Unsaponifiable Material.

We paid special attention to the nature of the unsaponifiable matter in these marine oils, for the following reason: One of us (C. L. C.) has already demonstrated that "mutton-bird oil" consists mostly of cetyl-oleate and other esters of cetyl alcohol. For some time we have been investigating the fate of cetyl alcohol in the body, and, incidentally, its origin in the mutton-bird. One possibility that presented itself to our minds is that the oil may be an indigestible (*i.e.*, unsaponifiable) residue of the fat present in the food of the bird. The finding of cetyl alcohol in whale-feed or other shell-fish, or in the fats of any form of marine life, would lend support to such a view, but, in spite of much time and labour spent in crystallizing out the unsaponifiable constituents, no trace of cetyl alcohol was found; on the other hand, there was clear evidence that the great mass of it consisted of cholesterol in more or less pure form.

In conclusion, we beg to thank the University of Otago for facilities given us for carrying on this work, and to acknowledge financial assistance received from the New Zealand Institute Research Fund.

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Food Values of New Zealand Fish.

Part 6: The Vitamin-A Content of Mutton-bird Oil and of some Fish-oils.

By John Malcolm, M.D.

Read before the Otago Institute, 9th December, 1924, received by Editor, 31st December, 1924; issued separately, 10th May, 1926.]

Since the beginning of the present century two great advances have been made in our knowledge of dietetics. One, originating in a better knowledge of the chemical constitution of the protein molecule, has led to the recognition of the importance of the quality as contrasted with the mere quantity of protein in diet; the other is the discovery of the need for accessory factors, or vitamins, in addition to the protein, salts, carbohydrate, and fat.

Of late years a large amount of work has been done on the presence of vitamins in various foodstuffs, and it seemed desirable to carry out similar investigations in New Zealand. This paper relates to the estimation of vitamin A in some marine products.

Method.

The mode of procedure commonly adopted in this kind of work is now so well established that it is unnecessary to set it out in detail, except so far as local conditions are concerned. Litters of young albino rats, weaned about the thirtieth day, were fed on a diet consisting of—Casein, 19·3 per

cent.; starch, 54·3 per cent.; lard, 16·1 per cent.; "Marmite," 4·4 per cent.; salt mixture (McCollum), 5·3 per cent. This diet is referred to in this paper as "diet 8." The rats were weighed twice or thrice a week till symptoms of lack of vitamin A set in and treatment began. Usually they were then weighed every second day, but, owing to pressure of other work, it was not practicable to have this done, and the food given, always at the same hour of the day. The temperature of the rat-room was kept at 17°–22° C. The experiments were begun in June, but it was not till early in September that difficulties in regard to the diet were overcome. These difficulties arose from the use of a dried yeast as source of vitamin B, faulty preparation of the salt mixture, and imperfect purification of the casein. The treatment ultimately adopted for the casein was a combination of that used by Sherman and by Hopkins—viz., extraction with boiling strong alcohol three times in succession, followed by heating for eight or more hours on an iron tray. In our case that could be done only in quantities of 400 grm. at a time, and, owing to the stage of growth of the litters, there was no time to stop for the purpose of accumulating a large stock of uniformly mixed, treated casein. Hence there is the possibility that some batches of diet may have been made with casein that still contained traces of vitamin A. That this did not occur to any appreciable extent is shown by the curves in charts 1 and 2, which are arranged so that the dates synchronize. Any fault in the purification of the diet would be shown more or less in all simultaneously, for the food was made up every four or five days, and all were fed alike. The only instance of an otherwise unexplainable rise is shown round the dates 18th to 21st October. (See rise in curve D, and possibly E_4 and E_1.)

The lard used was well aerated for at least eight hours on a boiling-water bath, and then filtered. Neither the other ingredients of the diet nor the mode of preparation call for any special comment at this stage. The oils tested were administered orally by means of small bulb-shaped pipettes, the delivery of which was determined by weighing. The smaller doses, such as 0·01 and 0·02, were given as dilutions in olive-oil so made that the amount administered was about 0·08.

MUTTON-BIRD OIL.

