SOUTH AFRICAN FISH OILS AS SOURCES OF VITAMINS A AND D.

THESIS

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by

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INTRODUCTION.

Fish oils are one of the most important sources of vitamins A and D. The medicinal value of cod liver oil, especially in the treatment of rickets, has been recognized many centuries, but it is really only within the last twenty-five years, since the discovery of the vitamins, that its use has been put on a scientific basis. To-day, although cod liver oil is still largely used, its place has been taken to some extent by other fish oils, such as halibut liver oil, which have a much higher vitamin content and require a correspondingly smaller dose, or by the more palatable vitamin concentrates prepared from the oil. The discovery of the relationship of vitamin D to ultra-violet light and the role it plays in the formation of the bone and in the prevention of rickets has made the use of vitamin D potent fish oils, artificial vitamin D and ultra-violet therapy an established practice in most parts of the world. In the same way, the appreciation of the importance of an adequate intake of vitamin A in the prevention of xerophthalmia and other conditions has resulted in a widespread use of vitamin A supplements in Europe and America. Fish oils containing vitamins A and D are also extensively used in animal feeding, especially by poultry farmers, for not only is vitamin D necessary to prevent 'leg weakness' in chicks, but the addition of the vitamins also results in increased egg production, improved hatchibility of the eggs and better shells. Some idea of the extent of the peacetime use of vitamins A and D can be gained from the fact that it has been estimated (119) that in 1939 the United States used 2.8×10^{16} I.U. of vitamin A for medicinal purposes and 3.65×10^{15} for poultry feeding.

The war has greatly increased the demand for vitamins A and D, adequate supplies of vitamin A being especially important in the prevention of night blindness. This condition is one of the first symptoms of a deficiency of the vitamin and presents an obvious danger during a 'black out'. In the last war both rickets and xerophthalmia were common in Europe. In Austria and Germany, where the diet in 1918 was particularly deficient in fat, infantile rickets assumed the proportions of an epidemic and juvenile rickets and osteomalacia were also common. Xerophthalmia, on the other hand, occurred chiefly in Denmark as a result of the extensive exportation of dairy produce to Germany and numerous cases were reported in Copenhagen. In the present struggle efforts have been made to compensate for the inevitable shortage of eggs and dairy produce by supplying the necessary vitamins from some other source. In Britain the fortification of margarine with vitamins A and D was made compulsory early in 1940, and as a further measure the Government has undertaken the free distribution of cod liver oil for all children.

Unfortunately the increased demand for vitamins A and D has found the Allied Nations cut off from most of their pre-war sources of supply of fish liver oils. The Norwegian output was lost when Norway was occupied by the enemy and British manufacture has practically ceased owing to the commandeering of the trawlers for mine-sweeping purposes. Japan's entry into the war has meant a still further reduction of supplies as she exported considerable quantities of high potency fish liver and fish liver oil to America. The seriousness of the position has been realised. Canada and the United States, which are now the chief sources of supply, are making every effort to increase their production of fish oils by the fullest utilisation of their resources and many types of liver, which had hitherto been discarded, are being processed. Other countries, like India and South Africa, which had previously done nothing in this field, are also playing their part and new industries are springing up.

The demand is principally for vitamin A. Although several workers have claimed to have synthesized this vitamin in the laboratory, it has not yet been possible to do so on an industrial scale and we are thus compelled to rely entirely on natural sources. In recent years whale liver oil has been used quite extensively as a source of vitamin A, especially in the fortification of margarines. The war, however, has stopped practically all whaling operations, so that no more vitamin A can be expected from this quarter. It has been suggested that carotene, either in the form of red palm oil (119) or as a concentrate prepared from carrots or alfalfa (11) might be utilized in the present emergency. Up to now, however, nothing has been done in this direction. The responsibility of supplying the whole of the Allied Nations' vitamin A requirements falls thus on the fishing industries. Fortunately the position with regard to vitamin D is much more satisfactory as fish oils form only one of several sources of supply. The antirachitic value of foods may be enhanced without the addition of vitamin D as such. For example, vitamin D milks, which are extensively used in America, may be prepared by irradiating the milk or by feeding irradiated yeast to the cow, as well as by the direct addition of vitamin D concentrates to the milk. Synthetic vitamin D - calciferol - was the first vitamin to be produced commercially and its manufacture on a large scale was well established in England and America long before the outbreak of the war. If necessary, enough calciferol could doubtless be produced to supply all the vitamin D needed for medicinal purposes. In America a beginning has also been made on the commercial production of vitamin D_3 by the extraction and irradiation of 7-dehydrocholesterol from whelks and periwinkles. No figures for the progress which has been made in this direction are available. Vitamin D from this source, however, would be in great demand for addition to poultry feed oils. The war has altered the relative demands for vitamins A and D. In 1939 livers of high vitamin D potency, especially those with a high chick efficiency, were the most sought after fishery material. To-day the demand is for vitamin A and livers like that of the soupfin shark, which are rich sources of the vitamin, command fabulous prices.

In view of the present demands for vitamins A and D, an examination of South Africa's national resources and her nutritional needs is of considerable interest. Since 1937 there has been a steadily growing awareness of the extent and seriousness of malnutrition in this country. The preliminary surveys carried out by the Union Health Department (45) (135) (161) have shown that the incidence of malnutrition amongst the schoolchildren, both European and Bantu, is very high. At least 40% of the children examined were below the standards for normal health and nutrition; this figure is probably an underestimation rather than an overestimation of the seriousness of the situation. Up to now the investigations have been of a general nature and very little information is available with regard to specific deficiency diseases. Work on this aspect of the subject has recently started and is still in active progress. There is no doubt, however, that the dietary of a large proportion of the population does not contain enough protective foodstuffs, and milk and milk products are particularly lacking. Radloff and Osborne (213), in a discussion of some South African diets, have shown that lack of vitamin A, vitamin D and calcium are amongst the most conspicuous faults of the diet of all but the wealthiest section of the population.

It might be thought that the abundant sunshine in South Africa would compensate for any deficiency of vitamin D in the diet and that rickets would be rarely seen. Actual experience has shown that this is not the case. In Cape Town mild rickets is not uncommon in the European and non-European children attending the municipal welfare centres; florid cases are seen more occasionally and a fair number of these are in native children (123). Cases of healed and healing rickets have also been reported amongst the Bantu school-children in Pietermaritzburg (136). Lack of calcium in the diet and an unfavourable Ca:P ratio are probably the chief causes of the disease. It is not likely that rickets among the natives is due to any inability to utilize the ultra-violet light on account of the pigmentation of their skin. One theory held to-day is that activation of the provitamin takes place on the surface and that the products of irradiation are absorbed through the skin (113). If this is the case, the natives should derive as much benefit from the sunshine as do the Europeans and any greater incidence of rickets amongst them is due to their lower economic status.

It is more difficult to form an estimate of the extent of vitamin A deficiency in this country. Neither Brock and Latsky (31) nor Kark and Le Riche (135) found any cases of keratomalacia or Bitot spots amongst the children they examined, but both groups of workers reported a considerable number of cases of skin changes which might be due to vitamin A deficiency. For example, the latter authors found that the incidence of the more advanced signs of Phynoderma (follicular hyperkeratosis presumably due to vitamin A deficiency) varied from 1% to 29% according to the district. Thus, although final proof is still lacking, it seems fairly certain that a considerable percentage of the population suffers from a deficiency of vitamin A.

While malnutrition in South Africa has its roots in the present economic system and any relief measures such as the distribution of vitamin supplements to school-children can only be of the nature of a temporary palliative, nevertheless the importance of adequate production of vitamins A and D in the form of fish oils can be appreciated, especially as it has been pointed out (106) that the country is at present unable to produce enough protective foodstuffs to supply the whole population. Until recently, South Africa had made no attempt to utilize the vitamin reserves of its fish. In 1939, for the first time, fish

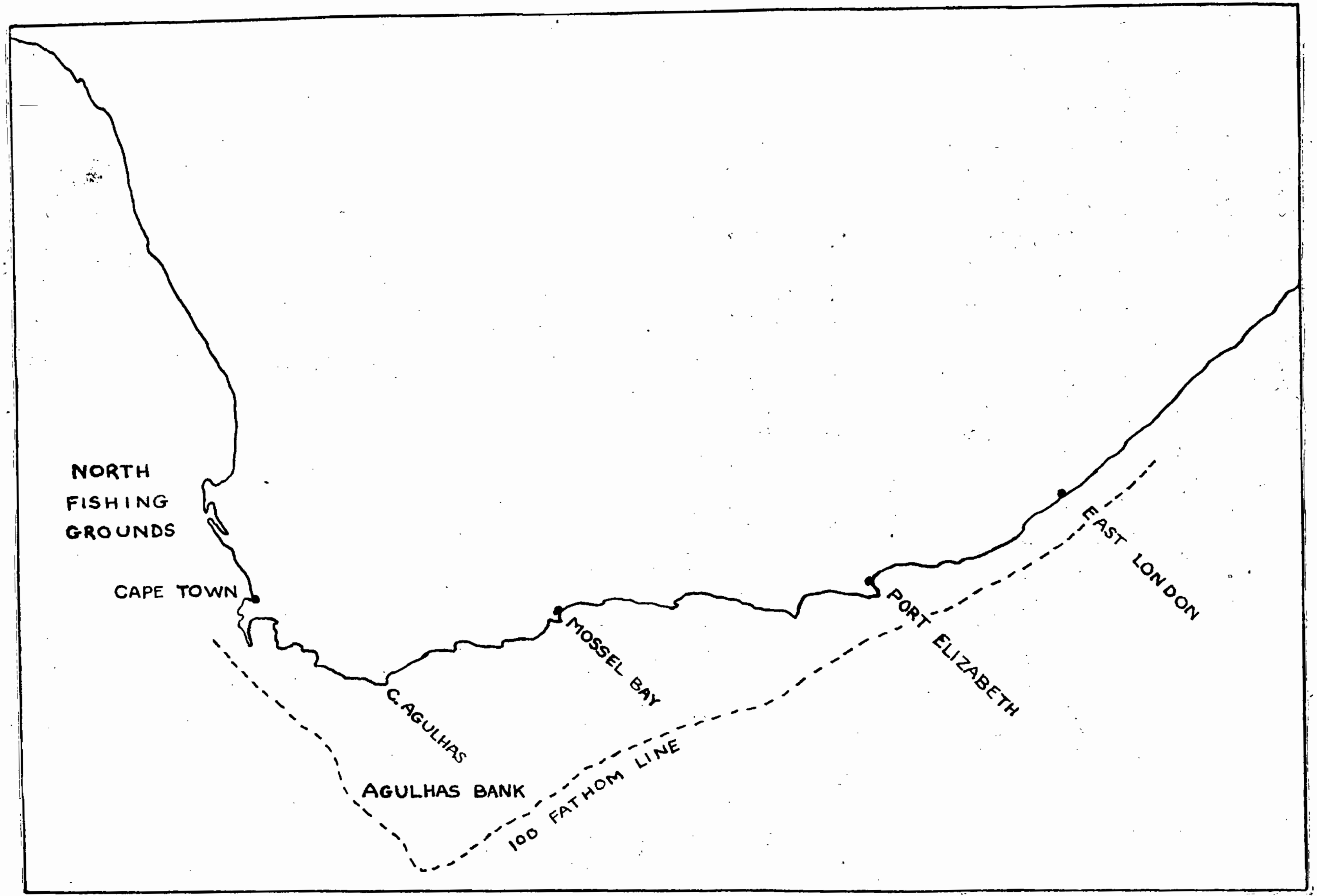


FIG. 1. Map of South African coastline showing principal fishing grounds.

livers were brought into port and processed for oil. Since then the industry has grown steadily and in 1941 several thousand gallons of high potency oil were exported to the United States for the manufacture of vitamin concentrates. From Table 1 it can be seen that the raw material available is considerable. Owing to difficulties in the collection of statistics, especially in the case of the inshore fisheries, the actual landings are probably rather greater than indicated by these figures. Moreover, they do not take into account the dogfish and other elasmobranchs taken incidentally in the trawl and previously dumped back into the sea.

TABLE 1.

Landings of some Species of Fish in Cape Town.

Local Name	Quantity	Potential Quantity of Liver	Year
Stockfish	24,096,742 lb.	1,100,000 lb.	1938 (201)
Kingklip	1,182,109 ''	47,000 ''	''
Kabeljou	3,326,591 ''	70,000 ''	''
Geelbek	586,876 ''	14,000 ''	''
Stonebass	41,976 ''	1,000 ''	''
Soles	2,627,605 ''	-	''
Silverfish	489,383 ''	4,900 ''	''
Yellowtail Ø	5,028 ''	56 ''	''
Snoek Ø	4,012,240 ''	60,000 ''	''
	8,510,000 ''	128,000 ''	1939 (202)
Maasbanker Ø	1,009,000 ''	25,000 ''	''

Ø Landed at Cape Town: Figures for other stations not available.

Except for the figures which were obtained by Molteno (188) (189) (190) in his preliminary survey of the vitamin A content of South African fish, and on the basis of which the collection and extraction of livers was started, no data were available for the oil content of the livers and viscera of the different species of fish or for the vitamin A and D potency of these oils. The present studies were, therefore, undertaken to provide more information about the seasonal and other variations in the oil content and vitamin potency in the most important commercial species landed at Cape Town. Almost all the work was subordinated to the object of providing essential operating data for the new South African industry. Nevertheless, the results which have been obtained are of considerable general interest.

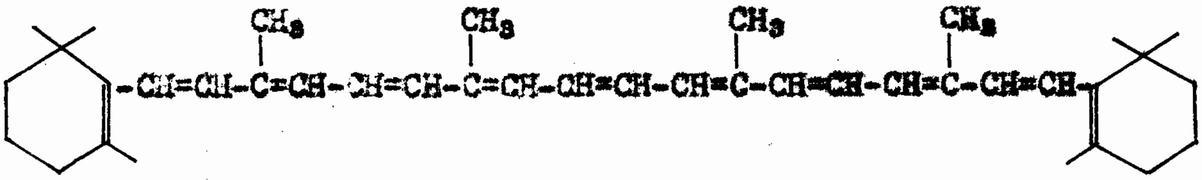
Much work still remains to be done. Studies similar to the present one should be carried out at other points along the coast; Walvis Bay and Durban, in particular, would make interesting stations, both from the point of view of studying the influence of the temperature of the water and the abundance of the food supply on the vitamin reserves of the fish and of following the annual migrations along the coast of fish such as the Snoek and the Yellowtail. To undertake work of this nature successfully, the present Division of Fisheries should be expanded to include a Fisheries Research Board such as exists in Canada and other parts of the world. This could then co-operate with the universities and the fishing industry to carry out a comprehensive programme of research on problems both of industrial importance and of academic interest.
The generosity of the various trawling companies in Cape Town and their willingness to help, which was experienced in the present undertaking, holds out the hope that this might be achieved in the near future.

THE CHEMISTRY AND PHYSIOLOGY OF VITAMINS A AND D.

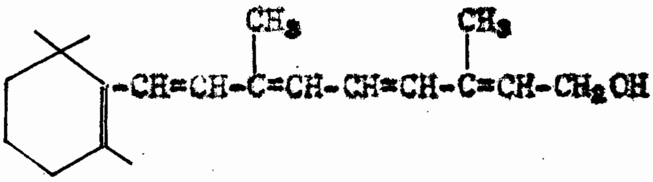
Vitamin A.

It is difficult to discuss the chemistry and physiology of vitamin A without dealing also to some extent with that of the carotenoids. This is inevitable because of the close relationship, chemical and physiological, between the vitamin and its precursors. The development of our ideas on the relation between vitamin A and the carotenoids has been traced by many writers (184) (176) and it will not be dealt with in detail here. As early as 1919, Steenbock (237) had observed that there was a parallelism between the occurrence of carotene and xanthophyll in plants and their vitamin A activity. His findings, however, were not confirmed until 1928 when von Euler and his associates (75) (76) (74) demonstrated the vitamin A activity of crystalline carotene. The connection between the orange-red plant pigment and the almost colourless vitamin present in fish oils was made clearer in the following year by Moore (191) (192) who showed that carotene acts as a pro-vitamin and is converted into vitamin A in the animal body. Finally the chemical basis of this relationship was established when Karrer and his co-workers determined the structure of both carotene and vitamin A.

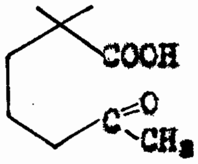
The constitution of both carotene and vitamin A rests chiefly on their behaviour on oxidative degradation. The structure of β-carotene was first established by Karrer (138) (139) in 1930 and has since been confirmed by many workers. The evidence on which it is based has been reviewed by Farmer (81) and Spring (236) and will not be dealt with here. In the following year, Karrer and his associates (140) prepared a very rich vitamin A concentrate from mackerel liver oil. They showed that the vitamin was an alcohol $C_{20}H_{29}OH$ or, less probably, $C_{22}H_{31}OH$. Like carotene, it gave geronic acid (III) on ozonisation. It also resembled carotene in its behaviour on oxidation with potassium permanganate and chromic acid. Karrer therefore proposed the structure II for vitamin A.



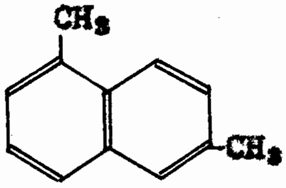
I β-Carotene.



II Vitamin A.



III Geronic acid.



IV 1,6 dimethylnaphthalene.

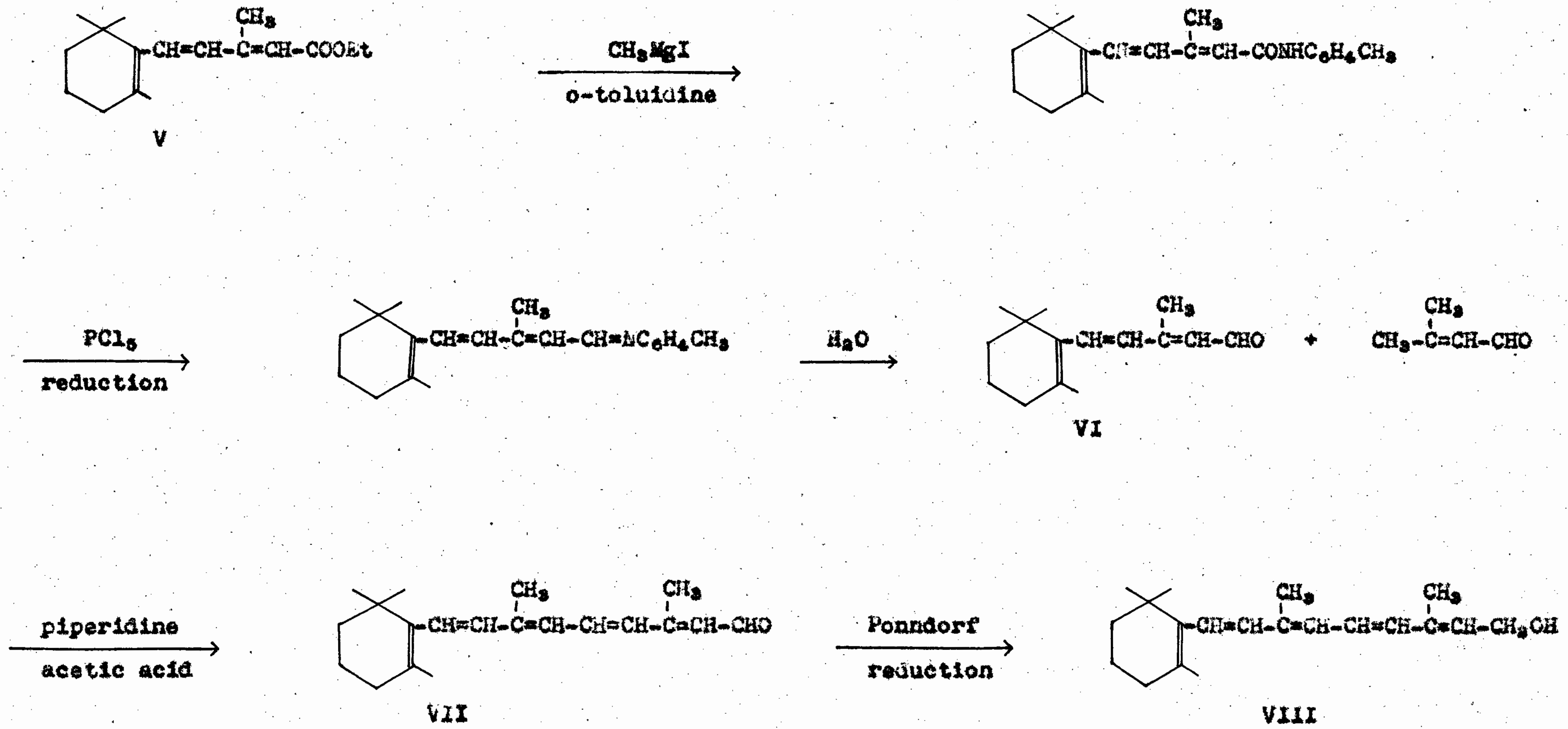
Partial confirmation of this formula was obtained by Heilbron, Morton and Webster (111)(108) who subjected a rich halibut liver oil concentrate to selenium dehydrogenation. 1,6 dimethyl naphthalene (IV) was obtained in good yield, indicating that the concentrate contained a substance whose constitution as far as the 14th carbon atom must be identical with that proposed by Karrer. Karrer himself completed the proof by the synthesis of perhydrovitamin A which he showed was identical with the product obtained by the complete hydrogenation of the natural vitamin (141).

