any significant effect on the parent oil with regard to its action on protein absorption or utilization.

(ii) *Calcium and phosphorus metabolism*

To determine the absorption of calcium and phosphorus in the presence of different fats like cow *ghee*, vegetable oils or *vanaspatis*, metabolism experiments had been carried out with adult rats fed on a synthetic diet similar to those used in the growth test. On a fat-free diet, there was little absorption of these minerals. When 5 per cent of cow *ghee* was added to the above ration the absorption rose to 45 per cent and 57 per cent for calcium and phosphorus respectively. When the different oils or *vanaspatis* replaced cow *ghee* in the above diet, the absorption of calcium ranged from 23 to 41 per cent and of phosphorus from 28 to 48 per cent. Hence, the replacement of cow *ghee* by vegetable oils or *vanaspatis*, reduced calcium and phosphorus absorption significantly.

Refining seemed to have no material effect on the ability of the oils to promote calcium and phosphorus retention. Hydrogenation depressed calcium and phosphorus retention by about 8 to 10 per cent as compared with the parent oils, which difference, however, was not significant statistically.

(iii) *Storage of vitamin A*

In all the experiments carried out to determine the comparative nutritive values of *ghees*, vegetable oils and *vanaspatis*, livers of the animals receiving cow *ghee* had been found to contain the maximum quantity of vitamin A. Among the different oils, mustard oil gave the maximum storage while the refined coconut oil group of animals recorded the lowest liver reserves. Refining as well as hydrogenation seemed to lower the capacity of the oil to promote the storage of vitamin A in the liver.

(iv) *Absorption and utilization of carotene*

It was found that different dietary fats differed in their ability to promote absorption and utilization of orally administered carotene. The digestibility of carotene, when administered with the refined oils, was found to be higher than when given with the corresponding raw ones. Cow *ghee* and lard resembled the refined oils in this respect. *Vanaspatis* behaved similar to the raw parent oils in this matter.

With regard to their efficiency to promote the utilization of the ingested carotene, as measured by the percentage recovered as vitamin A in the liver, cow *ghee* was found to be definitely better than all other fats and oils. Among the oils, mustard seemed to be the best and cotton-seed and refined coconut, about the worst. Contrary to the findings in the carotene absorption experiments, refining seemed to

decrease the efficiency of the oils for the hepatic storage of vitamin A. Hydrogenation too appeared to adversely affect the capacity of the parent oil to promote vitamin A storage in liver.

(v) *Vitamin B requirement*

It was observed that, at the sub-optimum level of thiamine intake of 5γ daily per head, cow *ghee*-fed rats made significantly better gains than those fed on the corresponding oils and *vanaspatis*. At other levels of thiamine ingestion, when there were either losses or fairly good gains in body weight, there were no significant differences between the various fats. Refining or hydrogenation did not seem to have any significant effect on the thiamine sparing action of the oil.

A similar study carried out to compare the riboflavin requirement in the presence of different fats showed that, when the daily allowance of riboflavin was 4γ or less per head, riboflavin-depleted rats getting cow *ghee* as the dietary fat showed significantly better growth than litter mates fed vegetable oils or 'Vanashpati'. The superiority of cow *ghee* disappeared when the average dose of riboflavin was increased to 8γ per head daily. No differences between the oils and *vanaspatis* were observed at any of the levels of riboflavin intake.

Summing up, it would seem that, with the sub-adequate dietaries that are widely prevalent in most regions of this country, the replacement of cow *ghee* by vegetable oils—raw, refined or hydrogenated—over long periods, is likely to lead to adverse effects such as decreased growth, higher vitamin requirements, lower Ca and P absorption, etc. However, when the deficiencies of the dietary are removed and the ration made adequate in all essential nutritional factors, the difference between cow *ghee* and the vegetable oils or *vanaspatis* tends to disappear and all of them seem to have about similar nutritive values. Between the different oils as also between the parent oil and its hydrogenated product, however, there seems to be no significant difference in most of these respects, under the conditions studied.