This oil, as is well known in New Zealand and Australia, is obtained from the stomach of the young mutton-bird (*Aestrelata lessoni*). When killed, the oil can be expressed from the crop, and it is commonly believed to be supplied to the young by the parent birds. Along with Mr. C. L. Carter, the writer has been engaged on an investigation into the biochemistry of this oil, and we have found that rats can tolerate and utilize doses that are large in comparison to their body-weight (0·5 grm. per day) with no serious effects, although they and other animals seem to be repelled by its flavour.

The sample used was obtained fresh in May, 1924. It measured about 4 oz., and probably represented the stomach-oils of about half a dozen birds. When kept undisturbed in the stock bottle it had a red colour; but the samples kept in the warm rat-room, in a small bottle, frequently uncorked, and stirred by the dosage pipette, soon became pale yellow, and were then renewed.

It was easily proved that this sample of mutton-bird oil contained vitamin A. What was, of course, more difficult to gauge was the minimal effective dose for cure of xerophthalmia and for the resumption of growth. No observations were made on its anti-rachitic properties.

The following are the essential features of the experiments :—

Litter B (chart 1), four in number—two bucks and two does—were weaned on the thirtieth day (7th August). Up to the 4th September they received some of the diets used earlier than diet 8, and their growth was slow, due probably to the insufficient supply of vitamin B. On diet 8, which was begun on the 4th September, their growth was steady and rapid till between the 23rd and 30th, when the weights decreased and eye troubles began. By the 3rd October all four suffered in this way, and on this date Nos. 1 and 4 (buck and doe) were separated from the other two and given doses of mutton-bird oil (0·08 grm. each). (See chart 1.) The eyes began to improve rapidly, but the weights did not immediately increase, and after about a week of this dosage, which, in the light of later experiments, appears unnecessarily large, the amount given was reduced to 0·04 grm. daily. On this dose the weights increased till, after about five weeks, the rats had gained 43 grm. and 36 grm. respectively. They then appeared to be in good condition, their eyes had completely recovered, their coats were good, and they felt "plump" in body when handled. The mutton-bird oil was then discontinued, and, as shown by the curves, the rats continued to grow or maintain their weight for about fifteen days, and then began to decline in health, to lose weight, and to develop eye trouble afresh. In about thirty days after receiving their last dose of mutton-bird oil they had lost 35 grm. and 26 grm. respectively. On the 4th December treatment was again begun on buck 1 with a dose of 0·01 grm., increasing to 0·02, 0·04, and 0·08, and, as shown by the curve, recovery again took place. Between the 8th and 18th December doe 4 was the subject of a class experiment which need not be described here. From the 18th December to the end she received the same treatment as buck 1, and showed a similar recovery.

Meanwhile the other two of this litter were made the subjects of an experiment on "oyster-oil," to be referred to later. Following the oyster-oil, mutton-bird oil caused a rise in weight and improvement in general health exactly comparable to that of Nos. 1 and 4. On stopping the mutton-bird oil they again lost weight, and on administering 0·04 grm. and later 0·08 grm. they again increased in weight. Their curves of weight were practically the same as those of B_1 and B_4.

Experiment C : In this, two does were used—of the same age, but not of the same litter. C_1 received 0·04 grm. mutton-bird oil ; the other, C_3, was used as a control. They were both kept in the same cage, and the control used greedily to lick the jaws of her companion after the latter received her dose ; possibly she also ate her companion's faeces, for, so long as they were together, the control's weight was maintained at a fairly level figure ; but when, on the 1st November, another control rat was substituted for the mutton-bird one she drooped and died. On the 3rd October both had well-marked eye symptoms, but, while the control's eye symptoms persisted in a subacute form for about a month and became aggravated shortly before death, the eyes of No. 1 improved within two days of commencement of the mutton-bird oil, and were completely cured in ten days. As shown in the curve, she also grew steadily while receiving the oil, and maintained her weight for ten days after the treatment was stopped. Then the weight fell and eye troubles began again. This rat was then used for an experiment on the effect of dried fish, to be referred to later.