The vitamin A concentrates used by Karrer and Heilbron in their investigations on the structure of vitamin A were clear yellow oils. Neither succeeded in isolating the crystalline vitamin. Although subsequent work has shown that the assumption that these preparations were fairly pure samples of vitamin A was justified, nevertheless, the failure to prepare crystalline derivatives rendered any claim to have isolated the vitamin in a state of purity somewhat insecure. This difficulty was also experienced in the subsequent synthetic studies and both the identity and the purity of the various products obtained has been based chiefly on the study of their absorption spectra. The first crystalline derivatives of vitamin A to be prepared were the β-naphthoate and anthraquinone-2-carboxylate (99) (183). The former melted at 76°C while the latter was obtained in two forms, yellow crystals M.P. 124°C and red crystals M.P. 118°C. The elementary analysis of these esters and their molecular weight were in agreement with the formula proposed for vitamin A by Karrer. Crystalline vitamin A was first obtained in 1937 by Holmes and Corbet (124) by recrystallization of the unsaponifiable matter of fish liver oil from methyl alcohol at low temperatures. The crystals melted at 7.5 - 8°C. This was much lower than was to be expected on the basis of Karrer's formula and it was suggested that the crystals consisted of a mixture of geometric isomers (12). Recently, however, Baxter and Robeson (13) have shown that these crystals contained solvent of crystallization. By using ethyl formate instead of methyl alcohol as a solvent, these workers have prepared crystalline vitamin A melting at 63-64°C. The same authors have also prepared a number of crystalline esters of vitamin A, including the acetate, the palmitate and the divitamin A succinate. This work should prove of considerable importance both as providing criteria of purity for natural and synthetic ~~pre~~preparations of the vitamin and also as offering an opportunity of replacing the present international standard of β-carotene by a crystalline preparation of vitamin A or one of its esters.

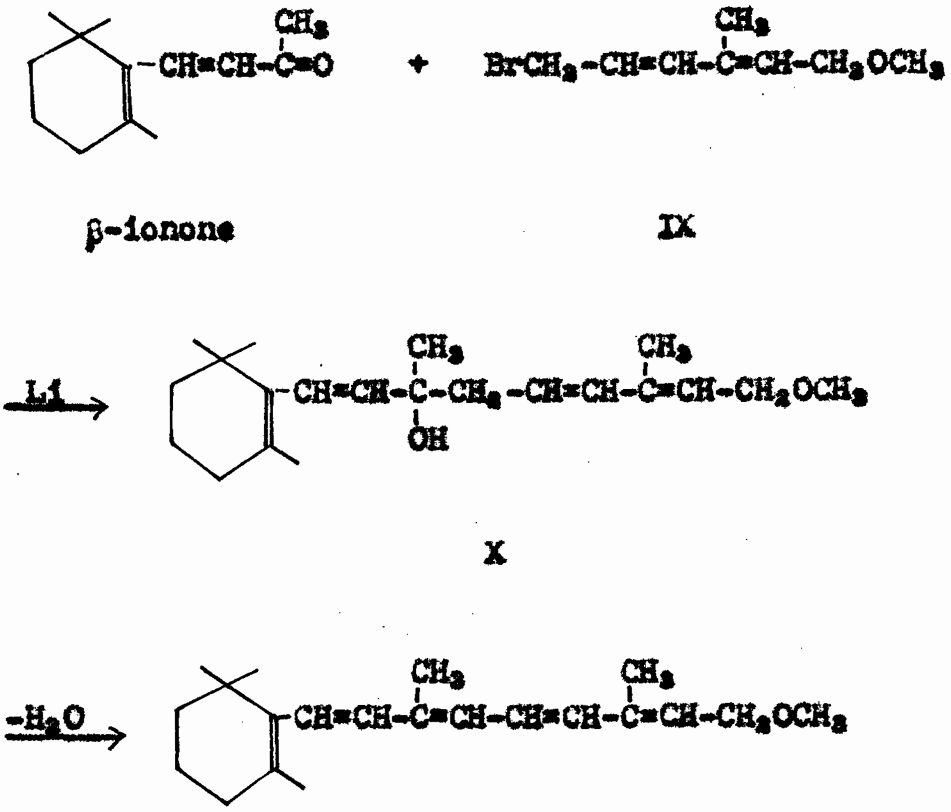
Following the publication of Karrer's structure in 1931, many attempts have been made to synthesize vitamin A. These have been reviewed recently by Jones (131) and only the two most important claims to have synthesized the vitamin will be discussed here. In 1937 Kuhn and Morris (158) claimed to have synthesized vitamin A by the method outlined below. The starting point of this synthesis was β-ionylidene acetic ester (V). By reduction of the imido chloride by the chromous chloride method of von Braun, the key substance β-ionylidene acetaldehyde (VI) was obtained. This was then condensed with β-methylcrotonaldehyde in the presence of a neutral peperidine-acetic acid catalyst to give the C_{20} aldehyde (VII). On reduction with aluminium *iso*-propoxide, vitamin A alcohol (VIII) was obtained. The final product, after purification by chromatographic analysis, contained 7.5% of vitamin A (colour test). When tested biologically on this basis, it gave a similar growth response to the natural vitamin. This work has recently been subject to criticism. Karrer and Rüegger (143) experienced difficulty in repeating Kuhn's synthesis. These authors state that the method yields a mixture of polyenes and that, moreover, the main product differs from vitamin A chromatographically and also in the location of the absorption maximum of the antimony trichloride coloration. Other workers (149) have also reported that Kuhn's synthesis gives biologically inactive products.

Synthesis of the methyl ether of vitamin A has been claimed by Kipping and Wild (147). Details of the method and of the biological activity of the product have not been published. The synthesis, however, is a novel one and deserves a brief consideration. Instead of the usual stepwise lengthening of the side chain of β-ionone or some similar substance, these workers built up the side chain first and attached it to the ionone residue en bloc. The essential step of the synthesis consisted of the condensation of the bromo ether (IX) with β-ionone using lithium as a condensing

Synthesis of Vitamin A (Kuhn and Morris)



agent. Dehydration of the resulting tertiary carbinol (X) yielded the methyl ether of vitamin A.



β-ionone

IX

X

XI methyl ether of vitamin A.

Spectrograhpic Studies of Vitamin A.

Spectrographic studies, both of the ultra-violet absorption spectrum and of the colour produced with antimony trichloride, have played an important role in the investigation of vitamin A and related substances. In the synthetic studies in this series, the identity and purity of the various products has been based chiefly on the study of their absorption spectra. In addition, the physical and chemical tests have provided a rapid and convenient method of estimating the vitamin and so have done much to advance our knowledge of its distribution in natural products.

With a solution of antimony trichloride in chloroform, vitamin A gives a transient blue colour. This is the basis of the well-known Carr-Price test (43). The reaction, however, is not peculiar to vitamin A, but is given by all carotenoids although in different degrees. Its chemistry is still obscure.

When the colour is examined spectroscopically, it is found, in the case of vitamin A concentrates, to be due to two bands, one with a maximum at about 620mu and the other at about 583 mu (107) (108). In low potency oils, these maxima are usually displaced to 603-606 mu and 572 mu respectively. In these oils also the presence of natural inhibitors frequently prevents the development of the maximum at 603 mu; the 572 mu band, on the other hand, is usually unaffected. On saponification of the oil, the inhibitors are removed and the full colour is able to develop. For this reason it is preferable to carry out the estimation of the vitamin by the colour test on the unsaponifiable matter.

In addition to the main bands at 620 and 583 mu, maxima at 635, 646, 656, 680 and 690-695 mu have been recorded (107). Of these the 693 mu band is the most important. In the case of oils from fresh water fish this band predominates and the maximum at 620 mu is absent or present merely as an inflection (89) (70 (88). The '693 mu chromogen' is now recognized as a second factor - vitamin A_2. In addition to the maximum at 693 mu, it also shows a second maximum at 660 mu. In concentrates rich in vitamin A_2 a considerable overlapping of the 650 and 620 mu bands occurs (160). It is thus difficult to determine the relative proportions of the two vitamins present.

In the ultra-violet vitamin A exhibits a broad absorption band with a maximum at 328 mu. In oils or concentrates rich in vitamin A_2, this band is replaced by maxima near 350 and 280 mu (71). The 328 mu band had been observed in 'biosterin' - a crude vitamin A concentrate - by Takahashi in 1925 (241). It was first investigated by Morton and Heilbron and their collaborators (196) (66) (50) who showed very clearly that it was a property of vitamin A. As a result of their work, the spectrographic method of estimating vitamin A was adopted in 1935 by the Medical Research Council who recommended the use of the provisional factor 1600 for converting

$E^{1\%}_{1cm.}$ 328 mu into I.U. per gm. (126) (127). This factor has been criticized by many workers (8) (51) (217), and in America, in particular, the factor 2000 is commonly employed instead. There is a grave suspicion, however, that the high values obtained in that country are due to a deterioration of the U.S.P. reference cod liver oil (68). It has been claimed also that the conversion factor is greater in the case of natural oils than in the concentrates prepared from them by saponification. This is apparently due to the higher biological efficiency of the vitamin A esters present in the former (91) (120) (187). Investigations with crystalline vitamin A alcohol and its esters, however, do not confirm these results (183) (13).

The extinction coefficient of pure vitamin A is probably in the neighbourhood of 1800. The highest figure recorded by Holmes and Corbet (124) was 2100. Mead, Underhill and Coward (183) and Baxter and Robeson (13), on the other hand, have reported the values 1800 and 1780 respectively. These are probably nearer the correct figure. For calculating the vitamin A content of fish oils, etc., however, it is conventional to use the value $E^{1\%}_{1cm.}$ 328 mu = 1600 which was obtained by Carr and Jewell (42) in 1933 for their richest vitamin A concentrate.

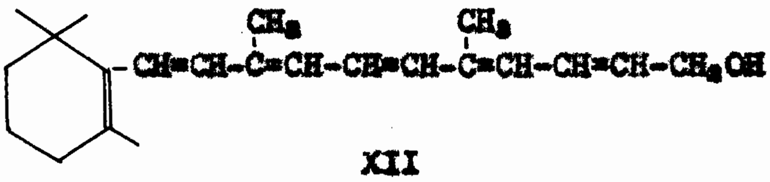
In addition to vitamins A_1 and A_2, other chromogens have been observed in fish and mammalian liver oils. These include 'cyclized vitamin A' and the '290 mu chromogen' of whale oil concentrates. 'Cyclized vitamin A' has been prepared by the action of alcoholic hydrogen chloride on vitamin A or by the action of heat in the presence of acid. Its ultra-violet absorption spectrum is characterized by a number of bands at 392, 369 and 350 mu. With antimony trichloride it gives a blue colour with a maximum at 620 mu which is practically identical with that of ordinary vitamin A (69). Cyclized vitamin A is also reported to occur naturally in fish liver oils and is found in the lower boiling fractions obtained by molecular distillation. It is stated to be biologically inactive (72).

In mammalian liver oils, and in whale liver oil concentrates in particular, the maximum at 325 mu is frequently absent and a band at 290-310 mu is observed instead (97). This is due to the presence of a chromogen which exhibits an absorption maximum at 285-290 mu and which gives with antimony trichloride a blue colour with bands at 594 and 496 mu instead of at 620 and 583 mu (208). The '290 mu chromogen', as it has been called, is also seen occasionally in minor quantities in fish oils (66). It is insoluble in 83% ethyl alcohol and may be separated from vitamin A by exhaustive extraction with that solvent. It possesses definite biological activity which, however, is lower then that of vitamin A. It has been suggested that it may represent a third factor, vitamin A_3, which contains one double bond less than vitamin A. Its insolubility in 83% alcohol and the fact that, unlike vitamins A_1 and A_2, it is not affected by alcoholic hydrogen chloride, makes it doubtful, however, whether it is the C_{18} analogue of vitamin A (220).

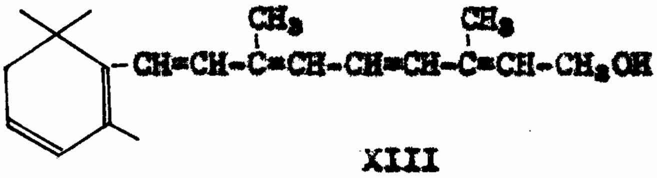
Vitamin A_2.

The recognition of a second factor, vitamin A_2, in fish oils has been based largely on spectrographic studies of the antimony trichloride reaction. Vitamin A_2 is found in largest amounts in the tissue of fresh water fish. It has also been observed in the liver and visceral oils of marine fish. In mammals and birds itis absent, except where, as in the case of sea birds, vitamin A_2 is present in the diet. A very interesting study of the distribution of vitamins A_1 and A_2 has been made by Wald (248) who suggests that in fish the ratio $A_1:A_2$ is determined genetically and that the 'transfer from vitamin A_1 to A_2 metabolism appears to be associated phytogenically with the migration of marine teleosts into fresh water'. It has not been possible as yet to isolate pure vitamin A_2. For this reason its chemical constitution and its biological activity are still uncertain. Feeding tests suggest that vitamin A_2 contributes to the total vitamin A activity of concentrates in which it occurs. (88). Up to the present, however, there is no evidence that A_2, although it may have a biological value equal to A_1, plays any significant role in mammalian nutrition. On the other hand, Wald's discovery (250) that vitamin A_2 replaces the vitamin A of the visual purple system in the eyes of fresh water fish suggests that here, at any rate, it may have the same functions as the 'classical' vitamin A.

The fact that vitamin A_2 cannot be separated from vitamin A_1 by chromatographic analysis or by molecular distillation indicates that it is very closely related to the latter. From its spectrographic behaviour it would appear to contain one extra conjugated double bond. The two most likely formulae for vitamin A_2 therefore are:

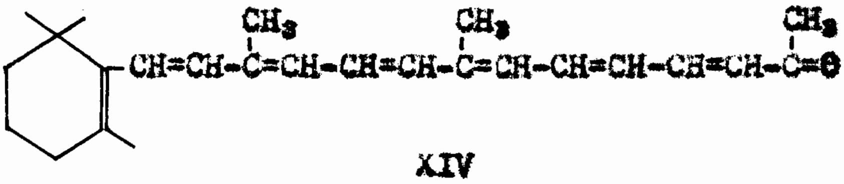


XII



XIII

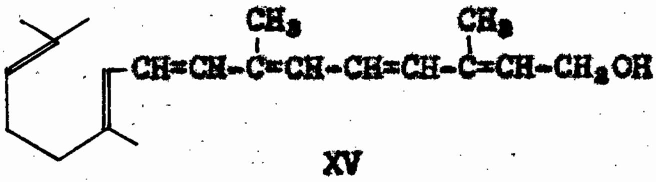
On ozonolysis XII should yield geronic acid while XIII should give α,α-dimethyl succinnic acid. On ozonolysis of a concentrate from fresh water fish oils Heilbron and his collaboraters (88) obtained practically the same yield of geronic acid as from an equivalent quantity of halibut liver oil concentrates. No α,α-dimethyl succinnic acid could be detected in the ozonolysis product. These workers, therefore, concluded that vitamin A_2 must have the formula XII. This was partially confirmed by treating the concentrate with aluminium *tert*.-butoxide and acetone. As in the case of vitamin A_1, a ketone was obtained which readily yielded a crystalline p-chlorobenzoylphenylhydrazone. The spectrographic behaviour and the elementary analysis of the latter were in agreement with the assumption that the ketone had the structure XIV, from which it followed that the original alcohol must have had the formula XII.



XIV

On the other hand, Gray (93) (94) has studied the elimination curves of vitamins A_1 and A_2. The elimination maximum is only 3°C higher than that of vitamin A_1. From analogy with the distillation curves of certain fatty acids he concludes that vitamin A_2 has the same number of carbon atoms as A_1, but that it contains one more conjugated double bond, i.e. he favours formula XIII.

Karrer has also criticized formula XII. Using his nomenclature, formula XII represents β-*apo*-5-carotenol (c.f. p. 22). From the known absorption spectra of vitamin A_1 and of β-*apo*-2- and β-*apo*-4-carotenols, the position of the absorption maximum in β-*apo*-5-carotenol may be calculated. This should be at 356-358 mu (145). The absorption band of vitamin A_2, however, is rather lower (350 mu). Karrer, therefore, considers it is questionable whether the latter really is the C_{22} homologue of vitamin A. Karrer has also repeated the ozonolysis of a vitamin A_2 concentrate (137). He claims to have obtained acetone in 50-70% yield; the geronic acid obtained in Heilbron's experiments he believes to have been derived solely from the vitamin A_1 present. He considers therefore that vitamin A_2 has the structure XV, i.e. it bears the same relationship to vitamin A_1 as lycopene to β-carotene.



XV

To confirm these results, an attempt was made to synthesize a substance of this formula by condensing *pseudo*-ionylidene acetaldehyde with β-methyl crotonaldehyde in analogy with Kuhn's synthesis of vitamin A. The product obtained, however, was non-homogeneous and there was no fraction which gave the absorption maxima typical of vitamin A_2 in the Carr-Price reaction.

This important piece of work requires confirmation. The formula proposed by Karrer would be expected to show an absorption band in the ultra-violet with a maximum at 345 mu, which is very close to that actually observed. This formula would also account for the similarity in the boiling points of vitamins A_1 and A_2. On the other hand, a compound of this structure should be biologically inactive. According to Karrer, the biological activity of his concentrate was fully accounted for by the vitamin A_1 present. This, however, was not the experience of Heilbron and his collaborators Neither does it appear probable that all the geronic acid obtained by these workers on ozonolysis of their concentrate was due to vitamin A_1. Further work on this problem would, therefore, be desirable.

Structure and Vitamin A Activity - The Provitamins.

The chemical basis of the relationship between vitamin A and its precursors was first indicated by Karrer when he established the constitution of both β-carotene and vitamin A. In the same year, Kuhn (151) (157) showed that specimens of β-carotene from certain sources contain another form of carotene which he called α-carotene. Shortly after this, a third isomer, γ-carotene, was isolated (153). The elucidation of the structure of these new compounds soon followed. The relationship of α- to β-carotene was shown by its behaviour on ozonization, when both geronic acid and isogeronic acid were obtained (142), indicating the presence of one β-ionone ring and one ring of the α-ionone type (XVI). γ-Carotene (XVII) was also found to contain one β-ionone ring while the other end of the molecule possessed the open chain structure of lycopene. Like β-carotene, α- and γ-carotene were both found to possess growth-promoting properties. Quantitatively, however, their activity was only half that of β-carotene (129) (155). The importance of the β-ionone ring in the molecule as a necessary prerequisite for vitamin A activity was thus established. Substitution of the β-ionone ring in any way destroys the activity. Thus zeaxanthin, which is a dihydroxy-β-carotene, is inactive. On the other hand, cryptoxanthin (XVIII), which is a monohydroxy-β-carotene and contains one unsubstituted β-ionone ring, acts as a provitamin A; its biological activity, however, is of the same order as that of α- and γ-carotene, i.e. only half that of β-carotene (156).

α-, β- and γ-Carotene and cryptoxanthin are the most important naturally occurring provitamins. In addition to these, five other biologically active carotenoids have been isolated. These are echinenone, the pigment from the sexual glands of the sea urchin, leprotene, myxoxanthin, and the two latest additions to the list of provitamins, aphanin and aphanicin. For an account of the isolation and structure of these compounds, reference may be made to the reviews of Morton (194) and Rosenberg (220). As their constitution is based largely on the fact that they are biologically active, they will not be considered further here.

