XI. ON THE PROPERTIES OF CERTAIN S. AFRICAN OILS WITH RESPECT TO THEIR CONTENT OF VITAMIN A.

By ELLEN MARION DELF.

(Received October 31st, 1923.)

Of late years much evidence has accumulated pointing to the value of vitamin A as a dietary factor, especially in connection with healthy growth in young animals.

By the labours principally of English and American workers, the distribution of the vitamin has been ascertained in a considerable number of natural and commercial products, principally, however, those belonging to the northern hemisphere. Comparatively little is known of the vitamin content of the local produce of other countries, and in a visit to S. Africa in the years 1920-1921 the author became interested in this question from the dietary point of view. In England and America, it has been found that milk and butter are active in growth-promoting properties, but that their value varies much with the previous diet of the cow, being greatest in the case of milk from pasture-fed cows and least in the case of cows on the usual winter feeds [McCollum, Simmonds and Pitz, 1916; Drummond, 1918; Drummond, Coward and Watson, 1921].

To what extent milk and butter may be a source of vitamin A in S. Africa is quite unknown. Over a great part of the interior, the dry climate makes it impossible for the cattle to obtain green grass for more than a small proportion of the year. Shortage of green vegetables is also common in the dry weather. It is conceivable that the brilliant sunshine may in part compensate for the shortage of green food, both for cattle and man. It seems probable, however, that a deficiency of vitamin A may become significant in certain cases, such as that of the native mine workers who spend many hours underground, and whose diet is subject to various restrictions [Delf, 1922]. It is almost certainly also of importance in the case of young white children, brought up on artificial foods, or on such milk as can be obtained in the more remote districts. For such reasons it seemed desirable to test the growth-promoting properties of some common S. African oils.

The method of testing has been that employed for several years as a routine in this kind of work by Drummond, Zilva, Coward and others. Used with due precautions, it has repeatedly been found capable of giving approximately quantitative results [Drummond and Coward, 1920; Zilva and Miura, 1921]. The young rats used were at first obtained from a dealer, but, in accordance with general experience, better results were gained later by the use of rats bred under laboratory conditions upon a fairly liberal diet.

Young rats about 45–50 g. in weight were placed upon an artificial diet consisting solely of ingredients in the following proportions:

Purified rice starch	...	...	150 g.
„ casein	...	...	60 „
Mixed mineral salts	...	...	15 „
Marmite	...	...	15 „
Hardened vegetable fat	...	...	30 „
Fresh lemon juice	...	...	15 cc.
Water	...	...	150–170 cc.

The salt mixture had the following composition:

Sodium chloride	...	...	52 g.
Magnesium sulphate	...	...	80 „
Sodium acid phosphate	...	...	104 „
Potassium phosphate	...	...	162 „
Calcium lactate	...	...	390 „
Ferric citrate	...	...	35 „

The work of many investigators has repeatedly shown that after 3–4 weeks on such a diet young rats mostly cease to grow. When the body weight is steady, addition of even a fraction of a drop of cod liver oil¹ to the diet daily causes growth to be resumed. If the hardened vegetable oil is replaced wholly or in part by some other active fat, growth is also resumed with more or less rapidity. The activity of the added fat is thus judged with a fair degree of accuracy.

(1) *Vegetable oils.* The three vegetable oils examined—sesame, mafurreira and castor oil—were found to be entirely devoid of growth-promoting capacity. Castor oil cannot be tolerated by young rats, but after saponification, the remaining unsaponifiable fraction was fed successfully to a group of four young rats which had ceased to grow on the standard diet. No improvement was seen and growth was not resumed. Certain other vegetable oils (examined in the whole nut) were found by Drummond and Coward to possess slight growth-promoting powers, viz. Brazil, Barcelona, walnut, pea, almond and butter nuts [Drummond, 1919, 2].

(2) *Seal oil, Nos. 1 and 2.* These oils were received early in January, 1922, but owing to difficulty in obtaining suitable experimental animals, the tests were somewhat delayed. The oils had been prepared by boiling at atmospheric pressure for about 10 hours. The subsequent storage, prior to delivery, was about two months.