Litter D (four does) : These, like the foregoing, did not grow well on the defective diets used during the month of August. Then, from the 4th September onwards, the growth was rapid till early in October, when a decrease in weight set in (see chart 1 ; the curve shown is the average for all four to begin with). On the 13th October one (No. 3) died, and the

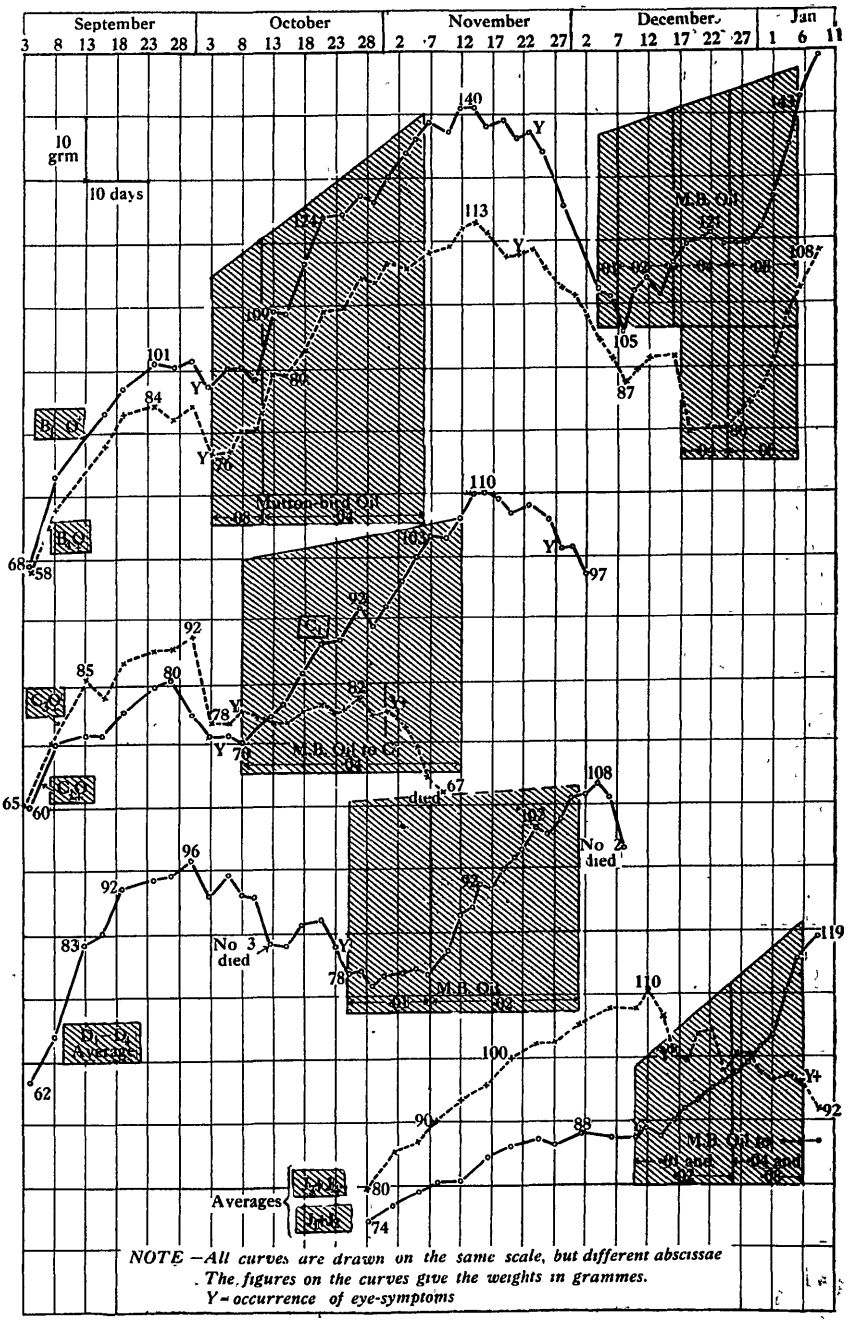
September
October
November
December
Jan
10 grm
10 days
M.B. Oil
Mutton-bird Oil
M.B. Oil to C.
died
No 2 died
No 3 died
Averages
NOTE.—All curves are drawn on the same scale, but different abscissae.
The figures on the curves give the weights in grammes.
Y = occurrence of eye-symptoms

CHART 1.