The work of Kuhn and Karrer and their respective collaborators on the stepwise degradation of α- and B-carotene has also provided much information concerning the relation between structure and vitamin A activity. By mild

Some Provitamins A

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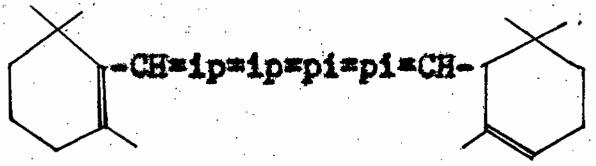
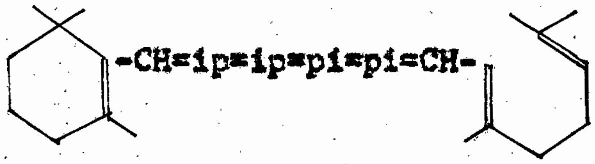
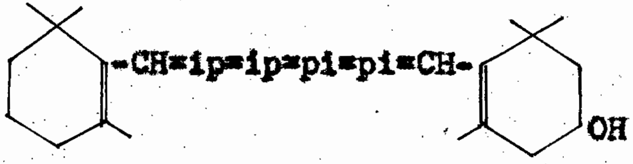


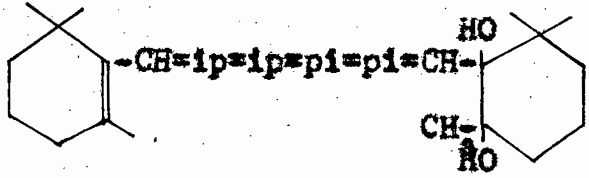
XVI α-carotene



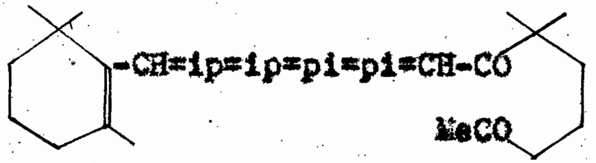
XVII γ-carotene



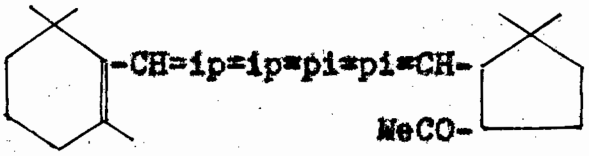
XVIII cryptoxanthin



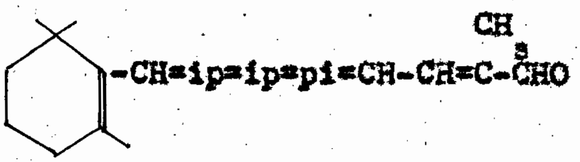
XIX dihydroxy-β-carotene



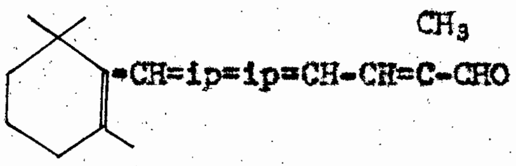
XX semi-β-carotenone



XXI anhydrosemi-β-carotenone



XXII β-*apo*-2-carotenal



XXIII β-*apo*-4-carotenal

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* The convenient abbreviation 'ip' for an isoprene unit $=CH-\overset{CH_3}{C}=CH-CH=$ is due to Morton (194).

oxidation of β-carotene with chromic acid, Kuhn and Brockmann (150) (152) (154) obtained first dihydroxy-β-carotene (XIX) and then semi-β-carotenone (XX), both of which were biologically active. On ring closure with alkali, the latter gave anhydrosemi-β-carotenone (XXI) which was still active. All degradation products contain an intact β-ionone ring. When, however, the second β-ionone ring is opened by further oxidation to give β-carotenone, the activity disappears. α-Carotene, under the same conditions, gave a similar series of degradation products; these, however, exhibited no vitamin A activity, indicating that the β-ionone ring had been attacked first.

By the oxidation of β-carotene andother carotenoids with alkaline permanganate under carefully controlled conditions, Karrer and his co-workers have obtained a series of very interesting aldehydes. Oxidation of β-carotene itself gives a mixture of products from which β-apo-2-carotenal (XXII) and β-apo-4-carotenal (XXIII) have been isolated in a pure state (146). As is to be expected from their constitution, these compounds, as also their oximes, act as provitamins A (77). β-apo-3- and β-apo-5-carotenals are also formed in this degradation, but the yields of these products are so small that they have not yet been obtained pure. On the oxidation of α-carotene, the corresponding α-apo-carotenals which are biologically inactive are formed (79); here, as in the case with chromic acid, the β-ionone ring is preferentially attacked.

These studies have very clearly demonstrated that the presence of an unsubstituted β-ionone ring in the molecule is one of the necessary conditions for biological activity. So many examples of this specificity are available that the failure of a carotenoid to promote growth in vitamin A deficient animals is frequently interpreted as evidence of an unmodified β-ionone ring. The system of conjugated unsaturation however, is also important. Hydrogenation of any one of the double bonds destroys the vitamin A activity. Thus the completely hydrogenated products, perhydro-β-carotene and perhydrovitamin A are biologically inactive. So also are β-dihydro-carotene (144) and the synthetic products dihydro- and tetrahydrovitamin A (90) (224). On the other hand, β-carotene diiodide possesses growth-promoting activity; this, however, is due to the fact that β-carotene is regenerated in the animal body (78).

It was assumed by the early workers that the conversion of β-carotene into vitamin A involved the fission of the central double bond according to the equation

$$C_{40}H_{56} + 2H_2O \longrightarrow 2C_{20}H_{29}OH.$$

β-carotene vitamin A alcohol.

The fact that α- and γ-carotene are only half as active as β-carotene was strong evidence in support of this theory. Within recent years, however, difficulties have arisen in connection with the assays of crystalline vitamin A alcohol and its esters. By definition, β-carotene possesses a potency of 1.66 million I.U. per gm. If it were converted quantitatively into vitamin according to the above equation, pure vitamin A alcohol would be expected to have a potency of 1.56 million I.U. per gm. The actual figures obtained by Holmes and Corbet (124) and Mead, Underhill and Coward (183), however, were in the neighbourhood of 3.0-3.3 million I.U. per gm.; more recently still, Baxter and Robeson (13) have prepared a sample of crystalline vitamin A which assayed at 4.3 million I.U. per gm. The superiority of vitamin A as such over carotene might possibly be explained on the basis of incomplete utilization of the carotene by the animals used for the assay. On the other hand, it is more likely that quantitative conversion of the carotene molecule into two molecules of vitamin A does not occur. Morton (194) has thus been led to suggest that carotene may undergo asymmetrical fission with the production of two fragments, one of which is already too small to yield vitamin A:

$$C_{40}H_{56} \longrightarrow C_{20}H_{29}OH + \text{decomposition products.}$$

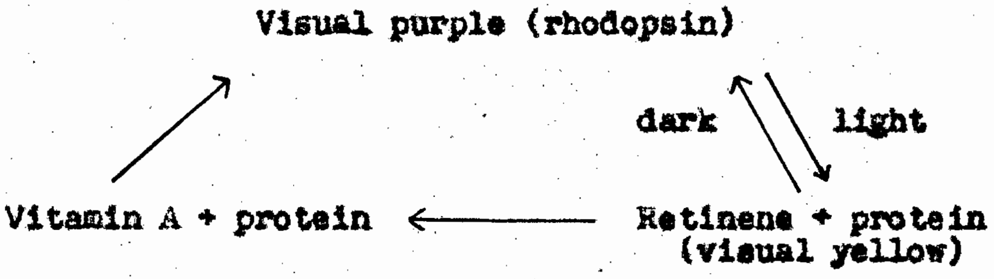
This, however, has been criticized by Hunter (128), who points out, amongst other things, that, since it is the β-ionone ring which is preferentially attacked in the oxidation of α-carotene with alkaline permanganate, α-carotene might well be expected to be biologically inactive on the basis of this mechanism. According to Hunter, 'a more satisfactory picture of the process is that the conversion takes place by fission of the central double bond of the β-carotene molecule, but that intermediate stages are involved in the ultimate production of vitamin A alcohol which lead to the loss of a certain proportion of the carotene in side reactions.'

It is generally assumed that conversion of carotene to vitamin A occurs in the liver. There is, however, very little direct evidence to support this hypothesis. In 1931, Olcott and McCann (203) reported the presence in the liver of an enzyme 'carotenase' which converts carotene into vitamin A. They also claimed to have effected the conversion in vitro by incubating the minced liver tissue with a colloidal solution of carotene. Other workers (1) (65) (73), however, have failed to confirm these results. Thus, although we have a very detailed knowledge of the relationship between chemical constitution and provitamin A activity, we know very little of either the site or the mechanism of the conversion of these provitamins into vitamin A.

The Nutritional Significance of Vitamin A.

A variety of symptoms follows the depletion of vitamin A in the animal organism. These practically all reflect, as Wolbach and Howe (261) (262) (263) have shown, a common effect of lack of vitamin A on the epithelial structures of the organs involved. Atrophy of the epithelium occurs and later substitution of a stratified keratinized epithelium for the normal epithelial structure. Cysts of keratinized epithelial cells accumulate in various parts of the body, especially in the salivary glands, the lungs, the stomach and the intestine. These cysts were regarded as abscesses by the early workers and hence the idea arose that vitamin A had a specific anti-infective action. The fact that animals on a vitamin A deficient diet are very susceptible to respiratory infections (61) (175) and frequently succumb to broncho-pneumonia strengthened this idea, as did the observation that children suffering from xeropthalmia are liable to severe respiratory disease (25). The relationship between vitamin A deficiency and occurrence of infections may be traced to the characteristic metaplasia of the epithelial tissue. The position is admirably summarized in the following statement: 'Under the ordinary conditions of vitamin experiments, the breaking down of the local defences allows access to the comparatively avirulent bacteria which normally inhabit the intestine, the respiratory tract or the conjunctival sac, so that the resulting infections are as a rule avirulent in type. When organisms of a more virulent type are present, the condition of the local defences rather than the presence of these organisms may be the factor which determines the onset of a virulent infection.' (52). There is no indication that animals on a vitamin A deficient diet are less able to produce antibodies than normal animals (102). Neither does the experimental evidence justify the belief that vitamin A therapy is effective clinically in infections caused by specific highly pathogenic organisms (44). Except, therefore, in so far as relates to the maintenance in a healthy condition of what has been called 'the body's first line of defence', vitamin A probably has no specific anti-infective action.

One of the most important manifestations of lack of vitamin A, particularly in experimental animals, is the occurrence of xerophthalmia. This was early recognised as a specific symptom of vitamin A deficiency (177). Xerophthalmia occurs most commonly in young animals. In adults one of the earliest signs of a deficiency of vitamin A is the development of night blindness. The relationship between malnutrition and night blindness in rats was studied by Frederica and Holm (85) and Tansley (242). These workers showed that the regeneration of visual purple after bleaching by a bright light was slower in vitamin A deficient animals than in the controls. In extreme cases no visual purple could be found in the retinas at all. The relation between vitamin A and the visual purple system is of particular interest. This has been investigated extensively by Wald (249) (250) who pictures the visual cycle as follows:



Rhodopsin (visual purple) is a complex carotenoid albumin in which retinene, a yellow pigment related to the carotenes, is the prosthetic group. On exposure to bright light it is bleached to an orange colour (visual yellow). During this process retinene is liberated. This may either be converted back to visual purple or may decompose, giving vitamin A. Part of the vitamin A thus formed may, in its turn, be resynthesized into visual purple; a certain proportion, however, diffuses away into other tissues. Unless the eye continuously receives a supply of vitamin A from the circulation, therefore, the regeneration of the visual purple is incomplete. The visual purple system described above is found in mammals, birds, amphibians and most marine fish. In fresh water fish, the visual pigment, porphyropsin, exhibits an absorption band in the ultra-violet with a maximum at 522-526 mu as compared with 500 mu in the case of rhodopsin. It enters into a visual cycle similar to that of rhodopsin except that vitamin A_2 replaces vitamin A_1 as a component (49). In certain fish, especially those which spend part of their life in fresh water and part in salt water, both rhodopsin and porphyropsin may be found in the retina. The very direct relation between vitamin A and the visual purple system, together with the fact that nightblindness is one of the earliest symptoms of a lack of the vitamin in man, has led to the widespread use of the dark adaptation test in nutritional surveys as an indicator of incipient vitamin A deficiency.

Another manifestation of vitamin A deficiency in man is a dry scaly condition of the skin. This is followed by papular eruptions due to hyperkeratosis of the hair follicles. These lesions usually occur in sexually mature persons between 16 and 30 years of age and not in infants. They have been reported frequently in China (83) (84); they have also been observed in South Africa (135) (171).

Lack of vitamin A further results in abnormal tooth structure and an unhealthy condition of the gums (260). Changes in the central nervous system have also been reported in experimental animals, and it has been suggested by Mellanby (185) that the atropy and metaplasia of the epithelial cells may be secondary to changes in their afferent nerve supply. This aspect of the subject has been discussed by Bessey and Wolbach (15) who advise caution in the acceptance of Mellanby's hypothesis.

In the early days of vitamin research, emphasis was laid on the growth-promoting activity of vitamin A. This property is still the basis of the biological assay of the vitamin. Failure of young animals to gain weight, however, will result from a deficiency of any of the vitamins and is not specific for vitamin A. It has been shown, moreover, by Orr and Richards (204) from measurements of the body length and limb bones that growth in a true sense is not arrested by lack of vitamin A; skeletal growth continues though the animals fail to gain weight. To call vitamin A 'the growth-promoting vitamin' is, therefore a misnomer.

Vitamin D.

There are few more romantic chapters in the history of biochemical research than that which concerns our ideas on the relation of vitamin D to bone formation in young animals and to rickets. The development of the understanding of the parts played by vitamin D and ultra-violet light in the cure of rickets has been traced in a number of excellent reviews (21) (159) (184) (206) and will not be dealt with in detail here.

It had long been known that rickets would respond to either of two apparently unrelated treatments, namely exposure to sunlight and administration of cod liver oil. The connection between the two was made clearer in 1924 when Hess (114) (115) and Steenbock (238) independently and almost simultaneously showed that irradiation of a rickets-producing diet with ultra-violet rays conferred upon it antirachitic properties. Irradiation of the various constituents of the diet in turn proved that it was the unsaponifiable fraction of the fats which became activated. Because of the widespread occurrence of cholesterol in fats, the irradiation of this substance was investigated. It was found to be activatable and for some time it was thought to be the provitamin. It became apparent, however, that, although samples of cholesterol can often be activated even after rather extensive purification, the results are irregular, and in 1926 it was proved simultaneously in three different laboratories (110) (207) (222) that the provitamin was not cholesterol, but a persistent impurity which was later identified by Windaus and Hess (253) and Rosenheim and Webster (221) as ergosterol.

The isolation of the antirachitic irradiation product of ergosterol in a pure form presented unusual difficulties and it was soon recognized that, under the influence of ultra-violet light, the sterol is transformed not into a single substance but into a mixture of isomers. Consequently it was not till 1931 that Windaus in Germany (256) and Bourdillon and his co-workers in England (6) succeeded in obtaining the pure crystalline vitamin. This was called vitamin D_2 in Germany and calciferol in England. The 'vitamin D_1' which was first isolated turned out to be a molecular compound of D_2 and lumisterol, one of the other products of irradiation; the old 'calciferol' was a similar addition product of calciferol proper and pyrocalciferol.

This was still not the end of the trail. Evidence from bio-assays accumulated to show that calciferol was not identical with natural vitamin present in fish oils. The discrepancy led in part to the study of the irradiation products of other sterols with the same ring B structure as ergosterol, and it is now established that the compound obtained by irradiating 7-dehydrocholesterol is identical with one of the natural vitamin D's. From the resumé above it is apparent that there are several substances with vitamin D activity which may be produced by artificial means, and from comparative assays on rats and chicks, there is evidence that fish liver oils contain more than one natural vitamin D. The question of the multiple nature of vitamin D will be discussed in more detail later.

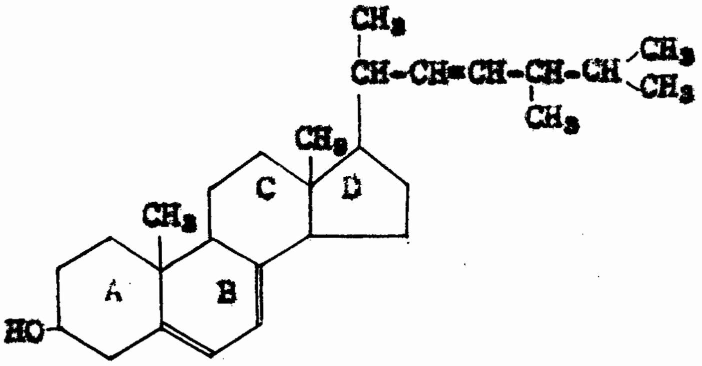
Irradiation products of Ergosterol.

The changes which take place when ergosterol is irradiated may be summarized by the diagram below due largely to Windaus (259) and Setz (227). The irradiation of any substance in the list leads to the formation of a mixture of all the substances below it and there is no indication that any of the changes are reversible. The final products of irradiation, Suprasterol I and suprasterol II, are not altered by further exposure to ultra-violet light and are not interconvertable.

In addition to this succession of products of irradiation, two additional isomers have been obtained by thermal treatment of calciferol: pyrocalciferol and isopyrovitamin.

$$\begin{tikzcd}
 & \text{Ergosterol} \arrow[d] & \\
 & \text{Lumisterol} \arrow[d] & \\
 & \text{Tachysterol} \arrow[d] & \\
 & \text{Calciferol} \arrow[d] & \\
 & \text{Toxisterol (Substance 248)} \arrow[dl] \arrow[dr] & \\
\text{Suprasterol I} & & \text{Suprasterol II.}
\end{tikzcd}$$

All these substances are isomeric with ergosterol and consequently their structures were worked out in a comparatively short time. This aspect of the subject has been reviewed by Fieser (82) and Strain (240) and only the broad outline will be given here.

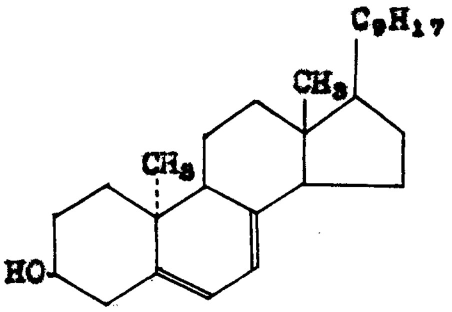


Ergosterol.

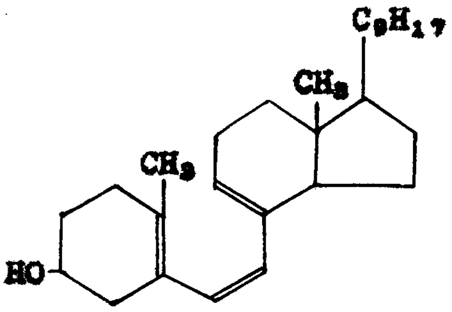
That the double bond in the side chain at C_{22}-C_{23} is not involved in the transformations is shown by the fact that all the isomers yield methyl-isopropylacetaldehyde on ozonization (95). It can be inferred that the absorption of ultra-violet light is attended by changes in the highly unsaturated ring B of ergosterol. In consequence, it is important to determine the degree of unsaturation of the different compounds, and this has been accomplished, where possible, either by hydrogenation experiments, particularly by the quantitative micro method of Kühn, or by perbenzoic acid titrations. Lumisterol, like ergosterol, has three double bonds, whereas, in both tachysterol and calciferol, the presence of an additional double bond has definitely been established, from which the conclusion must be drawn that one of the four original rings has opened in the course of photo-isomerisation. Overirradiation, at least in the case of suprasterol I, appears to result in the closing of a ring since only three double bonds are found in this compound. The same is true of the two pyro compounds.

Another method of determining the number of rings is by dehydrogenation experiments. Lumisterol, like ergosterol, yields Diels hydrocarbon, methylcyclopentanophenanthrene, on treatment with selenium. It must, therefore, contain the same ring structure as ergosterol. In confirmation of the evidence that the four ring structure is no longer present in tachysterol and calciferol, these substances yield no crystalline dehydrogenation products. The same is true of suprasterol I so that, although it contains a four ring structure, this is evidently different from that of ergosterol. In the pyro compounds on the other hand, the original ring system has apparently been reformed.