REFERENCES

- Abbot, O. D., Ahmann, C. P. and Overstreet, M. R. (1939). *Amer. J. Physiol.*, 126, 254.
 Abramson, E. and Brunius, E. (1942), *Acta. Physiol. Scand.*, 3, 164. (*Nutr. Abstr. Rev.* 13, No. 124, 1943-44.)
 Ahmad, B., Chand, R. and Mansoor-ul-Hassan (1946), *Indian J. med. Res.*, 34, 75.
 Arnold, A. and Elvehjem, C. A. (1939), *Amer. J. Physiol.* 126, 289.
 Arup, P. S. (1930)—*J. Dept. Agric.*, Irish Free State, 29, 236.
 Association of Official Agricultural Chemists, Official and Tentative Methods of Analysis, Washington, IV Edn. (1935).
 Association of Official Agricultural Chemists, Official and Tentative Methods of Analysis Washington, IV Edn. (1940).
 Atkinson, F. W., Hughes, J. S., Kuerth, B. L., Peterson, W. J. and Kramer, M. (1937), *J. Nutri.*, 14, 62.
 Augur, V., Rollman, H. S. and Deuel, H. J. (Jr.) (1947), *J. Nutrit.*, 33, 177.
 Baldwin, A. R. (1940), Univ. Pittsburg Bull., 40, 28.
 Banerjee, B. N. and Sonawala, S. D. (1935), *Agric. Livestock India*, 5, 382.
 Banerji, G. G. (1940), *Biochem. J.*, 34, 1329.