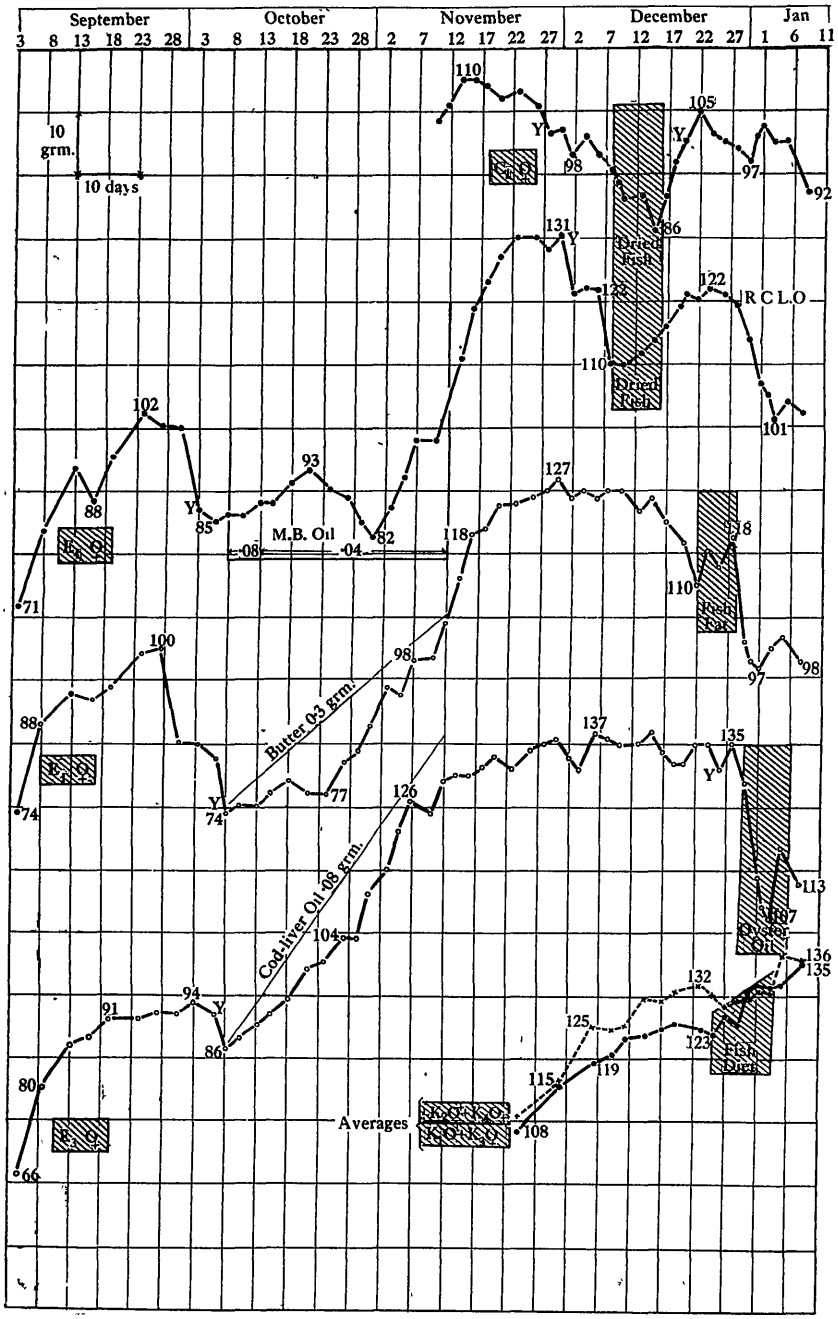
September
October
November
December
Jan
3 8 13 18 23 28 3 8 13 18 23 28 2 7 12 17 22 27 2 7 12 17 22 27 1 6 11
10 grm.
10 days
M.B. Oil
Butter 0·3 grm.
Cod-liver Oil 0·8 grm.
Averages
Dried Fish
Fish Diet
Oyster Oil
R C L O