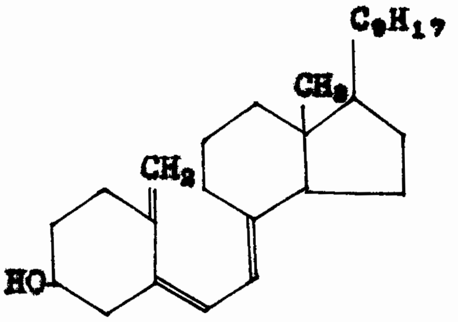
On the basis of these and other results, it is assumed that ring B opens in the change lumisterol ⟶ tachysterol, and the photoisomers of ergosterol are formulated as follows:



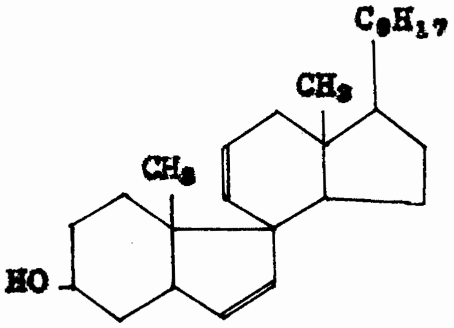
Lumisterol.



Tachysterol.



Calciferol (Vitamin D_2).



Suprasterol I.

Toxisterol has not yet been isolated and is known only from its absorption spectrum and toxic effects. The structure of suprasterol II is not known; it has four double bonds, whereas suprasterol I has only three.

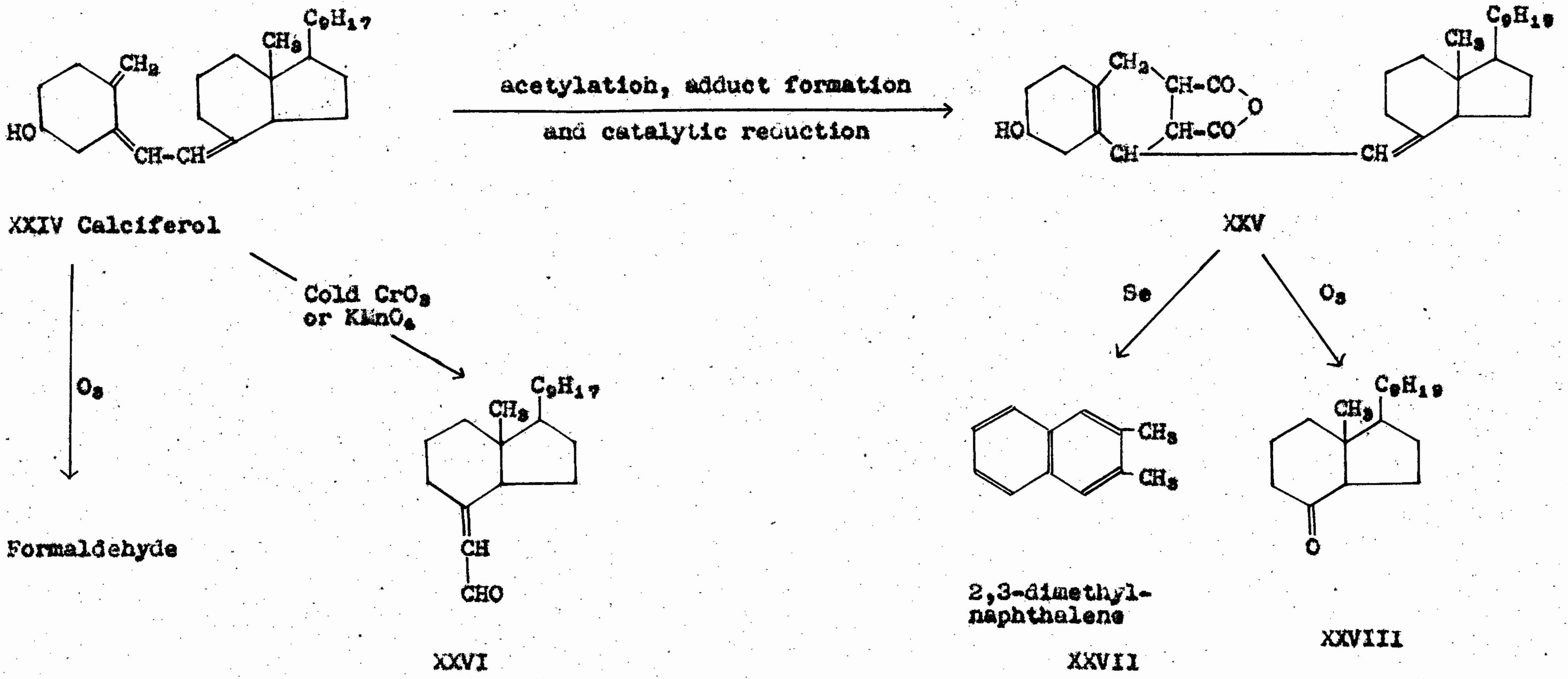
Lumisterol has the same ring structure as ergosterol and, since, when treated with mercuric acetate, it gives a dehydrolumisterol very similar to dehydroergosterol, the double bonds in lumisterol are considered to be in the same positions as ergosterol [Heilbron (112)]. Like all other irradiation products, lumisterol is not precipitated with digitonin, so that it must differ from ergosterol in the spatial relationship of the C_3-OH or the C_{10}-CH_3. At one time, it was thought that the change from ergosterol to lumisterol was simply epimerisation of the C_3-OH. It is now known that this is incorrect and that the initial effect of irradiation is a spatial rearrangement of the C_{10}-CH_3. The evidence for this comes from a study of the dehydro- and perhydrodehydro derivatives of ergosterol and lumisterol (57).

Little is known about the structure of tachysterol. The evidence for the opening of ring B in this compound has already been discussed. The present formulation is based on the fact that tachysterol and calciferol yield identical dihydro derivatives and consequently possess the same carbon skeleton (197); the great ease of formation of adducts with maleic and citraconic anhydrides indicates that the double bonds must be arranged in a particularly active system of conjugation.

Calciferol is, of course, the most interesting member of the series. Its structure has been established by the study of the products of oxidative degradation of the vitamin or of the dihydrocalciferol maleic anhydride adducts. With maleic anhydride calciferol acetate forms two isomeric adducts which are easily reduced to dihydro compounds where the side chain is saturated. Windaus and Thiele (257) proved that these compounds must have the structure XXV (page 35) by the following degradations. On ozonolysis, there was obtained a saturated ketone which, from the composition ($C_{19}H_{34}O$ bicyclic), must have the structure XXVIII. This observation establishes a double bond in the 7-8 position. The nature of the other half of the molecule was revealed by the isolation of 2,3-dimethyl naphthalene (XXVII) as product of the selenium dehydrogenation of the dihydro addition compounds. This kind of dehydrogenation was unique at the time, but the result has been checked by Thiele and Trautmann (244) by the study of a number of model dehydrogenations. The identification of degradation products characterising both parts of the molecule establishes rigidly the structure of the maleic anhydride addition product. Provided there is no rearrangement in the reaction of calciferol (as the acetyl derivative) with maleic anhydride at the temperature of the steam bath, these observations also completely establish the structure of the vitamin itself.

Further evidence for the structure XXIV was obtained by Heilbron, Jones, Samant and Spring (109) by direct oxidation of calciferol with cold chromic acid or permanganate to an oily aldehyde $C_{21}H_{34}O$ (XXVI) whose αβ unsaturated structure was deduced from analytical data and from a study of the absorption spectrum of the semicarbazone. This aldehyde could only arise from the cleavage of a double bond in the original 5,6-position of a substance with a structure XXIV.

Oxidative Degradation of Calciferol



Heilbron and his co-workers also showed that the ozonolysis of calciferol gives formaldehyde in yields as high as 30% of the theoretical amount required for the rupture of the methylene linkage at $C_{10}:C_{19}$. This last bit of evidence, although it is not entirely free from objection, however, since similar treatment of ergosterol gives small amounts of formaldehyde, definitely establishes the presence of the exocyclic methylene group postulated by Windaus and Thiele (loc. cit.).

The structure of calciferol may thus be taken as being firmly established by degradative evidence though attention must be drawn to the fact that Bernal and Crowfoot (14) have criticised formula XXIV on the basis that it does not conform with the data obtained from X-ray analysis of crystalline calciferol. No solution of this difficulty has yet been reached (41).

Multiple Nature of Vitamin D.

As has been stated before, when the activation of ergosterol was first discovered, it was thought that calciferol was the only form of vitamin D and ergosterol the only provitamin. This belief was based on the fact that all other sterols which could be activated showed the 'ergosterol' absorption spectrum in proportion to their activatibility. The possibility had been overlooked that there might exist other D provitamins having the same absorption spectrum as ergosterol. It is now recognized that this is the case and more than ten different substances having antirachitic activity have been described. Of these, five are well characterized chemical entities. Knowledge of the others is fragmentary and they are of academic interest only.

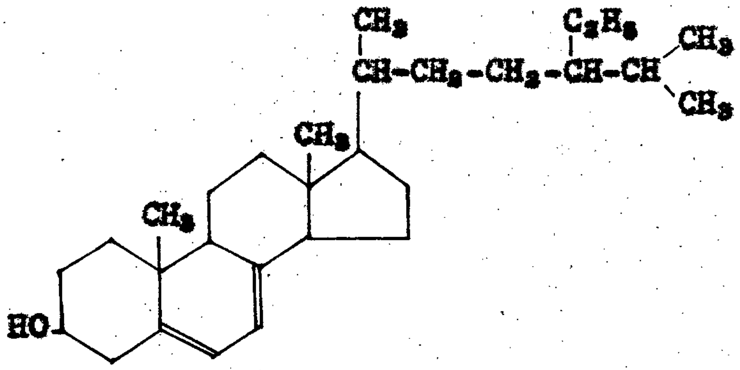
The chief mode of attack on the problem of the multiple nature of vitamin D has been the use of differential bio-assays with chicks and rats. Carrick [unpublished work quoted by Bills (17)] was the first to show that irradiated ergosterol was far less effective for chickens, rat unit for rat unit, than was cod liver oil. This was confirmed by Massengale (174) and numerous other workers. This provided the first definite proof that calciferol was not identical with the 'natural' vitamin D of fish oils and it greatly stimulated the study of the activatibility of sterols other than ergosterol, in particular of those with the same system of conjugated double bonds in ring B.

7-Dehydrocholesterol was prepared by Windaus, Lettre and Schenck (255) by the following semisynthesis. Cholesteryl acetate was oxidized by chromic acid to 7-keto-cholesteryl acetate. This was reduced by aluminium isopropylate to 7-hydroxy cholesterol, the dibenzoate of which, when strongly heated, gave the monobenzoate of 7-dehydrocholesterol. From this the free sterol was obtained by saponification. 7-Dehydrocholesterol has the same absorption spectrum as ergosterol and it is activated by irradiation in exactly the same way. Activated 7-dehydrocholesterol, or vitamin D_3, has been obtained crystalline by Schenck (225) and its structure has been shown to be analogous to that of calciferol from which it differs only in the nature of the side chain (252). Lumisterol-3 and tachysterol-3 have also been isolated.

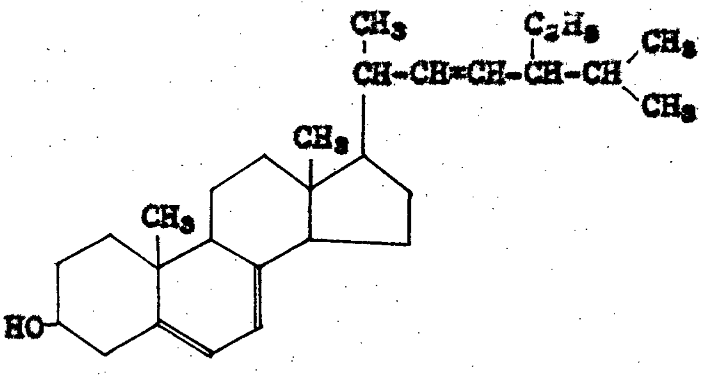
Vitamin D_3 has the same rat:chick efficiency ratio as cod liver oil, and its identity with one of the natural vitamin D's was established in 1936 when Brockmann (38) isolated the antirachitic vitamin of tunny liver oil as its dinitrobenzoate and found it to be identical with the dinitrobenzoate of vitamin D_3. Further proof of their identity was provided by comparative rat:chick assays by Grab (92) and by other studies (234) (105). Vitamin D_3 is thus a very important form of the antirachitic vitamin. Its importance is enhanced by the fact that 7-dehydrocholesterol has been isolated from hogskin by Windaus and Bock (251); it is thus in all probability the provitamin which is activated when human beings and animals are irradiated. As has been mentioned in the Introduction, it also occurs in molluscs, from which source it is extracted commercially.

Another well-defined form of vitamin D is 22-dihydrocalciferol or vitamin D_4. 22-Dihydroergosterol was prepared by Windaus and Lange (254) by the hydrogenation of the double bond of the side chain of ergosterol. On irradiation, it is activated to the corresponding vitamin which was obtained in the crystalline form in 1937 (258). It appears to be slightly less antirachitic for rats than either vitamins D_2 or D_3, while for chickens, its activity per rat unit is intermediate (179). It has not, as yet, been isolated from any natural source.

Two other 7-dehydrosterols have been prepared by the technique described for 7-dehydrocholesterol. 7-Dehydrositosterol was prepared by Wunderlich (265) and, as was expected, was found to be activated by irradiation. On the other hand, the irradiation product of 7-dehydrostigmasterol (163) proved to be inactive or, at the most, very feebly active. Exactly why this should be is not understood.

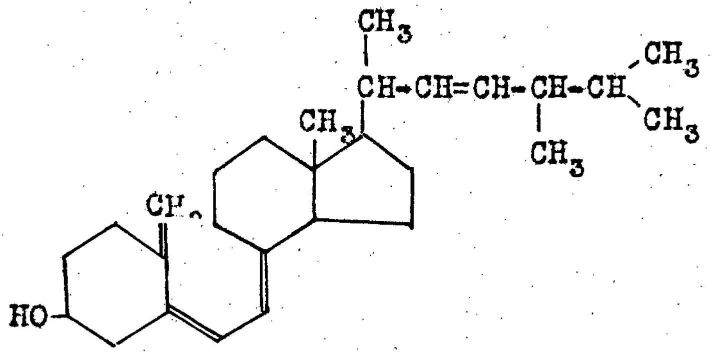


7-dehydrositosterol.

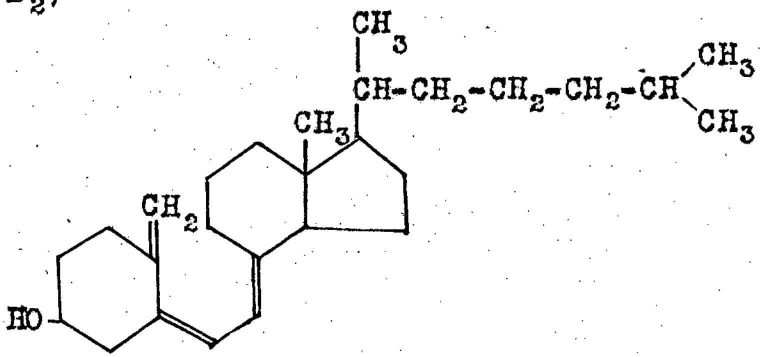


7-dehydrostigmasterol.

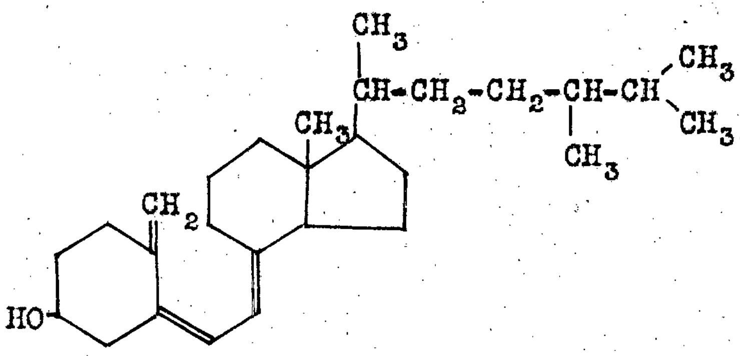
Some Forms of Vitamin D



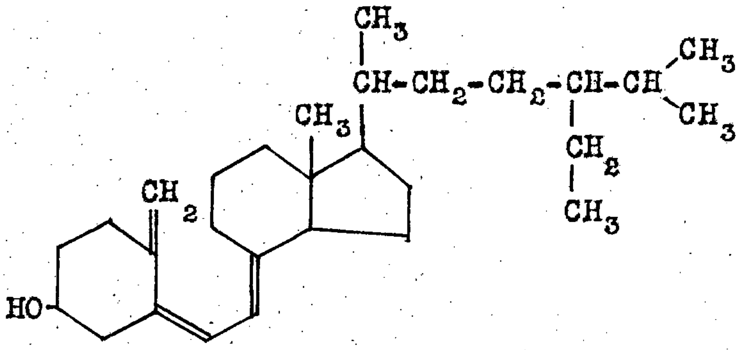
Calciferol (Vitamin D_2)



Activated 7-dehydrocholesterol
(Vitamin D_3)

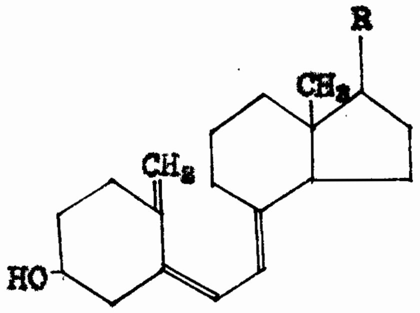


22-dihydrocalciferol
(Vitamin D_4)

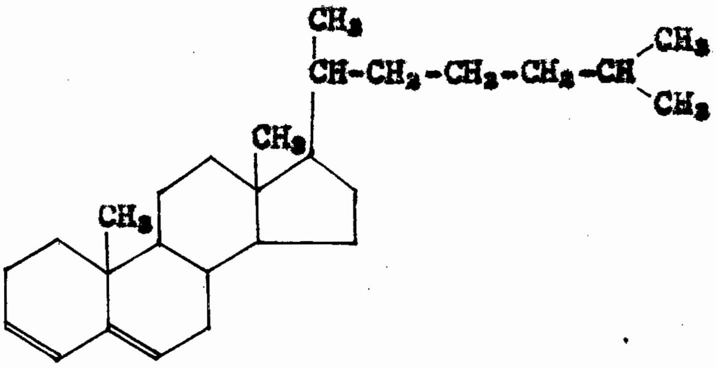


Activated 7-dehydrositosterol

The four forms of vitamin D already described, calciferol, irradiated 7-dehydrocholesterol, 22-dihydro-calciferol and activated 7-dehydrositosterol, differ merely in the nature of the side chain and may be represented by the formula



Of a rather different nature is the vitamin prepared by chemical means by Bills and Yoder. Although only a laboratory curiosity, it is particularly interesting from the point of view of the relation of structure to antirachitic activity. In 1926 Bills (19) treated cholesterol with fullers earth and obtained a product which was about as active as cod liver oil. According to Yoder (266) who confirmed Bills' findings, the fullers earth brings about a complete dehydration of the cholesterol, resulting in the formation of the doubly unsaturated hydrocarbon cholesterilene, which is converted to cholesterilene sulphonic acid by reaction with the sulphur of the clay.



Cholesterilene.

Cholesterilene itself is inactive, but the sulphonic acid and its salts have a slight but definite antirachitic potency; it is more effective for chickens, per rat unit, than is cod liver oil.

Besides these five well-defined forms of vitamin D, there are some nine others about which comparatively little is known. These have been reviewed by Bills (17) (18) and Reed (214) and will not be discussed further here.

Fish oils contain more than one form of vitamin D. Bills (23) carried out extensive rat:chick assays on oils from a number of different species of fish. He found a wide range in their relative effectiveness towards chickens. Some of his results are given in Table 2. From this work he concludes that two or more kinds of vitamin D exist in fish oils, the proportions varying in different oils.