- Banerji, G. G. (1941), *Biochem. J.*, **35**, 1354.
 — and Yudkin, J. (1942), *Biochem. J.*, **36**, 530.
 Basu, K. P. and Nath, H. P. (1946), *Indian J. med. Res.*, **34**, 13.
 — — (1946a), *Indian J. med. Res.*, **34**, 19.
 — — (1946b), *Indian J. med. Res.*, **34**, 27.
 Beck, S. and Peacock, P. R. (1941), *Brit. J. exp. Path.*, **21**, 227.
 Berry, L. J., Davis, J. and Spies, T. D. (1945), *J. Lab. Clin. Med.*, **30**, 684.
 Bertram, S. H. (1937), *Chem. Abstr.*, **31**, 7680.
 Bhalerao, V. R., Venkatappiah, D. and Anantakrishnan, G. P. (1947), *Indian J. vet. Sci.* **17**, 221.
 Boelter, M. D. D. and Greenberg, D. M. (1943), *J. Nutrit.*, **26**, 105.
 Boer, J. (1941), *Acta brev. neerl.*, **11**, 180.
 — and Jansen, B. C. P. (1942), *Arch. Neerland. Physiol.*, **26**, 1.
 — and Kentie, A. (1947a), *J. Nutrit.*, **33**, 339.
 — and Knol, H. W. (1947b), *J. Nutrit.*, **33**, 359.
 Boer, J. E. H., Groot and Jansen B. C. P. (1948), *Voeding*, **9**, 60.
 Bolton, E. R. (1928), *Oils, Fats and Fatty Foods*, J. & A. Churchill, London. II Edn.
 Booher, L. E. (1938), *J. Amer. Med. Assoc.*, **110**, 1920.
 Booth, R. G., Kon, S. K., Dann, W. J. and Ti Moore (1933), *Biochem. J.*, **27**, 1189.
 Booth, R. G., Henry, K. M. and Kon, S. K. (1942), *Biochem. J.*, **36**, 445.
 Boutwell, R. K., Geyer, R. P., Elvehjem, C. A. and Hart, E. B. (1945), *Arch. Biochem.*, **7**, 143.
 Boyd, E. M. (1933), *J. Biol. Chem.*, **101**, 328.
 Brenner, S., Brookes, M. C. H. and Roberts, L. J. (1942), *J. Nutrit.*, **23**, 459.
 Brenner, S. and Roberts, L. J. (1943), *Arch. Int. Med.*, **71**, 474.
 Brown, E. F. and Bloor, W. R. (1945), *J. Nutrit.*, **29**, 349.
 Bult, A. R. and Sorgdrager, C. J. (1933), *Acta. brevneerl. Physiol.*, **8**, 114.
 — — (1940), *Neerland. Tijdsch. Geneesk.*, **84**, 1963.
 Caldwell, A. B., McLeod, G. and Sherman, H. C. (1945), *J. Nutrit.*, **30**, 349.
 Callaway, J. L. and Noojni, R. O. (1940), *J. Invest. Dermatol.*, **3**, 71.
 Callison, E. C. and Knowles, V. H. (1945), *Amer. J. Physiol.*, **143**, 444.
 Campbell, H. L., Udiljak, M., Yarmolinaky, H. and Sharman, H. C. (1945), *J. Nutrit.*, **30**, 343.
 Cerecedo, L. R. and Vinson, L. J. (1944), *Arch. Biochem.*, **5**, 157.
 Council on Foods and Nutrition (1942), *J. Amer. Med. Ass.*, **119**, 1425.
 Coward, H. K. (1936), *Biochem. J.*, **30**, 1878.
 — (1942), *Brit. Med. J.*, **1**, 435.
 — Key, K. M., Dyer, F. J. and Morgan, B. C. E. (1931), *Biochem. J.*, **25**, 511.
 Cowgill, G. R. (1945), *Physiol. Revs.*, **25**, 664.
 Crockett, M. E. and Deuel, H. J. (Jr.) (1947), *J. Nutrit.*, **33**, 187.
 Datar Singh (1949), *Indian Dairyman*, **1**, 23.
 Datta, N. C. (1945), *Ann. Biochem. Exp. Med. (Calcutta)*, **5**, 109.
 — (1948), *Ann. Biochem. Exp. Med. (Calcutta)*, **8**, 69.
 Davies, A. W. (1933), *Biochem. J.*, **27**, 1770.
 Davies, W. L. (1939), *The Chemistry of Milk*, Chapman and Hall Ltd., London, p. 92.
 Day, P. L. and Darby, W. J. (1947), *Estimation of the vitamins*, Biological Symposia, Vol. XII, The Jaques Cattell Press, Lancaster, PA.
 De, N. K., Ranganathan, S. and Sundararajan, A. R. (1946), *Indian J. med. Res.*, **34**, 3.
 Deuel, H. J. (Jr.) and Holmes, A. D. (1921), *Amer. J. Physiol.*, **54**, 479.