CHART 2.

others were in very poor condition, with "staring" coats, "hunched-up" attitudes, and slight eye symptoms. This was followed by an improvement, which could be accounted for by imperfect purification of the casein used for a five-day quantity of diet. Then the weights dropped again, and on the 24th October the three survivors showed the typical symptoms of avitaminosis in eyes, weight, coat, &c. On the 25th October treatment with 0·01 grm. mutton-bird oil was begun. This was continued for a fortnight, during which the eye symptoms gradually disappeared, so that on the 6th November it was reported in the notes that all the eye symptoms had gone except for the appearance of a film on the right eye of No. 2. During this period, however, the average weight maintained a uniform level. What actually occurred was that No. 1 lost 1 grm., No. 4 lost 6 grm., and No. 2 gained 6 grm. The dose was then increased to 0·02 grm. daily, and a rapid rise followed in each of the three. By the 30th November—*i.e.*, after three weeks' dosage with 0·02 grm. oil—all were in good condition; their coats were still a little shaggy, but the eyes were normal, and they felt plump on handling. The oil treatment was now stopped, and for several days the weights were maintained or continued to rise. Then a rapid fall in weight set in, and two died, No. 2 on the 8th December and No. 1 on the 11th. The average curve is not carried beyond the 8th December, but it may be mentioned that the last survivor (No. 4) received further treatment with 0·02 grm. mutton-bird oil and recovered. When chloroformed on the 8th January she weighed 127 grm.

Litter E (four does): In the case of this litter also a marked effect was obtained by administration of mutton-bird oil in the two cases where it was administered. The curve of one of these, E_4, is given on chart 2. The other two rats were given butter (0·3 grm.) and cod-liver oil (0·08 grm.) respectively, in order to compare the results with that of the mutton-bird ones. The interesting feature of the result was that while there was little or no difference in the rate of growth during the time of administration of these sources of vitamin A, yet on ceasing the treatment the butter-fed and cod-liver-oil-fed ones continued to grow or to maintain themselves, without any eye symptoms appearing, for at least six weeks, while the mutton-bird-oil ones began to show eye troubles and loss of weight about eighteen days after the oil was stopped. (Compare the curves of E_4, E_1, and E_3 on chart 2.)

Litter J (four does): Two received gradually increasing doses of mutton-bird oil, and two were used as controls. The whole litter in this case had been too well fed before weaning, and were long in showing symptoms of avitaminosis (ten weeks); and the approach of a date when it was necessary to terminate all the experiments led to the mutton-bird-oil treatment being begun before the average weight had dropped much, although slight eye symptoms were evident. On the 10th December one doe began to receive 0·01 grm. oil, the other 0·02 grm., and the average weight began to increase, due mostly to an increase of the one receiving the smaller dose. The eyes soon recovered. Meanwhile the average weight of the controls showed variations, but on the whole continued to decline, and their eye symptoms became worse. On the 26th December the doses were increased to 0·04 grm. and 0·08 grm. respectively, and the result was a greatly increased rate of growth, both curves being practically the same. (Chart 1.)

When the experiment ended, on the 8th January, the oil-fed ones were in good condition and weighed 119 grm. and 120 grm. Of the controls, one died on the 7th January—weight at death, 88 grm.; the other was in very poor condition, with eyes deeply ringed with congestion, and flakes of haemorrhage—she weighed 95 grm.

The following observation may be recorded here, although it does not come under the title of the paper. Four rats were being fed on a diet deficient in vitamin B. Their weight was maintained at a fairly level figure. Mutton-bird oil was administered for over a week without effect, but the substitution of 5 c.c. of milk for the mutton-bird oil caused an immediate increase. From this it may be inferred that mutton-bird oil contains little or no vitamin B.

From these experiments it is obvious that mutton-bird oil can furnish sufficient vitamin A for rats when given in doses as low as 0·01 grm. to some, for others 0·02 grm. is required, and probably 0·04 grm. produces maximum effects in the majority.

Fish-oils.