Recently a cod liver oil has been subjected to molecular distillation by Hickman (22) (122) who concluded from the elimination curve obtained that the oil contained two principal and two minor vitamins D as well as traces of still two more forms. This offers physical evidence in confirmation of Bills' hypothesis. Knowledge of the nature of these vitamins is still lacking. Brockmann (38) (39) has isolated vitamin D_3 from both tunny and halibut liver oils. He has also shown the presence of a small amount of admixed calciferol (40) It is possible that calciferol which has an efficacy ratio of 1.0 to 3.2 may predominate in oils such as bluefin tuna and toluava which have very low efficacy ratios. Vitamin D_3 has an efficacy ratio of approximately 100; no artificial form of the vitamin is known which has as high a ratio as the liver oils of the sablefish (160), the dogfish (230) or the white sea bass (314). These oils must contain a hitherto unknown form of the vitamin which is characterized by an exceedingly high chick efficiency. Further progress in separating and identifying the vitamin D of fish oils may be expected from the use of molecular distillation and chromatographic absorption technique.

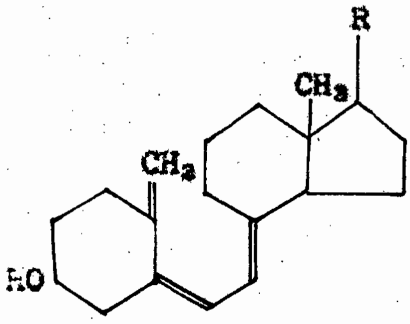
TABLE 2.

The relative effectiveness of vitamin D from different sources for rats and chickens.

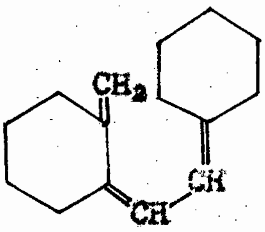
Type of Liver oil.	Efficacy ratio (Cod liver oil = 100)
Bluefin tuna (Thunnus saliens)	16 - 19
Totuava (Eriscion macdonaldi)	21
Striped tuna (Katsuwonus pelamis)	25
Pollack (Pollachius virens)	51
Cabrilla (Epinephelus analogus)	65
Halibut (Hippoglossus hippoglossus)	85
Chili pepper (Sebastodes goodei)	89
Ling cod (Ophiodon elongatus)	99
Wolffish (Anarhichas lupus)	102
Sardine body oil (Sardinia caerula)	109
Hake (Urophycis chuss)	133
Boccaccio (Sebastodes paucispinis)	146
Sablefish (Anoplopoma fimbria)	160
Basking shark (Cetorhinus maximus)	162
Dogfish (Squalus suckleyii)	230
White sea bass (Cynoscion nobilis)	265, 314
Calciferol	1.0 - 3.2
Irradiated 7-dehydro-cholesterol	90

Synthesis of Vitamin D Analogues

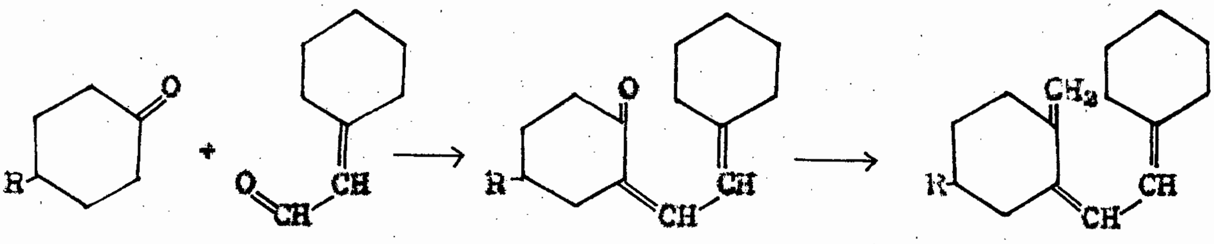
As has been pointed out above, the four most important forms of vitamin D, calciferol, irradiated 7-dehydrocholesterol, 22-dihydrocalciferol and activated 7-dehydrositosterol may all be represented by the formula



This similarity of structure has aroused interest in the possibility of the synthesis of simple vitamin D analogues containing the characteristic grouping

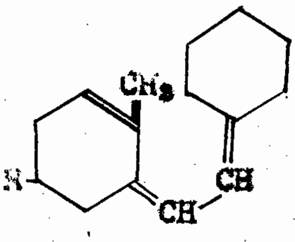


Work along these lines has been begun by Dimroth in Germany, (58) (59) and by Burkhardt and his collaborators in England (2) (3). Up to the present, however, very little progress has been made. The method of attack employed in both laboratories is indicated below.

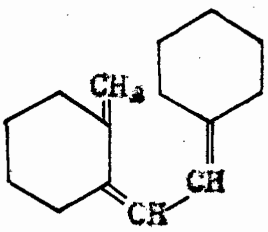


XXIX

XXX



XXXI



XXXII

By the condensation of cyclohexylidene acetaldehyde with substituted cyclohexanones, ketones with the structure XXIX have been obtained. Attempts to convert these to the corresponding vitamin D analogues (XXX) by the Reformatsky reaction or by the action of methyl magnesium iodide have not been successful. Trienes of the type XXXI have been obtained instead. Very recently, however, it has been reported (148) that Dimroth has announced the synthesis of a triene hydrocarbon(XXXII) to which must be ascribed the correct constitution of a calciferol analogue. Details of this work are, unfortunately, not available. These studies it is hoped will not only advance our knowledge of the relations between structure and antirachitic activity, but will also lead to the complete synthesis of vitamins D_2 and D_3, thus finally establishing their constitution, particularly from the point of view of their stereochemistry (58) (148).

The Physiology of Vitamin D.

Vitamin D plays an important part in the regulation of calcium and phosphorus metabolism and is indispensable for the normal calcification of bone. Its absence from the diet is followed by the development of rickets in the young animal and of osteomalacia in the adult. The clinical symptoms of these diseases are too well-known to need further description here.

The changes which occur in the bone in rickets are essentially defective calcification of the growing bone and compensatory hypertrophy of the epiphyseal cartilage. The histology of experimental rickets has been studied by many workers and an excellent account of this aspect of the subject is given by Shohl and Wolbach (230).

The characteristic bone changes are usually accompanied by a lowering of the Ca x P level in the blood serum and the hypothesis has been put forward that vitamin D acts essentially by raising the blood Ca x P by improving the absorption of these minerals from the intestine. In infantile rickets it is usually the inorganic phosphorus level of the blood which is lowered, the calcium being normal or even slightly raised. This is not always the case, however; low-calcium rickets, often associated with nutritional tetany, is not uncommon. The serum of a rachitic rat is also characterised by the fact that its phosphatase content is higher than normal. The significance of this change is unknown, but nevertheless an increase in the level of the phosphatase in the blood provides the earliest reliable evidence of the development of rickets.

Excessive doses of vitamin D are toxic and the symptoms of hypervitaminosis D are an exaggeration of the normal effects of the vitamin. The calcium and the phosphate level in the blood are raised above normal and overcalcification of the newly formed bone occurs ultimately at the expense of the shaft. Calcarious deposits are also formed in the arteries, kidneys and other soft tissues. Finally the animal loses weight rapidly and dies. A good account of hypervitaminosis D in rats, and also a review of the literature up to 1931, is given by Harris and Innes (103); more recently the subject has been reviewed by Reed, Stuck and Steck (214).

Various factors in the diet, other than vitamin D, also play a part in the development of rickets. This aspect has been dealt with in detail by Shohl (229) and will only be discussed briefly here. In children and in some experimental animals, for example dogs (186) and chickens (173), a deficiency of vitamin D alone, without any great distortion of the diet, is sufficient to produce rickets. Rats, on the other hand, do not seem to need vitamin D if their diet is properly constituted with respect to calcium and phosphorus and, in order to produce satisfactory experimental rickets, it is necessary either to restrict the calcium and phosphorus in the diet or to disturb the balance between the two. In practice it would appear that the relative proportions of calcium and phosphorus are more important than the absolute amounts. The standard rachitigenic diets, such as the Steenbock and Black No. 2965 or McCollum's No. 3143, thus have a Ca:P ratio of about 4:1. Excess of either element appears to act by interfering with the absorption of the other from the intestine and, as both are necessary for the formation of bone, rickets results. Any other factor which interferes with the absorption of either calcium or phosphorus acts in the same way, and it has been found with low phosphorus diets that excess of metals which form insoluble phosphates, for example beryllium, magnesium, strontium, iron, lead and thallium, cause bone lesions similar to rickets. The acidity of the diet, both actual and potential, must also be considered here. If the contents of the intestinal tract are too alkaline, the absorption of calcium and phosphorus is hindered. Excess of anions, on the other hand, facilitates the absorption of these minerals from the intestine, but increases their excretion. Rickets has been produced (100) by feeding a non-rachitogenic diet to which was added ammonium carbonate and ammonium chloride. This was alkaline in reaction, but provided an excess of anions for excretion; both absorption and retention of calcium were thus diminished. The fact that rickets can be caused by interference with the assimilation of calcium and phosphorus is evidence in favour of the hypothesis, already mentioned, that vitamin D acts by improving the absorption and retention of these minerals by the body.

Actually, however, it is not possible to say with certainty how the vitamin exerts its effect in the animal body. The study of the mode of action of vitamin D has been approached from many different view-points and has led to varied conclusions. The different theories which have been proposed may be classified under three main headings:

(1) That the vitamin acts by stimulating the parathyroid glands.

(2) That it acts merely to increase the net absorption of calcium and phosphorus from the gut.

(3) That it has a more specific action, either on the epiphyses or elsewhere.

The parathyroid theory was first advanced by Bloch and Faber (25) and has been supported by many workers, especially Taylor and his associates at Toronto. Taylor et al. (243) stressed the similarity in the effects of <u>excessive</u> doses of vitamin D and of parathormone; not only were the symptoms and postmortem findings almost the same in the two cases, but there was also a marked parallelism in the differences in sensitivity to parathyroid extract and to vitamin D in different species. Furthermore, they claimed that, in the dog, complete removal of the parathyroids rendered the animal very resistant or immune to excessive doses of vitamin D. Hence they argued that the vitamin acts by stimulating the parathyroid glands. On the other hand, Dale, Marble and Marks (54) have failed to obtain full confirmation of Taylor's work. Moreover Collip and his co-workers (47) found that the histological changes in the bone in hypervitaminosis D were different from those observed in experimental hyperparathyroidism; in the former case, decalcification of the shaft occurs without the appearance of osteoclasts, whereas this is characteristic of the latter. They, therefore, found it 'difficult to believe that irradiated ergosterol acts merely by stimulating production of the parathyroid hormone'. Lastly, injections of parathyroid extract do not heal rickets, but rather aggravate the disease since they withdraw calcium from the bone to raise the level in the blood. It would thus seem that the parathyroid theory of vitamin D action is most unlikely.

Some mention of the second theory of vitamin D action, namely that it acts by improving the absorption and retention of calcium and phosphorus by the body, has already been made. There is no fundamental pathological condition in the bone in rickets which prevents its calcification, since slices of rachitic bone will become calcified in vitro when placed in an inorganic salt solution of suitable calcium and phosphorus concentration (219) (228); histological examination of the bones shows no difference in the mode of calcification in vivo and in vitro. Harris (101) (103), who originally proposed this hypothesis, argued that vitamin D thus acts essentially by raising the blood calcium and/or phosphorus by improving their absorption from the gut, the deposition of the minerals in the bone following automatically on the correction of the blood abnormalities. This theory has been supported by the work of Nicolaysen (198) (199) and more recently by Smith and Spector (235). The latter authors found that the ingestion of mineral oil interfered with the action of vitamin D not only when it was administered as cod liver oil, but also when the animal was irradiated with ultraviolet light. As mineral oil is not absorbed from the intestine and must, therefore, cause its interference there, these results appear to indicate that the vitamin formed by irradiation reaches the alimentary canal where at least part of its role is to increase the absorption of calcium and phosphorus. Nicolaysen goes further; he considers that the influence of the vitamin is primarily on the calcium absorption, any effect on the absorption of phosphorus being indirect. He found that the absorption of calcium from isolated loops of the intestine was far slower in rachitic rats than in controls receiving vitamin D. On the other hand, phosphorus, either as potassium phosphate or as sodium glycerophosphate, was absorbed equally well in both cases. Other studies of the absorption and excretion of calcium and phosphorus led him to the same conclusions.

It may, therefore, be considered to be beyond doubt that vitamin D does play a big part in improving the absorption of calcium and phosphorus from the intestine, but it is less certain that this is its only sphere of action. Many workers have felt that mere increased intestinal absorption cannot explain all the known facts. For example, it has been pointed out that healing of rickets will often occur when the animal is still in a negative calcium balance (132), and also that significant changes in the concentration of phosphorus and calcium in the blood frequently do not take place until well after the clinical signs of rickets have appeared (214). Studies of the direct action of the vitamin on the epiphysis in vitro have led to conflicting results. Robison and Rosenheim (218), for example, could detect no effect, beneficial or otherwise, of radiostol, calciferol or irradiation; Venar and Todd (247), on the other hand, claimed that addition of an aqueous extract of vitamin D had a favourable influence on calcification of bone slices in vitro. Again, the use of radio-active phosphorus in the study of this problem has not helped as much as it was hoped. The first workers in this field, Dols et al. (60), could draw no conclusions as to the mode of action of vitamin D from their results. Later, however, Cohn and Greenberg (46) and Morgareidge and Manly (193) obtained results which they interpreted as indicating that the vitamin influences the intermediary metabolism of the bone-forming elements, in particular phosphorus. A similar hypothesis had been advanced earlier by McGowan (180) (181) who suggested that the essential action of the vitamin is to set free inorganic phosphate from the tissue phospholipids. Further support for a theory of this nature has also been given by Schneider and Steenbock (226) who found that with rats on a diet very low in phosphorus, vitamin D appeared to direct the phosphorus away from the soft tissues to the bone with the result that growth was inhibited or retarded. All that can be said of these results, however, is that they give some indication that the vitamin may have a specific localized action on calcium and phosphorus metabolism which may be supplementary to its more general influence on the absorption of these elements. Conclusive evidence on this point is still lacking.

From this very brief review of the subject, it can be seen that, in spite of the prodigous amount of work done on this problem, our knowledge of the fundamental mode of action of vitamin D is still very scanty. In view of the complex nature of the problem, however, this is not unexpected. On the other hand, if we consider that the existence of an antirachitic vitamin has been generally recognized for rather less than twenty-five years, the progress made is by no means inconsiderable.

The Origin and Function of Vitamins A and D in Fish.

The abundance of vitamins A and D in fish liver oils, in contrast with the relatively small amounts stored in the liver of other vertebrates, has led many workers to speculate on the origin and function of these vitamins in fish. Very little, however, is known on this subject. One theory is that the vitamin A and D reserves of fish are built up by the gradual accumulation of the small amounts of vitamin or provitamin present in the food. These are assumed to be ultimately derived from the plankton. In the case of vitamin A, for example, it has been shown that the diatom, Nitzschia closterium, is capable of synthesizing carotene (130), and attempts have been made to trace the transference of vitamin A from the diatoms, through the zooplankton and the small fish to the livers of the bigger fish such as the cod (67). There is a considerable amount of indirect evidence in support of this hypothesis. For example, it fits in well with the fact that the vitamin A reserves of the bigger fish are proportionately far greater than those of the smaller ones. It also explains why, in the halibut, there is a close relationship between the seasonal changes in the vitamin A potency of the liver oil and the diatom cycle (167).

On the other hand, there are many difficulties in the acceptance of this theory. In the first place, it has repeatedly been shown that the zooplankton, one of the essential links in the chain between the carotene of the diatoms and the vitamin A of fish, are devoid of vitamin A activity (62) (63). It has also been found that the diet of the cod is much poorer in vitamins A and D than was first suspected and it is doubtful whether the fish could derive sufficient vitamin from its food to account for the vitamin A and D reserves of the liver (20) (64). In the halibut, the position is even more acute. A very large halibut may contain up to 90 gm. of vitamin A, of which at least 70 gm. may be deposited within a month. Yet, according to Lovern (166), 'there is nothing unusual in the diet of the halibut - mixed species of fish - to account for this enormous deposition of vitamin A as compared with, say, a dogfish eating the same diet, and depositing only very small quantities of vitamin A'. The possibility that fish can synthesize both vitamins A and D must, therefore, be considered.

Very little direct evidence of synthesis of vitamins A and D in fish has been obtained. Bills (20), in an experiment with young catfish (Ictalivus punctatus), found that the vitamin D content of the liver was not decreased by keeping the fish in the dark on a vitamin D-deficient diet for 6 months. This he considers to be evidence, inconclusive to be sure, of the synthesis of vitamin D in fish. How the vitamin is elaborated is unknown. Hess et al. (116) gave codfish ergosterol intramuscularly and by mouth, but they failed to get any indication that the fish were able to activate this sterol. Neither does it appear likely that irradiation of the body surface plays any part in the synthesis of the vitamin as very little of the ultra-violet portion of the spectrum penetrates more than 2 metres below the surface of the water (7). If fish synthesize vitamin D, therefore, they must do so by some method other than the activation of the provitamin by irradiation with ultra-violet light.

It is also possible that fish may be able to synthesize vitamin A from carotenoids which have no provitamin activity for rats. The most important of such compounds to be considered is astaxanthin which is the predominant pigment of the crustacea and which is ingested in large quantities by many fish (194). That fish have powers of utilising carotenoids peculiar to themselves is indicated by their ability to elaborate vitamin A_2 which is only found in fish and animals such as sea birds which feed chiefly on fish (88). The origin of vitamin A_2 is also an interesting problem. It has been shown (195) that fresh water fish can convert carotene into both vitamin A_1 and vitamin A_2, but the reason why the latter should be found in relatively large quantities in fresh water fish and not in marine fish is completely unknown.

Very little also is known about the function of vitamins A and D in fish. Both vitamins A_1 and A_2 have been found in the visual purple of the fish's eye and it is assumed that in fish vitamin A plays the same part in the visual cycle as it does in other mammals (249) (250). The discovery of the presence of large amounts of vitamin A in the intestine of many species of fish has led to the suggestion that, in addition to its functions as a vitamin in the accepted meaning of the term, vitamin A acts as an intermediary in the absorption of fat from the intestine. This suggestion has been put forward largely as the result of the work of Edisbury and Lovern and their collaborators (71) (169) (170) on the distribution of vitamin A in the intestine of the halibut. These workers have shown that vitamin A is found in relatively large amounts only in those parts of the intestine which play an active part in the absorption of fat. This parallelism holds not only for the alimentary canal as a whole, but also for the various intestinal coats, and the theory has been tentatively advanced 'that vitamin A assists in the dispersal and translocation of the fat through the intestinal membranes or through the walls of the intestinal capillaries by esterification with fatty acids, the esters forming a labile complex with the tissue proteins' (166). A number of fish, however, do not store large quantities of vitamin A in their intestines and pyloric caeca, and it is necessary to postulate an alternate mechanism of fat absorption in these cases. Thus the suggestion that in fish vitamin A assists in the absorption of fat is only an attractive hypothesis. It seems that the time has come when controlled feeding experiments whould be carried out on aquarium fish to try and throw more light on the problems of the origin and functions of vitamins A and D in fish.

THE PRODUCTION AND UTILIZATION OF FISH OILS.

Cod liver oil has been held in esteem as a domestic remedy, particularly in the treatment of rickets, for many centuries. The discovery that its remarkable therapeutic properties are due to the presence of vitamins A and D led to investigation of other fish oils as sources of these vitamins. To-day the use of a wide variety of oils has been established. It is proposed to give here a short account of the various types of fish oils which are of importance as sources of vitamins A and D, and to deal briefly with their methods of production. This forms an essential background to the present studies which were undertaken primarily to promote the efficient utilization of South African fish oils as sources of vitamins A and D. A comprehensive review of this aspect of the subject is to be found in Brocklesby's monograph on the chemistry and technology of marine animal oils (82).