- Deuel, H. J. (Jr.) and Holmes, A. D. (1922), *Bull. U. S. Dept. Agri.*, No. 1033.
- , Movitt, E. and Hallman, L. F. (1943), *Sci.* 98, 139.
- and Mattson F. (with E. Wur) (1944), *J. Nutrit.*, 27, 107.
- and Wu, E. (1944a), *J. Nutrit.*, 27, 335.
- (with E. Brown) (1944c), *J. Nutrit.*, 27, 509.
- (1945 a), *J. Nutrit.*, 29, 309.
- Deuel, H. J. (Jr.) and Movitt, E. (with E. Brown) (1945), *J. Nutrit.*, 29, 237.
- Deuel, H. J. (Jr.), Hendrick, C. and Crockett, M. E. (1946), *J. Nutrit.*, 31, 737.
- Deuel, H. J. (Jr.), Hendrick, C., Movitt, E. Crockett, M. E., Smith, I. M. and Winzler, R. J. (1946a), *J. Nutrit.*, 31, 747.
- Deuel, H. J. (Jr.) and Meserve, E. R. (1946b), *Oils and Soaps*, 23, 211.
- Deuel, H. J. (Jr.), Meserve, E. R. Straub, E. E., Hendrick C. and Scheer, B. T. (1947), *J. Nutrit.*, 33, 569.
- Deuel, H. J. (Jr.), Cheng, A. L. S. and Morehouse, M. C. (1948), *J. Nutrit.*, 35, 295.
- Deuel, H. J. (Jr.), Johnson, R. M., Calbert, C. E., Gardener, J. and Thomas, B. (1949), *J. Nutrit.*, 38, 369.
- Deuel, H. J. (Jr.), Greenberg, S. M. and Savage, E. M. (1950), *J. Nutrit.*, 42, 239.
- Doctor, N. S., Banerjee, B. N. and Kothavalla, Z. R. (1940), *Indian J. vet. Sci.* 10, 63.
- Dutt, N. C. (1945), *Sci. and Cult.*, 10, 390.
- Dyme, H. C., Nelson, P. M., Lowe, B. and Nelson, V. E. (1941), *Iowa State Coll. J. Sci.*, 15, 189.
- Ershoff, B. H. and Adams, A. D. (Jr.) (1946), *Proc. Soc. Exp. Biol. Med.*, 62, 154.
- Escudero, P., Escudero, A. and Herraiz, M.L. (with Mosto, D., Pico, R. J., Radice, J. C. and Polak, M. (1939), *Nutrit., Abs. Rev.*, 14, No. 484, (1944-45.)
- Esh, G. C. and Sutton, T. S. (1948), *J. Nutrit.*, 36, 391.
- Euler, B. V., Euler, H. V. and Saberg, I. (1941), *Ark. Kemi. Min. Geol.*, (B), 14, No. 34.
- (1942), *Ark. Kemi. Min. Geol.*, (B), 15, No. 8.
- Euler, B. V. and Euler, H. V. (1945), *Ark. Kemi. Min. Geol.*, (B) 18, No. 12.
- Euler, B. V., Euler, H. V. and Ronnestam-Saberg, J. (1947), *Ark. Kemi. Min. Geol.*, (A) 24 No. 15.
- (1947a), *Ark. Kemi. Min. Geol.*, (A) 24, No. 20.
- Euler, B. V. and Euler, H. V. (1948), *Ark. Kemi. Min. Geol.*, (B), 25, No. 2.
- Euler, B. V., Euler, H. V. and Lindeman, G. (1948), *Ark. Kemi. Min. Geol.*, (B), 26, No. 2.
- Evans, H. M. and Lepkovsky, S. (1928), *Science*, 63, 298.
- Forbes, E. B., Swift, R. W., Elliott, R. F. and James, W. H. (1946), *J. Nutrit.*, 31, 203.
- (1946a), *J. Nutrit.*, 31, 213.
- Forbes, E. B., Swift, R. W., James W. H., Bratzler, J. W. and Black, A. (1946b), *J. Nutrit.*, 32, 387.
- Forbes, E. B., Swift, R. W., Thacker, E. J., Smith, V. F. and French, C. E. (1946c), *J. Nutrit.*, 32, 397.
- French, C. E. (1942), *J. Nutrit.*, 23, 375.
- French, C. E. and Elliott, R. F. (1943), *J. Nutrit.*, 25, 17.
- Galanini, A. (1939), *Rend. Ist. Sanit. Publ.*, 2, 312, (*Nutr. Abstr., Rev.* (1940) 9, 375).
- Geyer, R. P., Geyer, B. R., Derse, P. H., Zinkin, T., Elvehjem, C. A. and Hart, E. B. (1947), *J. Nutrit.*, 33, 129.
- Gillam, A. E., Heilborn, I. M., Morton, R. A., Bishop, G. and Drummond, J. G. (1933), *Biochem. J.* 27, 878.
- Gray, E., Leb., Hickman, K. C. D., and Brown, E. F. (1940), *J. Nutrit.*, 19, 39.
- Grewal, K. S. (1933), *Quart. J. Pharm.* 6, 650.
- Guerrant, N. B. and Dutcher, R. A. (1934), *J. Nutrit.*, 8, 397.
- Guggenheim, K. (1944), *Biochem. J.*, 38, 260.