As in other vertebrates, fat or oil occurs in fishes in the liver, and deposited under the skin, and mingled with some of the superficial muscle-fibres. That the liver-oils of all fishes contain vitamin A may be taken as established, but the fat usually eaten is that found in the other situations, and it is not at all certain that the latter is invariably a rich source of vitamin. Probably there are differences in this respect among different classes of fish, depending on the food of the fish, just as there is a difference between lard and beef-suet.

In this paper the writer can record only a few short experiments, but they are sufficient to show the lines on which further work may be done.

Tarakihi (Chilodactylus macropterus).

The tarakihi was chosen because of the high percentage of fat in its flesh. After boiling for twenty minutes the flesh was separated from the bones and spread in the sun to dry. Within twenty-four hours it was dry enough to grate finely in a mill. A sample was taken for fat-extraction (Soxhlet) and yielded 22 per cent. of fat, corresponding to 6 per cent. of the fresh material.

Experiments were made in the following ways: (1) Rats fed on diet 8 were offered some of the fish-powder; (2) rats were given doses of the ethereal extract of the powder; (3) a special diet containing the fish-meal was given.

(1.) *Addition of Fish-powder to Diet 8.*—Two does were used (C_1 and E_4). They had been used for mutton-bird-oil experiments, had recovered, and had again developed symptoms of lack of vitamin A. Although they belonged to different litters, the eye symptoms and loss of weight happened to occur in both about the same dates—29th November to 8th December. On the 8th December, whilst temporarily transferred to a clean empty cage, they were given the opportunity to eat some of the fish-powder. Within two hours 2 grm. was eaten. On subsequent days 4 grm. quantities were offered, and the residue weighed each day. By means of a separate test on two occasions it was found that each had eaten about the same quantity. This procedure was followed for eight days, and the average amount eaten by each per day was found to be 1·37 grm., which would contain 0·3 grm. of the tarakihi flesh-fat. The effects were not very striking. The curves (chart 2) show that C_1 decreased in weight during the treatment but increased afterwards, while E_4 showed moderate increase in weight. During the fish-meal period the eye symptoms improved, but only very gradually, and recovery was incomplete. They still showed signs of scratching and photophobia at a time when their weights were increasing. Within six days in C_1 and twelve days in E_4 the weights again fell, and all the eye symptoms were accentuated.

From this it may be concluded that 0·3 grm. tarakihi flesh-fat contains barely sufficient vitamin A to cure xerophthalmia or allow of much increase in weight; but it would have been better if the feeding could have been continued for a longer time.

(2.) *Administration of the Ethereal Extract of the Fish.*—This was used in one case only, doe E_1. She had recovered from avitaminosis by treatment with butter, and, about five weeks after stopping the butter, began again to lose weight and show eye symptoms. On the 19th December the right cornea was opaque, and there was a wide ring of inflammation with signs of scratching round both eyes; the skin area around the genitals was also wet and coloured brown. On the 22nd a dose of 0·06 grm. tarakihi flesh-fat was given, and this was repeated daily till the 27th. At first there was some improvement in weight, but the eye symptoms grew worse, and on the 27th the dose was trebled (0·18 grm.). This was followed by a rapid decrease in weight and aggravation of all the symptoms. No further doses were given, and, although she improved slightly in weight, her condition was very bad when the experiment terminated.

In this case, therefore, the extracted oil was insufficient to give a good effect, if it did not actually do harm.