Cod liver oil itself is still largely used in medicine. A typical cod liver oil contains 1200 I.U. of vitamin A and 100 I.U. of vitamin D per gram. Its place has been taken to some extent by other fish liver oils which have a much greater vitamin content and require a correspondingly smaller dosage. Halibut liver oil was one of the first to be introduced. The average halibut liver oil on the market contains from 10,000 - 100,000 I.U. of vitamin A per gram and from 900 - 3,000 I.U. of vitamin D. This range of potency is typical of a number of oils used for medicinal purposes. Amongst the most valuable liver oils are those of fish of the percomorph group and in particular those of the tuna family. These oils are a very potent source of vitamin D and may contain from 20,000 - 70,000 I.U. of vitamin D per gram (126). Owing to their relatively low vitamin A content (20,000 - 150,000 I.U. per gram), however, the natural unblended oils are rarely used. They are usually blended with other oils, for example halibut liver oil, cod liver oil or cod liver oil concentrates, in order to secure a better balance between the two vitamins. They are also used extensively in the manufacture of vitamin D concentrates. In contrast with these oils are those of the elasmobranch fishes which are characterized by their negligible vitamin D content (21). The liver oils of certain species are, however, rich in vitamin A. This is the case in the soupfin shark which is to-day one of the most important sources of vitamin A. Soupfin shark liver oil contains 76,000 to 100,000 I.U. of vitamin A per gram. It is chiefly used in the manufacture of vitamin A concentrates, particularly for the fortification of foodstuffs (205). Before the war, it was also employed extensively as a source of vitamin A in animal feeding oils. Dogfish liver oil is now used for this purpose instead.

In 1937, it was reported that the oil from halibut intestines greatly exceeded that from the liver in vitamin A potency (168). Since then, the visceral oils of other species of fish have also been shown to be rich in vitamin A (212). As a general rule, however, their vitamin D content is low. The production of visceral oils has been begun on a small scale, chiefly in Canada, and there is little doubt that, once the difficulties of extracting this type of material have been overcome, visceral oils will supply an important part of the world's vitamin A requirements.

While fish liver oils remain pre-eminent as a source of vitamin A and vitamin D, fish body oils have been used to an increasing extent within recent years. Under this heading are included sardine or pilchard oil, menhaden oil, herring oil and the oil from salmon cannery waste. These oils find their chief application in industry, particularly in the paint and varnish industry. They are also, however, largely employed in animal feeding oils, especially as a base for making poultry oils. A typical sardine oil contains 100 - 500 I.U. of vitamin A per gram and 20 - 100 I.U. of vitamin D.

It is blended with shark or dogfish liver oil to provide the necessary vitamin A. It is also frequently fortified with high vitamin D potency oils, especially oils of high chick efficiency. The resulting poultry oils usually contain 1000 - 3000 I.U. of vitamin A and 100 - 400 I.U. of vitamin D. The investigation of the possibilities of producing fish body oils in South Africa has been undertaken very recently. No work on this aspect of the subject has, however, been carried out by the writer. The production and utilization of these oils will, therefore, not be considered further here.

The methods of extracting oils from fish livers and viscera are as numerous and varied as the types of oil produced. Fish livers of high oil content such as cod or shark livers are relatively easy to extract. Cod liver oil is usually obtained by treating the livers with live steam at a pressure of 60 - 100 lbs. per square inch for 30 minutes. The oil is then run off, filtered and stored. A second treatment yields a lower grade of oil suitable for poultry feeding. The production of cod liver oil has been described in detail by many workers (32) (64) (162). It is unnecessary, therefore, to deal with it further here.

High potency livers such as those of the halibut and the tunas usually contain from 5 to 30 percent of oil. Steaming methods are of no value in these cases, as the heat co-agulated protein has a strong tendency to reabsorb the liberated oil. Solvent extraction has been employed. It is, however, expensive and the oils are darker in colour and less palatable than those obtained by other methods. To-day solvent extraction has largely been superseded by various digestion techniques.

In the alkali digestion process (34) (35) the livers are minced or ground into a paste and heated with two or three times their weight of dilute aequous alkali for about 30 minutes at 80-90°C. Either plain caustic soda or buffer mixtures such as borax and caustic soda (28) may be used. The alkali is usually added in the proportion of 0.5 - 3 lbs. per 100 lbs. of liver. The exact amount used, however, depends on the type of livers being processed and the optimum conditions can only be determined by trial and error. Under these conditions, very little hydrolysis of the oil takes place. The proteins of the liver are thrown into colloidal solution and may be separated from the liberated oil by centrifuging. It is claimed that the yield of oil by this method is over 80% of the theoretical. Where the livers are old and the oil is high in free fatty acids, it is reported (36) (37) that there is danger of loss of vitamin A due to adsorption of the vitamin on the soaps formed during the digestion. In such cases, it is recommended that the 'stick water' should be washed with a low potency oil such as pilchard oil or with a vegetable oil. Owing to the enormous capacity of alkali digested proteins for adsorbing oil, this method, according to Brocklesby (34), is of doubtful value when applied to material containing less then 10% of oil. For livers belonging to this category, pepsin digestion is to be preferred This latter method has been used in the present work as a routine method of obtaining oil samples for analysis. The technique employed is described in detail in a later section of this thesis.

Much attention has been devoted recently to the discovery of a suitable method of extracting visceral oils. The chief difficulty here lies in the very low oil content of the material. Halibut intestines, for example, only contain 1-5% of oil. Solvent extraction cannot be used, owing to the high water content of the viscera. Modifications of the alkali and pepsin digestion processes have, therefore, been employed. These usually consist of digestion of the viscera in the usual way, followed by the addition of either a vegetable oil or a low potency fish oil which acts as a solvent for the vitamins (246). The disadvantage of this method is that the oils obtained in this way can never be as potent as the original visceral oil. This difficulty might be overcome to some extent by the use of an oil which can be readily separated from the vitamins by molecular distillation; the still residues from the commercial vacuum distillation of vitamin from fish oil, for example, have been proposed for this purpose.

The oils obtained by the modern methods of extraction require very little further refining. Cod liver oil for medicinal purposes is usually cold cleared before being put on the market. High potency oils, however, are used without further treatment.

The preparation of concentrates of vitamins A and D from fish oils has assumed considerable importance during the last few years and a large proportion of the oils produced are used for this purpose. The advantage of concentrates is that the doses required are very small and they may be easily administered. Furthermore, the fishy smell and taste which is associated even with the highest grade oils is to a large extent removed during concentration, so that the final product may be used for the fortification of food stuffs without detriment to their flavour. Numerous methods of preparing vitamin A and D concentrates have been proposed. The majority of them involve either partial or complete saponification of the oil. These methods are all based on the same principle and differ only in the technique employed in isolating the vitamin-containing unsaponifiable fractions. There is little to be gained therefore from considering them in detail here. In the last two or three years the use of molecular distillation in the production of vitamin A and D concentrates from fish oils has assumed increasing importance. This method which has been perfected by Hickman and his associates (29) (121) (245) has much to recommend it. By this means the vitamins are obtained in their natural esterified form. In the case of vitamin A particularly, this is an important advantage because, not only are the esters more stable than the free vitamin, but also it has been claimed that they are more easily absorbed and have a higher biological value (91) (120). Another advantage is that the bulk of the oil is not lost as in the case of saponification methods. The residual oil from which the vitamins have been distilled is of high quality and may be used for industrial purposes. There seems little doubt, therefore, that molecular distillation will, in the future, gradually replace the older methods of preparing vitamin concentrates.

GENERAL PLAN OF WORK.

The work presented in this thesis was undertaken as part of a collaborative study of the vitamin A and D content of South African fish products which has been in progress in the Department of Chemistry for the last five years. The writer's contribution to this programme may be divided into three parts.

(i) The development of a satisfactory method for the routine estimation of oil in fish tissues and its extraction in a suitable condition for subsequent analysis.

(ii) A study of the seasonal changes in the oil content of the livers and viscera of some of the most important species of fish landed in Cape Town and the influence of this on the vitamin A and D potency and the analytical characteristics of the oil. For this purpose samples were obtained at approximately monthly intervals for the determination of the oil content, the vitamin A potency of the oil, the iodine value and the percentave unsaponifiable matter. Facilities for vitamin D assays were not available when the work started and had to be built up ab initio. The capacity of the animal house could not be developed sufficiently, therefore, to carry out assays on monthly samples and blending of the samples collected in several consecutive months had to be resorted to.

In several cases, for example, in the stockfish, the kingklip and the snoek, this work was a continuation and extension of the preliminary survey of the field carried out by Dr. Molteno whose results are discussed and compared with those obtained by the writer. Wherever data on the life history and feeding habits of the species studied was available an attempt was made to relate the observed seasonal changes in the oil to these factors.

(iii) In addition to the above studies, random samples of the liver and in some cases the viscera of a number of species of fish were examined. This work was largely of an exploratory

nature/....

nature and serves to indicate those species which may be worth further study. In three cases, namely in the maasbanker, the vaalhaai and the jacopever, the results were sufficiently promising to warrant a more detailed study by other members of the department.

EXPERIMENTAL.

I. SAMPLING OF MATERIAL.

The method of sampling varied with the species of fish and the purpose for which the material was required. It is described more fully under each species. In comparatively few cases was the whole fish handled. Usually the fish were gutted on board the trawler and the livers or viscera were placed in two-gallon tins with press-on lids and kept on ice until they were delivered at the laboratory. In this way a larger number of livers was included in each sample than would otherwise have been possible. The method, however, has the disadvantage that no information can be obtained about the size, sex, condition, etc. of the fish concerned.

In all cases, the livers or viscera were minced in a large household mincing machine; the mince was well mixed and a representative portion taken for analysis.

II. EXTRACTION AND ESTIMATION OF OIL CONTENT.

Methods based on the preliminary pepsin or alkali digestion of the tissue proteins have been developed for the convenient, rapid and quantitative extraction of oil samples from fish tissues in suitable condition and amount for subsequent analytical operations. These are discussed in relation to other methods commonly employed for such oil extractions. The alkali and pepsin digestion procedures have been found superior to the sodium sulphate desiccation method for the extraction of vitamin A and D bearing oils from visceral tissues. The recovery of vitamin A and of unsaponifiable matter generally is quantitative, though that of free fatty acids is variable. The alkali digestion procedure has been found applicable also to the rapid and approximate estimation of the oil content of fish heads and fish flesh.

For the extraction of oil samples from high oil content materials such as fish livers, the method which has been most extensively used in the past is steaming (56) (133) (210). This method suffers from the disadvantages, however, that it does not give quantitative release of the oil from the tissues, and in particular releases no oil whatsoever from material of low oil content. Alternative procedures have been necessary in the case of such materials and also in all cases where a quantitative index of the amount of oil in the tissues has been required. These procedures have for the greater part involved the desiccation of the tissue in some manner, followed by solvent extraction of the oil (9) (34) (133). While suited to the estimation of the percentage oil in fish tissues, they have suffered from disadvantages when applied to the rapid and almost routine preparation of representative samples of oil as have been required for the present survey. For this it was desirable that the oil samples as prepared should not only give an accurate estimate of the amounts of vitamin A and D in the tissues, but that they should also be as representative as possible of the oils as they would be produced in practice by commercial alkali digestion and pepsin digestion methods. The quantitative laboratory extraction of oil samples by methods based on the preliminary digestion of the tissue proteins by pepsin or by alkali digestion has therefore been investigated and compared with the older method based on desiccation of the tissue followed by extraction.

(1) Desiccation of the tissues with anhydrous sodium sulphate followed by extraction of the oil with ether.

This technique was used in the early stages of the work and as a basis of comparison for the pepsin and alkali digestion procedures.

The minced tissue was ground with anhydrous sodium sulphate in a large mortar. For livers a ratio of 2 parts sodium

sulphate/....

sulphate to 3 parts of liver was found satisfactory. In the case of viscera, the proportion had to be increased to 5 parts sodium sulphate to 3 parts viscera; occasionally with these more hydrous materials even this was not sufficient. After being left to dry for not longer than 30 minutes, the material was reground and extracted with peroxide-free ether for 6-18 hours, depending on the oil content of the tissue.

This technique was found to suffer from a number of disadvantages:

(i) The addition of insufficient desiccant interfered with the progress of extraction. Evidence was obtained, in the case of viscera at least, which indicated that if the material was not properly dried, the oil and vitamin A were extracted at different rates. In general, vitamin A tended to be extracted preferentially to begin with. This is seen in Table 3.

TABLE 3.

Extraction of Stockfish Intestines.

Ratio intestines: sodium sulphate.	Time of Extraction.	$E^{1\%}_{1cm.}$ of extracted oil.				
		Exp.1	Exp.2	Exp.3	Exp.4	Exp.5
3 : 2	15 min.	386	361	336	218	112
	2 hours	338	296	305	248	81
	4 hours	312	233	222	218	82
		Exp.6	Exp.7	Exp.8		
3 : 5	15 min.	116	119	49.1		
	2 hours	118	121	53.7		
	4 hours	118	133	49.1		

Oils prepared by partial extraction of the tissue were therefore not representative in so far as vitamin A content was concerned, and the technique of extracting for 15 minutes to get material for estimation of vitamin A content, and to completion to determine the total oil content of the tissues was not applicable. Since a deficiency of sodium sulphate was not always obvious, trouble was encountered in the early application of this method to materials of widely varying water content. Using an adequate proportion of sodium sulphate, on the other hand, extraction proceeded uniformly (Table 3) and reliable results for the value $E^{1\%}_{1cm.}$ 328 mμ for the extracts could be obtained.

(ii) The high proportion of sodium sulphate necessary in the case of viscera, however, introduced another source of error. It was by no means easy to obtain a homogeneous mixture of tissue and desiccant, particularly as it was desirable that the time of mixing should be as short as possible because of the enormous surface at which the vitamin was exposed to oxidation. Highly discordant results for duplicate determinations of the percentage oil in the tissue by means of this method were occasionally obtained which could only be explained on this basis.

(iii) Finally the use of a high proportion of desiccant meant that an excessively large bulk of material had to be extracted in order to get even a small sample of oil. This disadvantage was particularly apparent in the case of low oil content material such as stockfish intestine.

In view of these disadvantages, a search was made for a more convenient method of extraction and estimation of the oil content of both livers and viscera and a modification of Brocklesby's pepsin digestion technique (35) was found to be very satisfactory. A similar technique using alkali digestion was also developed and a detailed comparison of the three methods of extraction was made.

(2) Pepsin Digestion of the visceral material followed by ether extraction of the oil from the digestion mixture.

The general principles of the pepsin digestion technique for obtaining oil samples in a qualitative manner have been described by Brocklesby and Green (35). These authors

describe/...

describe the digestion of the material at pH 1.2-1.5, peptisation of the more resistant material by final heating after addition of sodium carbonate, followed by separation of the oil in a centrifuge. By replacing the centrifugal separation by ether extraction, the method has been put on a quantitative basis and a detailed study of the recovery of oil, vitamin A and unsaponifiable matter generally has been made.

The procedure actually used in this work was as follows: 0.5 - 1 gm. of commercial pepsin was dissolved in 100 ml. of water and 2 ml. of hydrochloric acid (sp. gr. 1.2) was added. This solution was then added to every 100 gm. of minced tissue and the whole was digested at 45-50°C with occasional stirring for 12-36 hours. During this period the vitamin was protected from oxidation by replacing the air in the flask with carbon dioxide. When practically all the tissue protein had been rendered water soluble, a saturated solution of sodium carbonate was added to neutralize the acid and the mixture was heated in a boiling water bath for 10-15 minutes to destroy the lipolytic enzymes and to complete the digestion. The oil was then separated by extraction of the digestion mixture with peroxide-free ether. When persistent emulsions were encountered as often occurred in the early stages of the development of the technique, the extraction mixture was centrifuged in a large bucket centrifuge. The ethereal layer was pipetted off. Fresh ether was then added and the contents of the tube well mixed and centrifuged again. This device was also employed in the alkali digestion technique. No accurate control of the pH was found necessary under the above conditions and the amount of pepsin employed usually led to complete digestion within 15-20 hours.

(2) Alkali digestion of the visceral material followed by ether extraction of the liberated oil from the digestion mixture.

While the pepsin digestion procedure described above has been employed with success on many types of material, an alkali

digestion/....

digestion method based on the commercial procedure commonly used for the extraction of liver oils has been found to be more convenient in many respects. Its chief advantage is that the time of extraction is much less than in either the desiccation or pepsin digestion techniques. This means, amongst other things, that there is less danger of loss of vitamin A due to oxidation during this period. Alkali digestion also gives an oil of better colour and appearance than the other methods.

This procedure was briefly as follows. The minced liver or other material was digested with twice its weight of a 1% sodium hydroxide solution at a temperature of 80-90°C until complete disintegration of the tissue was effected, (usually 20-30 minutes). When cold, the solution was extracted with peroxide-free ether, alcohol being added if necessary to break any emulsions.

The application of this technique to livers and visceral tissues generally was studied by the writer. In addition, its use has been extended by other members of the department to include the extraction and estimation of the oil in fish heads and fish flesh, and it has been shown that the recovery of oil from this type of material did not differ materially from that obtained by the preliminary vacuum drying of the tissue followed by ether extraction.

(4) Comparison of the above three methods of extraction.

As far as the writer is aware, the use of the pepsin and alkali digestion techniques for the quantitative extraction and estimation of the oil in fish tissues has not been developed previously. A critical examination of the recovery of oil, vitamin A and unsaponifiable matter from livers and visceral tissues by these methods has, therefore, been made and the results have been compared with those obtained by the older desiccation technique. The applicability of the alkali digestion method to fish heads is also discussed briefly.

(a) Application to visceral tissues:

(i) Yield of Oil: The amount of hydrolysis of the oil which occurs during the actual pepsin or alkali digestion is negligible. The oil extracted from the material desiccated with sodium sulphate, however, contains all the free fatty acids present in the tissues, whereas that obtained by pepsin digestion contains only a variable proportion (a part of the fatty acids is lost during the final sodium carbonate treatment) and that derived from alkali digestion is neutral and free from fatty acids. For this reason close correspondence in the yields of oil obtained by the three methods is only to be expected when the material under examination is fresh and little post-mortem hydrolysis of the oil has occurred. These conditions were fulfilled in most of the liver samples examined and, as may be seen from Table 5, the yields of oil for the three methods did not differ materially from one another. With low oil content intestines in which autolysis occurs with great rapidity in storage, on the other hand, marked differences in the yield of oil by the three methods are found (Table 5). That these are mainly due to differences in the fatty acid content of the oils is indicated by the results in Table 4.

TABLE 4.

Influence of the Method of Extraction on the Yield and Acidity of Oil from Stockfish Intestines.

Method of Extraction.	Yield of Oil.	% F.F.A. (Average M.W. taken as 340)
Desiccation	2.61%	39.5
Pepsin digestion	2.35%	25.3
Alkali digestion	1.30%	<1

The three methods of extraction gave equally reproducible results. Duplicate determinations of the oil content by any one method did not differ by more than 5%, and in most cases the agreement was closer than this. Since the sampling error

was/.....

was probably considerably greater than 5%, there was no point in attempting to achieve greater accuracy.

TABLE 5.

Influence of Method of Extraction on the Yield, Vitamin A Potency and Unsaponifiable Matter Content of the Oil Obtained.

Material	Method of Extraction	% Oil	$E^{1\%}_{1cm.}$	% Unsap. Matter	$E^{1\%}_{1cm.}$ x % Oil	% Unsap x % Oil
Kabeljou livers.	Desiccation	26.9	46.5	5.82	1250	156
	Pepsin	26.6	43.2	6.95	1150	185
	Alkali	25.1	46.4	7.34	1160	184
Vaalhaai livers.	Desiccation	77.7	16.4	2.58	1270	200
	Pepsin	76.2	17.4	2.51	1320	191
	Alkali	76.4	17.6	2.52	1320	192
Stockfish intestines.	Desiccation	3.50	158	28.0	553	98.0
	Pepsin	3.16	176	25.6	556	81.0
	Alkali	2.06	277	37.7	571	77.7
Stockfish intestines.	Desiccation	5.03	83.0	-	418	-
	Pepsin	4.96	79.0	-	392	-
	Alkali	4.20	91.2	-	383	-
Snoek livers.	Desiccation	17.6	10.4	-	183	-
	Pepsin	18.2	9.4	-	171	-
	Alkali	15.2	12.0	-	182	-
Gurnard livers + intestines.	Pepsin	3.23	14.8	13.1	47.8	42.3
	Alkali	2.91	15.7	14.2	45.7	41.3

(ii) Recovery of Vitamin A: The desiccation, pepsin and alkali digestion techniques all achieve a quantitative extraction of the vitamin A. This is seen in Table 5. The partial or the complete removal of the free fatty acids in the pepsin or alkali digestion processes naturally enhances the vitamin A potency of the remaining oil, but the product "% Oil x $E^{1\%}_{1cm.}$ 328 mu" is the same for all three methods within the limit of experimental error. Contrary to the findings of Brocklesby (33), no evidence of any significant destruction of vitamin A in the sodium sulphate desiccation has been observed.