- Hagnell-Zethraeus, G., Neymark M. and Windmark, E. M. P. (1942), *Nutrit. Abstr., Rev.* 12, 92.
- Hartwell, G. (1922), *Biochem. J.*, 16, 78, 825.
- (1928), *Biochem. J.*, 22, 1212.
- Hassan, A., and Drummond, J. C. (1927), *Biochem. J.* 21, 653.
- Hassan, M., Ibrahim, M. and Khanna, L. C. (1949), *Indian J. med. Res.*, 36, 33.
- Heftmann, E. (1947), *J. Nutrit.*, 34, 455.
- Henry, K. M., Kon, S. K., Hilditch, T. P., and Meara, M. D. (1945), *J. Dairy Res.*, 14, 45.
- Hickman, K. (1943), *Ann. Rev. Biochem.* 12, 353.
- Hilditch, T. P. (1941) Industrial Chemistry of fat and waxes, Balliere, Tindall and Cox, London
- Hoagland, R. and Snider, O. G. (1940), *U. S. Dept. Agric. Tech. Bull.* No. 725.
- (1942), *U. S. Dept. Agric., Tech. Bull.* No. 821.
- Hoagland, R. and Snider, C. G. (1943), *J. Nutrit.*, 26, 219.
- Jacobs, M. B. (1938), Chemical analysis of foods and food products, Macmillan and Co., Ltd. London, P. 218.
- Jones, J. H. (1940), *J. Nutrit.*, 20, 367.
- Jones, J. H., Foster, C. Dorfman, F. and Hunter, G. L., (with Quincy, M. E. and Alexander, D. L.) (1945), *J. Nutrit.*, 29, 127.
- Karmarkar, G. (1944), *Indian med. Gaz.*, 79, 535.
- Kehar, N. D. and Chanda, R. (1948) *Sci. & Cul.*, 13, 426.
- (1948), *Sci. & Cul.*, 14, 33.
- Kehar, N. D. and Sahai, K. (1949), Report of the Vanaspati Scheme submitted to the Vanaspati Research Planning Committee, Food Dept.
- Kehar, N. D. & Mukherjee, R. (1949), *Indian J. vet. Sci.* 19, 113.
- Kemmerer, A. R., Fraps, G. S. and DeMottier, J. (1947), *Arch. Biochem.*, 12, 135.
- Kennelly, B. and Quackenbush, F. W. (1947), *Fed. Proc.*, 6, 266.
- Kentie, A. (1947), *Nederlands Melk Zuiveltijdschr.*, 1, 118. (*Nutrit., Abstr. Rev.*, 17 No. 4871, 1947-48).
- Kline, L., Hall, W. L. and Morgan, J. F. (1941), *J. Ass. Off. Agric. Chem.*, 24, 147.
- Kundson, A. and Floody, R. J. (1940), *J. Nutrit.*, 20, 317.
- Kon, S. K. and Booth, R. G. (1933), *Chem. & Industry*, 52, 844.
- Langworthy, C. F. (1923), *Industr. Eng. Chem.*, 15, 276.
- Lassen, S. and Bacon, E. K. (1949), *J. Nutrit.*, 39, 83.
- Lease, E. J., Lease, J. G., Steenbook, H. and Baumann, C. A. (1939), *J. Nutrit.*, 17, 91.
- Leichnger, H., Eisenberg, G., and Carlson, A. J., (1948), *J. Amer. Med. Ass.*, 136, 388.
- Lemley, J. M., Brown, R. A., Bird, O. D. and Emmnett, A. D. (1947), *J. Nutrit.*, 33, 53.
- Lewis, J. M., Bodansky, O., Falk, K. G. and McGuire, G. (1942), *J. Nutrit.*, 23, 351.
- Loosli, J. K., Lingenfelter, J. F., Thomas, J. W. and Maynard, L. A. (1944), *J. Nutrit.*, 28, 81.
- Macdonald, D. G. H. and McHenry, E. W. (1940), *Amer. J. Physiol.*, 128, 608.
- MacDonald, A. J. and Bose, S. (1942), *Indian J. med. Res.*, 30, 285.
- Maignon, F. (1933-34), *Nutr. Abstr. Rev.*, 3, 345.
- Majumdar, B. N. (1941), *Indian J. vet. Sci.*, 11, 329.
- Mannering, G. J., Lipton, M. A. and Elvehjem, C. A. (1941), *Proc. Soc. exp. Biol.*, 46, 100.
- Mannering, G. J., Orsini, D. and Elvehjem, C. A. (1944), *J. Nutrit.*, 28, 141.
- Mason, E. D., Theophills, F. and Frindot-Moller, J. (1945), *Indian J. med. Res.*, 33, 219.
- Mattil, K. F. and Higgins, J. W. (1945), *J. Nutrit.*, 29, 255.
- Mattil, K. F., Higgins, J. W. and Robinson, H. E. (1946), *Science*, 104, 255.
- Mattil, K. F. (1947), *Oil and Soap*, 28, 344.
- Maynard, L. A. and Raamussen, E. (1942), *J. Nutrit.*, 23, 385.