(3.) *Feeding on a Fish-meal Diet.*—A special fish diet was made up so as to resemble diet 8 in its proportions of protein, fat, &c., by substituting fish-meal for part of the lard and all of the casein. It consisted of fish-powder, 90 grm.; lard, 25 grm.; Marmite, 12 grm.; salts, 12 grm.; starch, 159 grm. It was made up with water in the usual way, and divided into a certain number of equal-sized rations. Assuming that a rat ate 10 grm. of the fish diet, it would receive about 0·6 grm. of the fish-fat. Litter K, consisting of four does, was used, and the results are shown in the curves on chart 2, last two curves. Owing, no doubt, to the liberal food-supply they received before weaning, they persisted in growing, although at a slow rate, for ten weeks after weaning. Eye symptoms did not occur in any, but from the experience obtained from the other litters it can be said that if the diet contained sufficient vitamin A the weights would have increased rapidly. Two of the does were given a mixture of equal parts of fish diet and diet 8, so that their dose of oil was the same as in the case of those rats that received the fish-powder in addition to diet 8—viz., 0·3 grm. As shown on the chart, this did not cause any distinct difference, and we may assume in this case also that 0·3 grm. does not contain appreciable quantities of vitamin A. Another litter, M, was fed partly on diet 8 + cod-liver oil, partly on full-strength fish diet, and some on diet 8 alone. The growth in the first two of these groups was maximal, but, unfortunately, some of the controls showed that the litter had, like K, been too well fed before weaning, or weaned too late, for they showed considerable powers of growth. Thus the average gain per rat for three weeks was 41 grm. on cod-liver oil, 46 grm. on the fish diet, and 23 grm. on diet 8 alone. These results are in marked contrast to the cases where ethereal extract was used.

Oyster-oil.

This was prepared as follows: Twenty-five Stewart Island oysters were strained, minced, and extracted several times with strong alcohol at room-temperature. The alcohol was distilled off in a partial vacuum at a temperature under 50° C. The residue, as well as the residue of oyster-flesh, was extracted by shaking up with ether several times and drawing off the ethereal extract. This was then washed with boiled water in a separating-funnel, and the oil was obtained by allowing the ether to evaporate. In

all these manipulations care was taken to avoid oxidation, but it was not possible to use elaborate precautions.

The oil was administered to two rats, B_2 and B_3, that had developed symptoms of lack of vitamin A. Unfortunately, they were first used for a class demonstration to show that cod-liver oil was curative, and distinct improvement had set in before the oyster-oil treatment was begun. On continuing with oyster-oil, given into the mouth as a smear on the end of a glass rod because it was too viscid for the pipette, their condition became much worse. Their eyes had recovered, but their coats were staring, the skin around their genitals was wet and discoloured, the buck developed a painful condition of the penis, and altogether they were so miserable that mutton-bird oil was substituted for the oyster-oil. In a few days there was a marked improvement in their whole appearance, and the weights rose steadily.

Another attempt to use this oil was made on E_3 (chart 2). She had shown a marked growth and recovery from avitaminosis under the influence of cod-liver oil, but about the 26th December, six weeks after the oil had been stopped, her weight began to fall and slight eye symptoms came on. From the 31st December to the 6th January she was given a daily dose of 0·06 grm. of the oyster-oil, diluted, for easy administration, with olive-oil. In spite of this her weight continued to decrease and the eyes became deeply affected.

These experiments, therefore, give no evidence of vitamin A in ethereal extract of oysters.

Red Cod (Lotella bacchus).

A few observations were made on the flesh-oil and liver-oil of the red cod, extracted in each case with ether in a Soxhlet apparatus. The oils were administered to rats that had previously been the subjects of other experiments and were in very poor condition (see curve of E_4, chart 2, R.C.L.O., on 28th December). No amelioration of the symptoms resulted, and although the conditions of experiment were such that one can hardly speak dogmatically, yet the impression received was that here also ethereal extracts were useless, if not actually harmful.

Conclusions.

1. Mutton-bird oil is one of the richest known natural sources of vitamin A. Daily doses of 0·04 grm. contain sufficient of it for the normal growth of rats; in some 0·02 grm., and in still others 0·01 grm., was found to cure xerophthalmia and cause some growth.

2. The vitamin A of mutton-bird oil does not appear to be stored to the same extent as that of cod-liver oil or butter, and vitamin B appears to be absent.

3. In the New Zealand fish examined (tarakihi) the fat of the flesh was not particularly rich in vitamin A—thus at least 0·3 grm. fat was necessary for maintenance or growth. It is suggested that there are probably considerable differences in the vitamin content of the fat of fish-flesh, depending on the food of the fish.

4. The ethereal extracts of tarakihi flesh, oysters, red cod (flesh and liver) were not found to contain vitamin A, and when given to animals suffering from avitaminosis seemed to aggravate the condition.

I beg to acknowledge with thanks the financial aid of a research grant from the New Zealand Institute.