Each of these oils was fed to animals to the extent of 10 % of their diet. All the animals receiving them were found to resume growth in a normal way (see Fig. 1). These oils therefore have a considerable value with respect to their vitamin content. Further experiments were not made with them,

¹ This is true only of a first-rate grade of cod liver oil; for variations in quality of cod liver oil, with season, and mode of preparation see Drummond and Zilva [1921, 1922]. An average grade appears to give perceptible growth on about 15 mg., i.e. half a drop administered daily.

however, as it appears unlikely that they would ever be sufficiently abundant commercially to be of any economic importance.

(3) *Whale oils, Samples 1, 2, 3.* These are crude whale oils graded according to their preparation. Like the seal oil, quality 1 was prepared by boiling with steam at atmospheric pressure for about 10 hours. The other two oils were extracted at a higher temperature. This grade was heated by steam at a pressure of 50 lbs., the time not being specified. According to Mr Bearpark, this is the only significant difference between the samples.

Young non-growing rats given the standard diet in which the fat had been entirely replaced by whale oil No. 1 or 2 grew extremely well (Fig. 2).

Fig. 1. Chart of growth of young rats at first kept on non-growing diet (broken line). The hardened fat was then replaced by an equal weight of seal oil No. 1, (rats 5, 2, and 10) and seal oil No. 2, (rats 7, 8 and 29) respectively.

Those receiving No. 3, however, were variable in behaviour. Out of nine such animals, four resumed growth at a normal rate; the others after showing a temporary improvement declined in weight, three of these ultimately dying of no apparent disease. This would lead one to suspect a real difference in the vitamin content of the oils, No. 3 being the least valuable in this respect.

When the fat was half replaced by one of the oils, the resulting experiments showed clearly that oil No. 1 was better than oil No. 2 (cp. Fig. 2, Nos. 76, 65 with Nos. 53, 60). With a diet in which oil No. 1 formed only 1 % of the total, three animals out of four showed improvement (cf. Fig. 2, No. 130), one losing in weight (Fig. 2, No. 129). At a later date, four animals were given three drops each of this oil daily as an addition to their non-growing diet, and little or no benefit could be detected, two animals dying and two barely maintaining their weight for the experimental period. This sample appears to have nearly as much activity in growth production as good English butter fat produced in the summer months¹.

¹ It was found by Drummond and Coward that the growth-promoting value of butter fat varies with the season in which it is produced [1920; Coward and Drummond, 1920]. Using a good grade of butter, these workers estimated that 0.2 g. of butter, daily, fed separately to non-growing rats, produces "slow but steady growth" [Coward and Drummond, 1920, p. 735]. With a rat of average appetite on a synthetic non-growing diet, this represents the equivalent of about 1 % of the total synthetic food taken.

If we assume that the whale oils were of similar origin, the extraction at atmospheric pressure (about 100°) appears to be by far the most satisfactory from the point of view of resulting vitamin content, in spite of the greater opportunity for oxidation by this method. Samples of whale oil from other sources previously examined by Drummond were also found to be rich in vitamin A, their relative activity being independent of the "low" or "high" grade [Drummond, 1919, 1].

(4) *Sperm oil (from the head, blubber and meat respectively)*. The samples of head and blubber oil were prepared by heating "head matter" and blubber respectively, in an open vessel at 100° for about 10 hours. The meat oil, on the other hand, was heated under pressure at the same temperature as were whale

Fig. 2. Chart showing behaviour of typical rats on non-growing diet (broken line) followed by diet containing whale oil.

Nos. 17, 19,	10 % whale oil I	Nos. 53, 60,	5 % whale oil II
" 20, 33,	" " II	" 65, 76,	" " I
" 37, 41, 47	" " III	" 129, 130, 1	" " I

No. 60 shows at P rapid recovery after transference to a perfect diet (the standard diet in which good butter replaces the hardened fat). Curve B represents growth (after a preparatory period on non-growing diet not shown) with a basal diet containing 2 % good English butter; this is constructed from figures kindly supplied by Dr K. H. Coward.

oils 2 and 3. Judging by the results obtained with the three samples of whale oil, much deterioration would be expected by this method.