- Maynard, L. A. (1946), *J. Nutrit.*, 32, 345.
- McCollum, E. V., Simmonds, N. and Pitz, W. (1916), *J. biol. Chem.*, 27, 33.
- Miller, H. G. (1943), *J. Nutrit.*, 26, 43.
- Misra, U. C. and Patwardhan, V. N. (1948), *Indian J. med. Res.*, 36, 27.
- Mitchell, H. H. (1923-24), *J. biol. Chem.*, 58, 873.
- Moore, T. (1929), *Lancet*, 1, 499.
- (1940), *Biochem. J.*, 34, 1321.
- Mukherjee, R. and Kehar, N. D. (unpublished results).
- Muthanna, M. C. and Seshan, P. K. (1941), *Indian med. Gaz.*, 76, 487.
- Myburgh, S. J. (1943), *Onderst. J. vet. Sci.*, 18, 157.
- Napier, L. E. and Das Gupta, C. R. (1945), *Haematological Technique*, III Edn., Revised by C. R. Das Gupta, U. N. Dhar and Sons Ltd., Calcutta.
- Nath, H., Barki, V. H., Elvehjem, C. A. and Hart, E. B. (1948), *J. Dairy Sci.*, 31, 793.
- Nhavi, N. G. and Patwardhan, V. N. (1946), *Indian J. med. Res.*, 34, 49.
- Nielson, E. and Elvehjem, C. A. (1942), *J. Biol. Chem.*, 144, 405.
- Norris, F. A. and Mattil, K. F. (1946), *Oil and Soap*, 23, 289.
- Olson, F. R., Hegsted, D. M. and Peterson, W. H. (1939), *J. Dairy Sci.*, 22, 63.
- Oser, B. L., Melnick, D. and Pader, M. (1943), *Ind. Eng. Chem. Anal. Ed.*, 15, 724.
- Popper, H. and Brenner S. (1942), *J. Nutrit.*, 23, 431.
- Quackenbush, F. W., Platz, B. R. and Steenbock, H. (1939), *J. Nutrit.*, 17, 115.
- Quackenbush, F. W., Kummerow, F. A. and Steenbock, H. (1942a), *J. Nutrit.*, 24, 213.
- Quackenbush, F. W., Steenbock, H., Kummerow, F. A. and Platz, B. R. (1942b), *J. Nutrit.*, 24, 225.
- Quackenbush, F. W. and Steenbock, H. (1942c), *J. Nutrit.*, 24, 393.
- Ram, Gupta and Athawala (1943), *J. Indian Chem. Soc., Indus. & News Ed.* 6, 23.
- Ramasarma, G. B. and Hakim, D. N. (1942), *Nature*, 149, 611.
- Rao, B. V. and Anantakrishnan, C. P. (1945), *Indian J. vet. Sci.*, 15, 231.
- Rao, S. D. (1945), *Nature*, 156, 234.
- (1945a), *Nature*, 156, 449.
- Ray Sarkar, B. C. and Sen, K. C. (1943), *Indian J. vet. Sci.*, 13, 219.
- Reader, V. and Drummond, J. C. (1926), *Biochem. J.*, 20, 1256.
- Richardson, L. R., Hogan, A. G. and Itschner, K. F. (1941), *Missouri Agri. Exp. Sta. Res. Bull.* No. 333, 3.
- Ritzman, E. G., Colovos, N. F., Keener, H. A. and Teeri, A. E. (1945), *New Hamp. Agr. Exp. Sta. Tech. Bull.* No. 87.
- Roy, A. (1944), *Ann. Biochem. Exp. Med.*, (Calcutta), 4, 17.
- Salmon, W. D. and Guerrant, N. B. (1930), *Alabama Expt. Sta. 41st Ann. Rept.*
- and Goodman, J. G. (1931), *Alabama Expt. Sta. 42nd Ann. Rept.*
- (1937), *J. Nutrit.*, 13, 477.
- Schantz, E. J., Boutwell, R. K., Elvehjem, C. A. and Hart, E. B. (1940), *J. Dairy Sci.*, 23, 1205.
- Schantz, E. J., Elvehjem, C. A. and Hart E. B. (1940), *J. Dairy Sci.*, 23, 181.
- Scheunert, A. (1940), *Klin. Wochs.*, 19, 342.
- Seshan, P. A. and Sen, K. C. (1942), *J. Agric. Sci.*, 32, 194.
- Sherman, W. C. (1940), *J. Biol. Chem.*, 89, xc Proc.
- (1941), *J. Nutrit.*, 22, 153.
- Sherman, H. C., Campbell, H. L., Udiljak, M. and Yarmolinsky, H. (1945a), *Proc. Nat. Acad. Sci., U. S.*, 31, 107.
- Shukers, C. F. and Day, P. L. (1943), *J. Nutrit.*, 25, 511.
- Smith, J. A. B. and Dastur, N. N. (1938), *Biochem. J.*, 32, 1868.