(iii)/.....

(iii) Recovery of Unsaponifiable Matter: The influence of the method of extraction on the percentage unsaponifiable matter in the oil is shown in Table 5. It was to be expected that, as in the case of vitamin A, the three methods should achieve a quantitative extraction of unsaponifiable matter generally. Good agreement for the figures for the pepsin and alkali digested oils was found in all instances. The recovery of unsaponifiable matter by the desiccation technique, however, was rather lower in the case of the kabeljou liver oil than that in the alkali and pepsin digestion methods; in the case of the stockfish intestinal oil, on the other hand, it was relatively higher. The reason for these differences is at present unknown. They only serve to emphasize the erratic behaviour of the desiccation technique.

(iv) Recovery of Vitamin D: Since the pepsin and alkali digestion techniques achieve a quantitative extraction of vitamin A and unsaponifiable matter generally, it is reasonable to assume that the extraction of vitamin D is also complete. It was not possible, however, to investigate this point owing to the limited capacity of the animal house.

(v) Influence of the Method of Extraction on the Iodine Value of the Oil: Where no post-mortem hydrolysis of the oil had occurred prior to extraction, the oils obtained by the three methods of extraction were found to have the same iodine value (Table 6).

TABLE 6.

Influence of the Method of Extraction on the Iodine Value of the Oil.

	Desiccation	Pepsin Digestion	Alkali Digestion
Vaalhaai liver oil	182	182	182
Skate liver oil	184	183	188
Stockfish liver oil	158	158	-
Kingklip liver oil	155	154	-
Kabeljou liver oil	-	94	97

No/....

No attempt was made to study the effect of the method of extraction on the iodine value in cases such as the stockfish intestinal oils where considerable post-mortem hydrolysis occurs, since it was felt that the loss in the alkali digestion of up to 50% of the fatty acids present so alters the balance between glycerides and unsaponifiable matter that the results would be of very little significance.

(vi) <u>Influence of the Method of Extraction on the Phosphatide Content of the Oil</u>: Many of the oils examined by the writer were found to contain a high proportion of phosphatides (up to 36%). The study of these is a complete problem in itself. The examination of the influence of the method of extraction on the phosphatide content of the oil has, therefore, been postponed until the problem as a whole is studied.

(b) <u>Application to Fish Heads</u>:

The alkali digestion method has been shown by other members of this department (215) to be applicable also to the routine extraction and estimation of the oil in fish heads.

In Table 7 are recorded data for the oil contents of various types of fish heads and, in one case, the body as determined by alkali digestion - ether extraction on the one hand, and by vacuum desiccation - ether extraction on the other. In all cases where there is appreciable oil in the heads there is reasonable correspondence between the oil yields by the two methods. As the alkali digestion method is much more rapid than the vacuum desiccation method and yields the oil in much better colour, odour and general condition, it is, therefore, to be preferred when an approximate index of the percentage fat in such material is required. It must be stressed, however, that alkali digestion cannot be expected to give accurate values for the oil content unless the material is fresh and the oil secreted is neutral in character. No figures for the recovery of non-saponifiable matter from this type of material can be

given/....

given; work on this aspect of the subject, however, is still in progress.

TABLE 7.

Comparison of Vacuum Desiccation and Alkali Digestion Methods for Extraction of Oil from Fish Heads.

Material.	% Oil.	
	Alkali Digestion	Vacuum Desiccation
John Dory heads	1.6	1.6
.. .. bodies	6.2	6.3
Kabeljou heads	2.2	2.2
.. ..	3.6	3.6
Snoek heads	14.6	15.2
.. ..	8.3	8.4
Geelbek heads	14.2	14.3
.. ..	15.1	16.6
Jacopever heads	10.6	10.6
.. ..	6.2	10.2
Gurnard heads	0.31	0.52
.. ..	1.00	0.92

(5) Use of the Pepsin Digestion Technique in the Present Studies.

The pepsin digestion technique has been used to a considerable extent in the present work for the extraction and estimation of the oil present in both livers and viscera. It has been found to be more convenient and more reliable than desiccation with sodium sulphate followed by ether extraction.

As has been mentioned before, the extraction of the desiccated material was very erratic, especially in the case of viscera containing a lot of water. On the other hand, pepsin digestion of this material always went smoothly. Again, with livers and particularly viscera containing only a small percentage of oil, the working up of sufficient material to get enough oil for subsequent analysis (at least 6-10 gm.) was far simpler by pepsin digestion than by desiccation.

The pepsin digestion method has been shown to give a quantitative recovery of vitamin A and unsaponifiable matter generally and hence almost certainly vitamin D. The yield of oil obtained by this method differs very little from that obtained by the more tedious desiccation technique. Pepsin digestion followed by ether extraction is therefore recommended in all cases such as the present where the rapid and routine estimation and extraction of oil in a suitable condition for subsequent vitamin studies is desired.

The alkali digestion technique has also been shown to be very suitable for work of this nature. It has the advantage over both the desiccation and pepsin digestion methods in its rapidity of extraction. It is, however, less suited to the application to visceral tissues in which considerable post-mortem hydrolysis occurs than is the pepsin digestion method. For this reason it was not used in the present studies.

III. METHODS OF EXAMINATION OF OIL SAMPLES.

Iodine Value.

The iodine value was determined by the Wijs method, the time of contact being one hour (30).

Saponification Value.

This was determined by the A.O.A.C. method (5).

Unsaponifiable Matter.

The procedure followed was that recommended by the Society of Public Analysts' sub-committee on the determination of unsaponifiable matter in fats and oils (216).

Vitamin A.

The vitamin A content of the oil was estimated spectrographically using a Hilger E3 quartz spectrograph in conjunction with a sector photometer. Cyclo-hexane was used as a solvent. The determination was carried out on the oil directly and not via the unsaponifiable matter, since in all but a few cases the vitamin A content of the oil was so high that extraneous absorption at 328 mu could be ignored.

The results are expressed as $E^{1\%}_{1cm.}$ 328 mu. In view of the wide differences of opinion as to the correct factor for converting $E^{1\%}_{1cm.}$ into I.U. per gm., this procedure was considered most satisfactory. Where it was wished to calculate the actual percentage of vitamin A in the oil (for example in the calculation of the ratio vitamin A : total unsaponifiable matter), use was made of the convention $E^{1\%}_{1cm.}$ 328 mu = 1600 for pure vitamin A (page 14).

Vitamin D.

The vitamin D content of the oils was estimated biologically using the 'line test' method. The procedure described by Coward (48) was followed. The rachitogenic diet used was Steenbock's No. 2965 (239). The length of the preparatory period was 21 days, while the actual test period was 10 days. The animals were not dosed daily, but were given the complete dose at the beginning of the curative period. A small

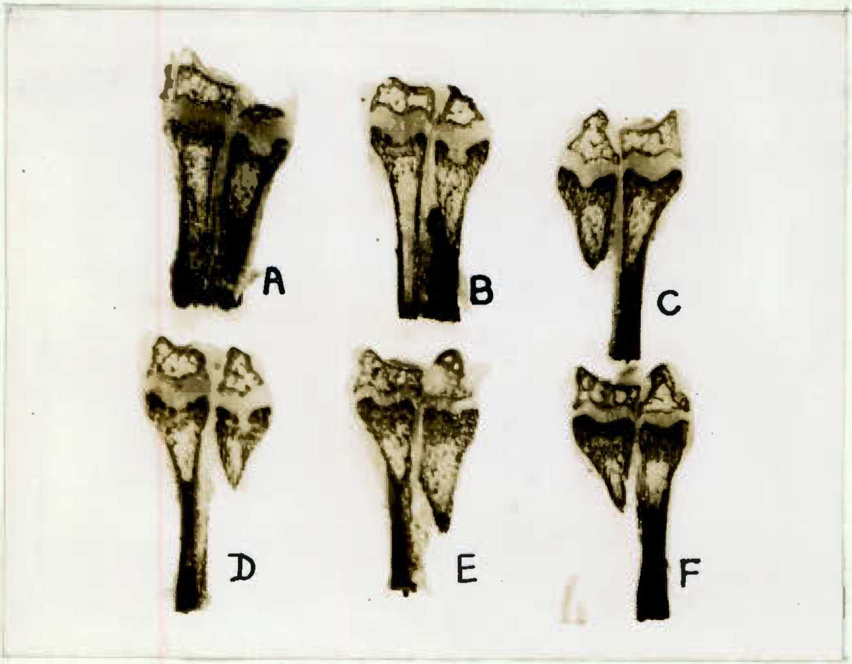


FIG. 2. Failure of Line Test.

Rats which had been reared on
University Stock Diet A.

The rats were all from one litter. The doses of vitamin D given were: A control, B 1.15 I.U., C 2.3 I.U., D 4.6 I.U., E 9.2 I.U., F 18.4 I.U.

modification was introduced in the treatment of the bones for the reading of the line test. The radius and the ulna were not separated and cut as described by Coward. Instead both bones were cut longitudinally in one operation (Fig. 2). This procedure was found to be simpler than Coward's while the sections obtained in this way gave exactly the same picture on development.

The normal stock diet of the rat colony of the Department of Physiology (Stock Diet A) has the following composition:

Mealie meal	32%
Sussex ground oats	32%
Lucerne meal	6%
Wheaten bran	5%
Peanut meal	5%
Linseed meal	5%
Fish meal	10%
Oat germ meal	2%
Bone meal	1%
Cod liver oil	2%

(Ca = 1.23%, P = 0.84%, Ca:P ratio = 1.46:1)

As this diet contains 2% of cod liver oil, it was probable that it would be too rich in vitamin D for use as a stock diet for animals required for vitamin D assays. Nevertheless, for the sake of convenience and simplicity in the management of the rat house, a preliminary attempt was made to use young rats reared on this diet for assay purposes. The test was carried out as described above and in all 8 litters, (48 rats) were used. Figure 2 is typical of the results obtained for the line test in this series. It should be noted that the control shows moderately severe rickets. In no case, however, was a 'line' observed. Instead, most litters showed a progressive narrowing of the rachitic metaphysis with increasing dosage of vitamin D.

Similar results have been reported by many workers. For example, in an early account of the line test, Coward (49) states 'If the degree of rickets has been severe ----- calcification ranges from a very narrow line across the metaphysis of the rat on the lowest dose of vitamin D to a thicker line on the higher doses ---- . <u>If the degree of rickets has been less severe, the metaphysis is less wide and calcification actually begins at the ends of the long bones and extends into the metaphysis towards the base of the epiphysis.</u>' She further reproduces camera lucida drawings of a line test of this type and these form a series which closely resembles that obtained in the present studies. Bourdillon et al. (27) also report essentially the same experience and they too stress that 'this is only common in litters where the development of rickets prior to dosing has been imperfect'. The failure to get a satisfactory line test in this preliminary trial may, therefore, be directly attributed to the high vitamin D content of stock diet A. The vitamin D reserves of the young rats were too high and consequently, although they developed rickets after three weeks on the rachitogenic diet, it was not severe enough, for, as McCollum et al. (178) pointed out in their original description of the line test, it is essential at the end of the preparatory period that the epiphyseal cartilage and metaphysis should be calcium free, i.e. it is not sufficient merely to produce rickets, an exaggerated form of florid rickets must be caused to develop without fail.

As stock diet A was thus unsuitable for the present work an alternative stock diet for the rats required for vitamin D assay had to be found. Fortunately this was not far to seek. The Biological Control Laboratories of the Union Health Department, Cape Town, have been carrying out vitamin D assays for a number of years and have developed a satisfactory stock diet, the composition of which is as follows:

Rolled oats	63%
Casein	20%
Bird seed	10%
Dried yeast	5%
Salt mixture	2%
(Steenbock's No. 40)	

This diet (stock diet B) was, therefore, used for further work. Actually it was not found necessary to rear a separate colony of rats for assay purposes. This was a considerable advantage as the rats bred far better on diet A than on diet B. Young rats are not born with large reserves of vitamin D even if the mother's diet is rich in the vitamin (21). Vitamin D is transferred somewhat more readily via the milk, but even from this source the quantity is not sufficient to affect the development of rickets later (118). The young first begin to build up significant reserves of vitamin D during the latter half of the suckling period when they start to eat their mother's food (117). The procedure adopted, therefore, was as follows. Female rats bred for assay purposes were fed on diet A during pregnancy and for the first 14 days of lactation. At this stage, i.e. just before the young open their eyes, diet B was substituted. After weaning the young rats were continued on diet B until they were heavy enough to be put on to the rachitogenic diet. Using this technique, severe rickets developed in 3 weeks and a satisfactory line test was always obtained. It has been found by other workers in the Department of Physiology that a satisfactory line test can be obtained in rats reared on Stock Diet A if the preparatory period is extended to 4 weeks. Under these conditions, the vitamin D reserves of the young rats become depleted and severe rickets develops. The treatment described above, i.e. the introduction of diet B and the use of a 3 weeks' preparatory period, is shorter and was, therefore, preferred in the present work.

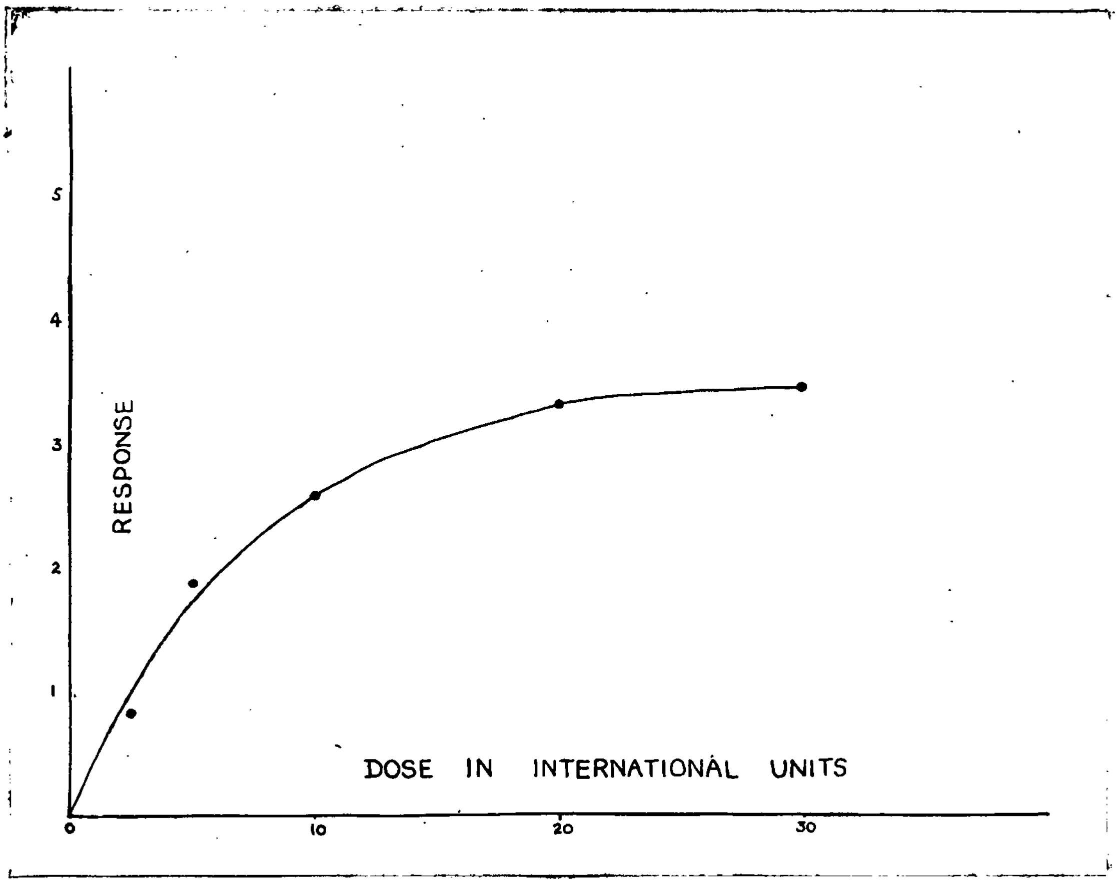


FIG. 3. Dose-response curve relating healing of rats of University stock to dose of vitamin D given.

As altogether about 20 vitamin D assays were contemplated and as, in most cases, the approximate range of potency of the oils was known, economy of labour and of animals was effected by constructing a dose-response curve. For this purpose 11 litters of rats were used, one rat in each litter receiving 0, 2.5, 5, 10, 20 and 30 I.U. of vitamin D respectively at the beginning of the test period. The curve obtained is reproduced in Fig. 3, while the data on which it is based are summarized in Table 9. This curve was then used in all the assays in the manner described by Coward (48).

TABLE 9.

Dose-Response Curve.

	Average value of degree of healing of rats receiving one dose of vitamin D					
Litter	No dose	2.5 I.U.	5 I.U.	10 I.U.	20 I.U.	30 I.U.
48	-	0	-	3	4	4
49	0	1	4	1½	4	3
50	0	0	1	2	2	3
51	0	0	¾	1½	3	3
52	0	0	1½	3	2½	3½
58	0	2	1½	4	4	4
59	0	2	3	3	3	4
60	0	1	2	2	4	3
61	0	0	1½	3	2	3
62	0	1	½	1½	5	3½
63	0	2	3	4	3	4
Average	0	0.82	1.88	2.58	3.32	3.45

Proof of the reliability of the results of the vitamin assays carried out in the present studies was obtained by the co-operation of Dr. H.A. Shapiro of the Union Health Department who kindly consented to check the assays of certain samples.

INDIVIDUAL SOUTH AFRICAN FISH AS SOURCES OF VITAMINS A AND D.

PART I.

1. THE STOCKFISH OR HAKE : MERLUCCIUS CAPENSIS (CAST.).

The stockfish is very closely related to the North Atlantic hake (Merluccius vulgaris). Gilchrist (87) considers that the two species are identical, but according to Norman (200) they differ in several important morphological characteristics. The stockfish is also allied to Merluccius gayi which is known as the 'English' hake in New Zealand and the pescada in Chile.

From the commercial point of view, the stockfish is the most important species of fish landed in the Union. On the average, about 20 million pounds of stockfish are landed every year. The actual figures for the years 1935-1938 are given below (Table 10); owing to the war later statistics are not available.

TABLE 10.

Landings of Stockfish in the Union (1935 - 1938) (4).

Year	Quantity	Value
1935	18,700,230 lb.	£124,644
1936	20,337,128 lb.	£132,446
1937	22,686,390 lb.	£151,759
1938	24,096,742 lb.	£165,320.

The stockfish is found all around the South African coast from Walvis Bay on the West coast to Natal on the East. The most important fishing grounds are those on the West coast where unlimited quantities may be procured at all times of the year. All the material examined in the present studies came from these grounds. In addition, considerable quantities of stockfish are also taken on the Agulhas Bank and off East London. The amount caught on the Natal coast, however, is negligible. By far the greatest proportion of the fish landed is caught by the trawlers operating at a depth of 40 - 300 fathoms. In the pre-war years the landings of trawl caught fish showed a steady increase year after year as a result of the gradual growth of the trawling fleet. The inshore fisheries only account for about a million pounds of stockfish a year and these are mostly small fish.

Because of its commercial importance, a study of the oil content and vitamin A and D potency of both the liver and intestine of the stockfish was one of the first objects of the present work. A short study of the seasonal variations in the yield and vitamin A potency of the liver oil had already been made by Molteno and Rapson (190). This work has been repeated here and extended to include information on the vitamin D potency of the oils; a complete seasonal study of the intestinal oils has also been made.

Experimental.

Sampling of Material.

The livers and intestines examined were in all cases taken from fish caught on the west coast. The fish were gutted on board the trawler and the livers or viscera were placed in 2-gallon tins and kept on ice until they were delivered at the University. As the trawlers operating on these grounds are only away from port for 3 days at a time, the material arrived in excellent condition. The livers were fresh and firm and the viscera, although they tended to undergo autolysis more rapidly than the livers, were nevertheless also in a satisfactory state of preservation. For the examination of the intestinal oil, the total viscera, excluding the liver were brought to port and the intestines were removed, freed from adhering mesentery and cleaned of their contents at the University. In the stockfish, the pyloric caeca are 2 in number and are present as very small outgrowths of the pyloric end of the intestine. In the present work, the 'intestine' was, therefore, taken to include both the pyloric caeca and the intestine proper.