Each of these oils was first given to experimental animals in the proportion of 10 % of their total diet. When the *head oil* was used as sole source of fat in the diet, a variable but slight improvement was seen (Fig. 3, Nos. 13, 14, 24 and 32). No. 14, however, died soon after the beginning of the experiment.

When either meat or blubber oil was used, however, striking improvement resulted (Fig. 3). The head oil was thus found to be distinctly the least effective of the three oils. Since the method of preparation was not calculated

Fig. 3. Chart showing behaviour of rats on non-growing diet (broken line) followed by diet containing 10 % of sperm oil from the head, meat and blubber respectively.

Nos. 22, 23, 34, 36. Blubber oil.
 „ 9, 11, 1. Meat oil.
 „ 24, 32, 13, 14. Head oil.

Fig. 4. Chart showing behaviour of rats on non-growing diet (broken line) followed by diet including sperm meat oil.

Nos. 62, 63, 77, 80. 5 % sperm meat oil.
 „ 66, 75, 83, 84. 2.5 % „
 „ 107, 108, 109, 96. 1 % „

to produce great destruction of the vitamin content, this oil is probably inferior in its original state.

The *sperm meat oil* was also given to groups of four animals in the proportions of 5 % and 2.5 % respectively of the total fat in the diet. In both cases growth was resumed, but those on the larger ration were heavier and more vigorous at the end of the experiment (Fig. 4). On a diet including only 1 % of the oil, three succeeded in maintaining their body weight, and one

Fig. 5. Chart showing growth of rats on non-growing diet (broken line) followed by diet including blubber oil.

Nos. 89, 111, 112. 1 % blubber oil.

„ 88, 118, 119. 3 drops blubber oil.

„ 146, 147, 148, 149. 3 drops new sample blubber oil.

„ 131, 143, 153, 155.

The basal diet for the last group was made without the addition of fresh lemon juice.

(No. 107) gained somewhat in weight. This oil is therefore very efficient in promoting growth, and appears to have about the same value as good English butter [Cramer, 1922 (cp. Fig. 2, curve B)]. Possibly if the method of preparation were modified, the meat oil could be obtained showing still more activity.

Sperm blubber oil was tested in the same way as the meat oil and with diets containing either 5 %, 2.5 % or even 1 % of the oil, good growth resulted. When three small drops were administered daily, in addition to the usual basal diet, a fairly satisfactory growth was obtained (Fig. 5, Nos. 88, 118, 119). When two drops were given daily, growth could still be detected, but when only one drop was given daily, the weight was barely maintained during the

experimental period. By direct determinations, it was found that with the dropping rod used, the drops averaged about .03 g. each. The drop method ensures that the animals receive their oil ration independently of their appetite for the synthetic food, a point which becomes of more importance as the ration diminishes. With animals of average appetite, a daily dose of three drops is the equivalent of a ration of 1 % of the oil. If the appetite falls off, however, the proportion of oil to the total food taken becomes appreciably greater.

In an ordinary sample of good cod liver oil (cp. footnote, p. 94) about half a small drop daily would give appreciable slow but steady growth [Drummond and Zilva, 1921, 1922].

Sperm blubber oil is thus less efficient than a good sample of cod liver oil in promoting growth, but it is the most active of all the whale oils hitherto examined.

In accordance with the convictions of Harden and Zilva [1918] and of Drummond [1919, 2] that "rats show a more satisfactory growth and development when their ration contains the antiscorbutic factor in addition to the other requisites," the standard diet adopted for the above experiments includes 5 % lemon juice as the source of vitamin C. Certain investigators [Cramer, 1922; Cramer *et al.*, 1922] however still assume that this vitamin is unnecessary for the rat. Cramer states that rats can be bred successfully in the laboratory on a diet of white bread, boiled maize (presumably yellow maize) and rice; he admits that better animals and bigger litters can be produced if cod liver oil and marmite are added to the rations, but does not suggest that fresh green food or carrots would also be beneficial. The antiscorbutic element seems to be in fact excluded from both his normal and experimental diets. This suggests that the need for vitamins A and C may be to some extent interchangeable in the diet of the rat, and in the course of the present experiments a few observations were made which point to the same conclusion.