- Smith, J. A. B. (1948), *Proc. Nutr. Soc., Abstr. Communications, Brit. J. Nutr.*, 2, 2.
- Stir, F. E., Arnold, A. and Elvehjem, C. A. (1939), *J. Nutr.*, 17, 485.
- Sullivan, M. and Nicholas, J. (1941), *J. Invest. Dermatol.*, 4, 181.
- Sure, B. (1941), *J. Nutr.*, 22, 491.
- (1941a), *J. Nutr.*, 22, 499.
- Treichler, R., Kermerer, A. R. and Fraps, C. S. (1942), *J. Nutr.*, 24, 57.
- Unna, K., Richards, G. V. and Sampson, W. L. (1941), *J. Nutr.*, 24, 63.
- Wagner, K. H., Gunther, L. and Schulze (1941), *Vitamin u. Hormone*, 1, 455.
- Wainio, W. W. (1942), *J. Nutr.*, 24, 317.
- Westerlund, A. (1947-48), *Nutrit., Abstr. Rev.*, 17, No. 4753.
- Whipple, D. V. and Church, C. F. (1936), *J. Biol. Chem.* 113, cvii (Proc.).
- Wilbur, J. W., Hilton, J. H. and Hango, S. M. (1933), *J. Dairy Sci.*, 16, 153.
- Yasuda, M. (1931), *J. Biol. Chem.*, 94, 401.
- Zialcita, L. P. (Jr.) and Mitchell, R. H. (1944), *Science*, 100, 60.