In Fig. 5 the weight charts of Nos. 146, 147, 148 and 149 represent growth on a diet including three drops daily of blubber oil. The oil was from a second supply received when the first lot had been finished. Nos. 131, 143, 153 and 155 received a similar oil ration, but their basal diet had no lemon juice or other source of antiscorbutic. Those without lemon were more restless and more variable in growth-rate than those with the lemon included. On the whole they made less satisfactory growth. When the same experiment was repeated using a higher ration (2.5 %) of oil, no difference could be detected between the two lots of animals. On the other hand, an increase in the supply of lemon juice does not appear to benefit the rats, whether the supply of oil is minimal or not. When a smaller ration of oil was used (two drops) maintenance of weight resulted without growth, whether lemon juice was supplied or not. Below a certain minimal amount of the fat-soluble factor, therefore, addition of antiscorbutic vitamin (in the form of lemon juice at least) does not appear to compensate for the deficiency of the fat-soluble vitamin.

SUMMARY.

The experiments reported above, have given the following results:

- (1) Sesame, mafurreira and castor oils have been shown to be deficient in vitamin A.
- (2) Two samples of seal oil are shown to have considerable growth-promoting properties.
- (3) A difference in value was found in three different samples of crude whale oil. Sample No. 1 was the most active, and had been extracted (unlike the other two) at 100°. The lower activity of the other two samples was correlated with their extraction at considerably higher temperatures.
- (4) The sperm whale gives oils of differing activity with regard to vitamin A. The oil from the head is least and that from the blubber most active in this respect. With more careful extraction, the blubber and meat oil would serve as a very valuable source of this vitamin.
- (5) The rat has little need of vitamin C in the diet. It has a much greater need of vitamin A; when the latter is limited however, the addition of vitamin C to the diet appears to have a really beneficial effect on growing rats. This suggests that the two vitamins are to some extent interchangeable in the diet of the rat.

The foregoing experiments were made at the Physiological Institute (Biochemical Department), University College, London, by the courtesy of Prof. J. C. Drummond. My thanks are due to him and to Dr K. H. Coward, for the generous way in which the facilities of the Department were placed at my disposal. The expenses involved were met by a grant from the Board of Scientific and Industrial Research in S. Africa; publication has, however, been delayed for various reasons. Samples of oils were supplied gratis by the respective firms producing them. From the Moçambique Soap and Oils Co. Ltd., of Lorenzo Marques, samples of castor, mafurreira and sesame oils were received; and from Irvin and Johnson, Ltd., of Cape Town, various samples of seal, sperm and whale oil. Particulars of these have been supplied by Mr A. H. Bearpark, their scientific oil expert to whom especially my thanks are due.

REFERENCES.

- Coward and Drummond (1920). *Biochem. J.* **14**, 734.
 Cramer (1922). *Brit. J. Exp. Path.* **3**, 298.
 Cramer, Drew and Mottram (1922). *Proc. Roy. Soc. Lond. B.* **93**, 449.
 Delf (1922). Publication XIV, *S. Afr. Inst. Med. Research*.
 Drummond (1918). *Lancet*, Oct. 12.
 — (1919, 1). *J. Physiol.* **52**, 344.
 — (1919, 2). *Biochem. J.* **13**, 81.
 Drummond and Coward (1920). *Biochem. J.* **14**, 661.
 Drummond, Coward and Watson (1921). *Biochem. J.* **15**, 540.
 Drummond and Zilva (1922). *J. Soc. Chem. Ind.* **41**, 280.
 Harden and Zilva (1918). *Biochem. J.* **12**, 408.
 McCollum, Simmons and Pitz (1916). *J. Biol. Chem.* **27**, 33.
 Zilva and Drummond (1921). *Lancet*, ii, 753.
 Zilva and Miura (1921). *Lancet*, i, 323.