For samples obtained in this way, no information was available as to the weight and condition of the fish concerned. The livers examined throughout these studies, however, were very uniform in size so that the size factor has little influence on the results. In the case of the intestines, a wider variation was encountered, but this was reduced by selecting as far as possible only the intestines of the larger fish.

In order to study the influence of the size of the fish on the vitamin A potency of the oil, and also to determine the percentage by weight of the different organs in the fish, a number of fish were brought ashore ungutted. Unfortunately it was found that under these circumstances the viscera, including the liver, tended to undergo autolysis much more rapidly than usual and the experiment had to be repeated several times before the information required could be obtained. The reason for this behaviour was not clear as kingklip handled in exactly the same way arrived in perfect condition.

By the courtesy of Dr. C.J. Molteno, a number of samples of liver oil were also obtained which had been extracted in a pilot plant from 100 - 300 lbs. of liver.

Extraction of Oils.

For the extraction of the livers at the University either ether extraction or pepsin digestion was used. The samples obtained from Dr. Molteno were extracted by alkali digestion followed by centrifuging. The method of extraction is not considered to have any influence on the results obtained; it has been shown that in cases such as this, i.e. where the material is fresh and the oil content is high, all three methods give the same results within the limits of experimental error.

Unless otherwise stated, the intestinal oils were all extracted by pepsin digestion.

Blending of Oils for Vitamin D Assay.

It was not possible to do vitamin D assays on all the oils extracted as neither the time nor the animals were available. It was, therefore, necessary to blend the samples of several consecutive months for each assay. For this purpose the oils were grouped as far as possible on the basis of their vitamin A content on the assumption that seasonal variations in the vitamin D potency of the oil would be parallel to changes in the vitamin A; the oil content of the livers was also taken into account. This procedure was followed, not only in the case of the stockfish, but in all the other fish as well. Where, as in the case of the stockfish and kingklip livers, the variations in the yield of oil were small, equal weights of the different samples were blended. In fish such as the snoek, on the other hand, where the differences in the oil content of the liver are relatively far greater, the amount of each oil taken was proportional to the yield. By working in this way it was hoped to get a fair picture of the average potency of the oil over a given period.

Seasonal Studies of Stockfish Liver and Intestinal Oils.

A considerable amount of information about the seasonal changes in the yield and vitamin A potency of stockfish liver oils is now available. The figures which have been obtained cover the greater part of the last 5 years. The work of Molteno and Rapson (loc. cit.) extended from March 1938 to March 1939; the present studies cover the period September 1939 to December 1940, and, lastly, since September 1941

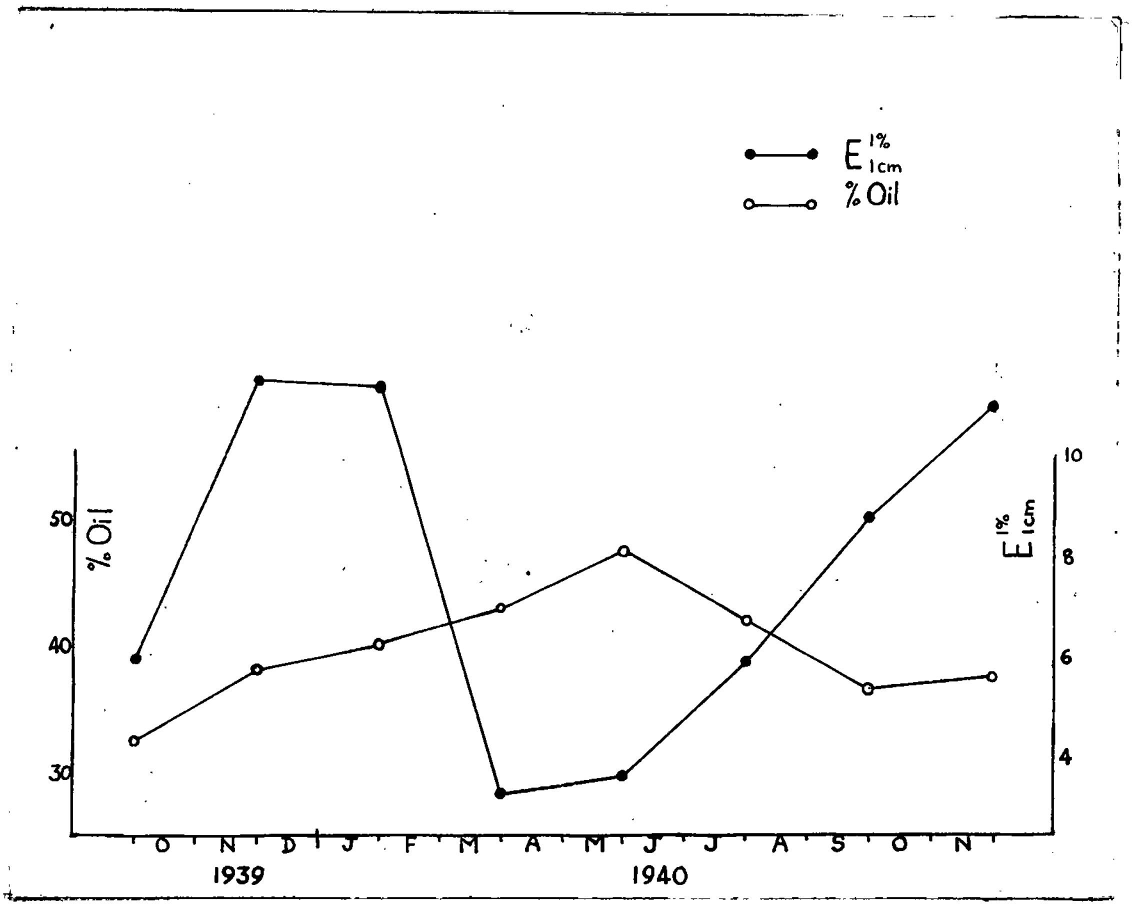


FIG. 4. Stockfish Liver Oils
Seasonal changes in the yield of oil and the vitamin A potency of bimonthly samples.

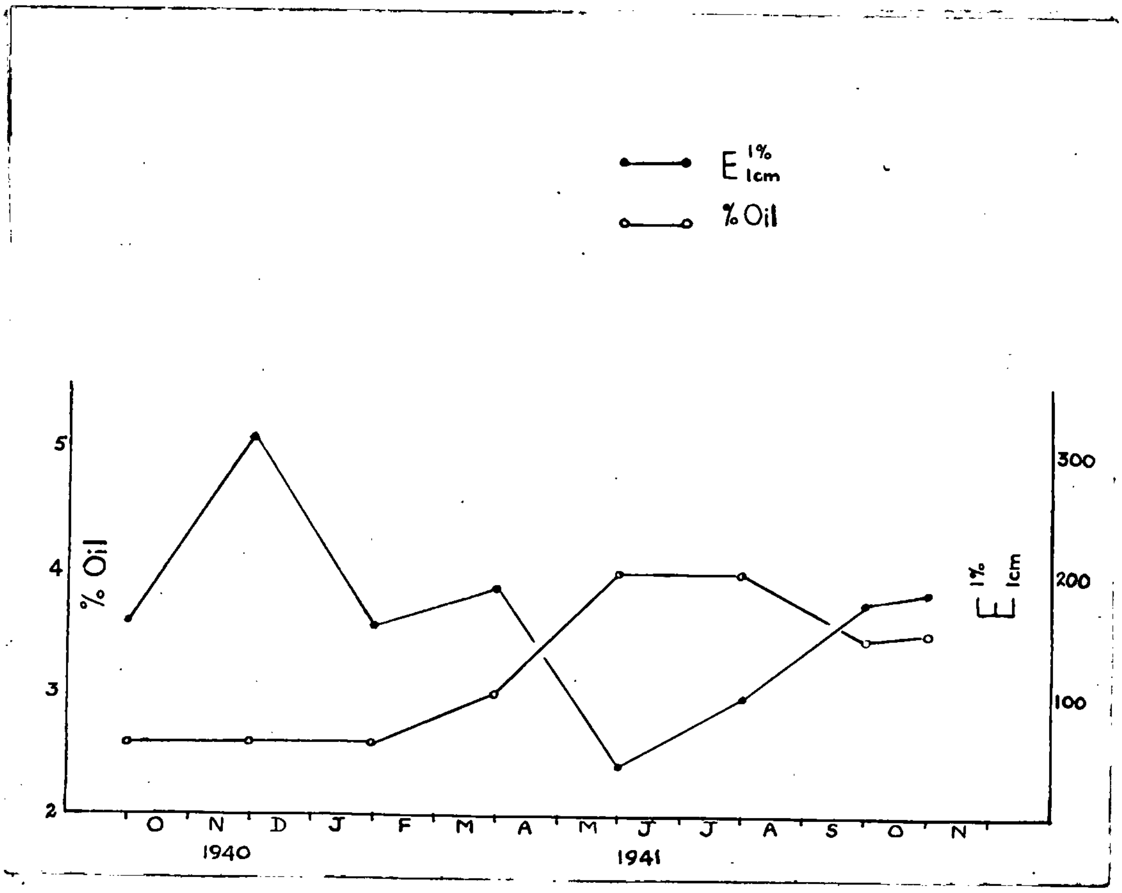


FIG. 5. Stockfish Intestinal Oils
Seasonal changes in the yield of oil and the vitamin A potency of bimonthly samples.

complete data on the yield of oil and the vitamin A content of all the stockfish livers landed at Cape Town are available from the records of Messrs. Vitamin Oils (Pty.) Ltd. In spite of the comprehensiveness of these studies, or perhaps because of it, a clear picture of the seasonal changes in the liver oil is still lacking. Information about the intestinal oils of the stockfish is far less complete and the figures given below (Table 12) are all that are available.

The results obtained in the present studies will be considered first. The data for the liver and intestinal oils are summarized in Tables 11 and 12 respectively; the relationship between the E value of the oils and the iodine value and percentage unsaponifiable matter is shown in Figs. 6 and 7. The seasonal changes in the oil content and the vitamin A potency are shown graphically in Figs. 4 and 5, where mean bi-monthly values have been plotted in order to get somewhat smoother curves.

It will be seen that the changes in the oil content of the liver are not great. Nevertheless a definite seasonal cycle was observed, the yield of oil being at its lowest from September to November, rising to a maximum in June and then falling off again. The vitamin A potency of the liver oil shows a similar trend, but in the opposite direction, i.e. the highest values were recorded from November to January and the lowest from April to June. As far as the vitamin D potency of the oil is concerned, no seasonal trend is obvious from the figures in Table 11. The results, as far as they go, seem to indicate that, as in the case of the cod (20), the vitamin D content of the oil remains more or less constant throughout the year, but more work on this aspect of the subject is necessary before any definite statement can be made. The study of the intestinal oils was not undertaken during the same year as that of the liver oils, but the seasonal changes observed were essentially the same as those described above, i.e. the yield of oil was at its lowest and the

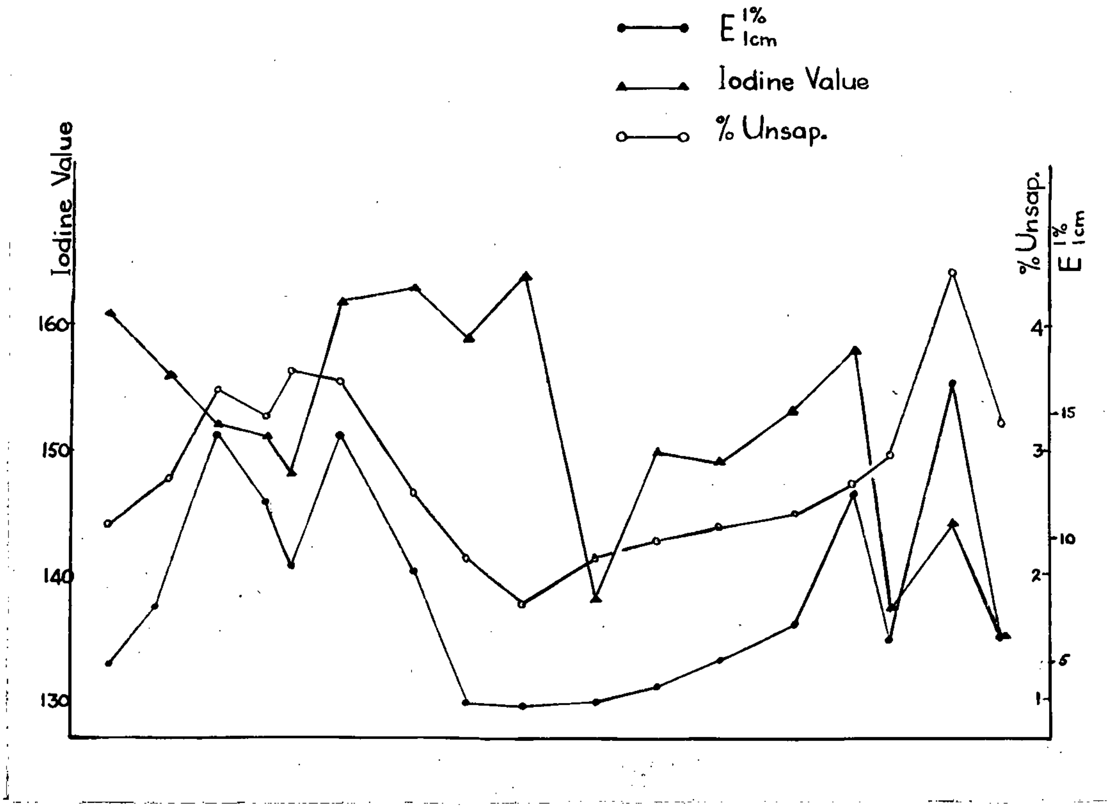


FIG. 6. Stockfish Liver Oils
Relation between E value, iodine value
and percentage unsaponifiable matter.

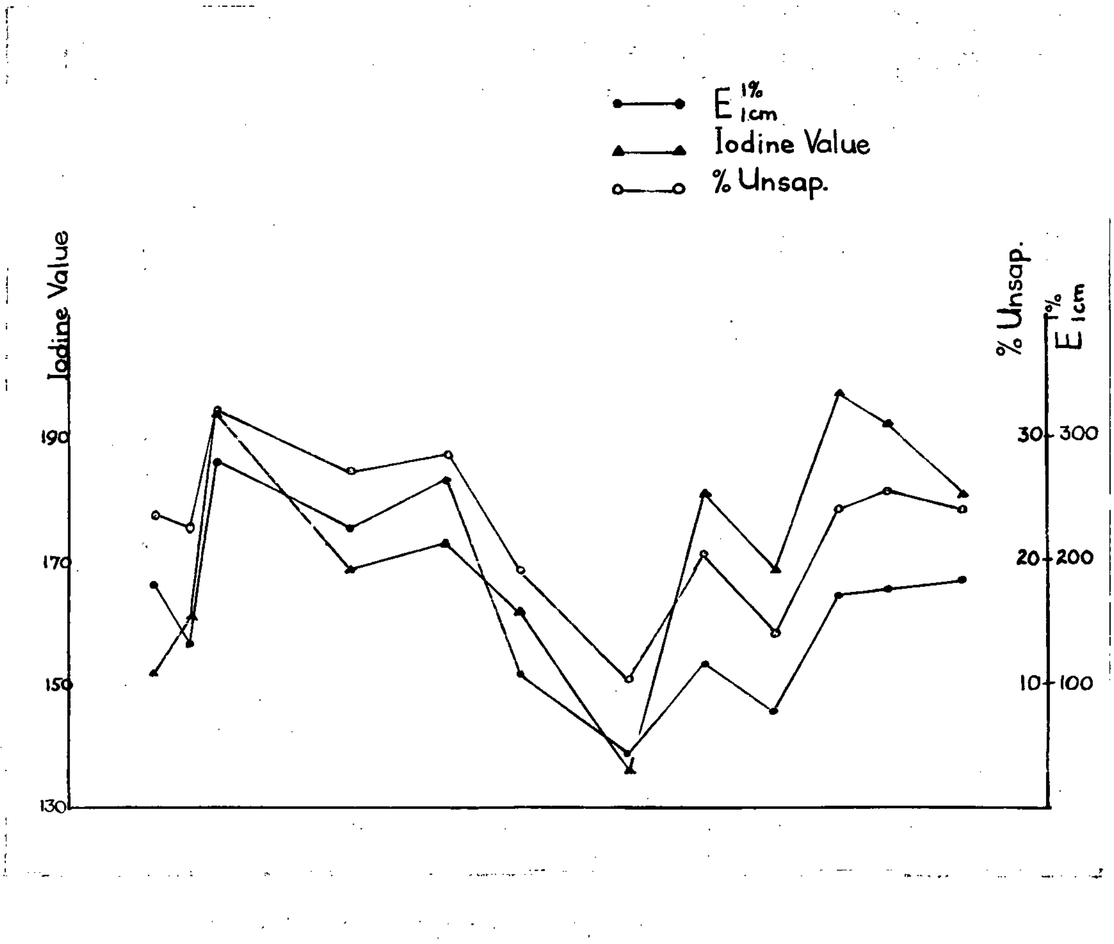


FIG. 7. Stockfish Intestinal Oils
Relation between E value, iodine value
and percentage unsaponifiable matter.

TABLE 11.

Seasonal Variations in the Oil and Vitamin Content of Stockfish Livers.

Date	Total wt. of sample (kg.)	Method of extraction	% Oil	Iodine value	Sap. value	Unsap. matter %	Vitamin A $E^{1\%}_{1cm.}$	Vitamin D I.U./gm.
16. 9.39	Bulk sample	Alkali digestion	ca 30	161	-	2.40	4.8	170
10.10.39	"	"	ca 35	156	186	2.77	7.2	
6.11.39	"	"	35-40	152	185	3.48	14.2	150
5.12.39	"	"	35-40	151	185	3.27	11.4	
20.12.39	"	"	ca 40	148	-	3.63	8.8	
8. 1.40	"	"	ca 40	162	186	3.65	14.1	
19. 2.40	"	"	ca 40	163	-	2.66	6.6	
-. 3.40	"	"	-	159	183	2.12	3.4	130
12. 4.40	1.8	Ether/Na_2SO_4	43	164	186	1.76	3.2	
19. 5.40	3.3	"	45	138	176	2.14	3.4	
20. 6.40	4.8	"	50	160	170	2.28	4.0	
18. 7.40	2.4	"	45	149	175	2.36	5.1	
29. 8.40	3.2	"	39	153	181	2.46	6.6	380
23. 9.40	5.4	"	34	158	185	2.71	11.7	
14.10.40	7.9	Pepsin digestion	39	137	189	2.98	5.8	
11.11.40	2.6	"	28	144	181	4.42	16.1	
5.12.40	7.8	"	47	135	182	5.21	5.8	

TABLE 12.

Seasonal Variations in the Oil and Vitamin Content of Stockfish Intestines.

Date	Weight of sample (gm.)	% Oil	Iodine value	Unsap. matter %	Vitamin A $E^{1\%}_{1cm.}$	Vitamin D I.U./gm.
14.10.40	1200	2.6	152	23.9	182	Not more than 3 I.U./gm.
28.10.40	1700	2.3	161	22.7	131	
11.11.40	720	2.3	194	32.4	282	
10.12.40	760 a	2.9	-	-	336	
16. 1.41	980	2.8	169	27.3	226	
3. 2.41	920 e	2.5	-	-	85	
3. 3.41	1070	3.0	173	28.6	265	
10. 4.41	1700	3.1	162	19.1	108	
5. 6.41	1350	4.0	136	10.1	41	
10. 7.41	2000	3.0	181	20.6	117	
15. 8.41	1360	5.0	169	14.0	79	
18. 9.41	1850	3.7	197	24.1	172	
13.10.41	1750	3.2	192	25.6	176	
17.11.41	1080	3.5	181	24.2	183	

e samples ether extracted. All other samples pepsin digested.

vitamin A potency at its highest during the summer months while the reverse was true during the winter.

The figures obtained during 1941 and 1942 by Messrs. Vitamin Oils also fit into this picture and the writer is very much indebted to Dr. E.R. Roux for permission to consult his records and discuss his results. The monthly averages are given below (Table 13) are based on the semi-weekly extraction of at least 2000 lbs. of liver.

TABLE 13.

Stockfish Livers extracted by Messrs. Vitamin Oils.
Seasonal Variations in the Oil Content and Vitamin A Potency.

Date	Yield of Oil	$E^{1\%}_{1cm.}$ 328 mu.
Sept. 1941	29.1	7.8
Oct. ..	28.0	9.1
Nov. ..	21.9	10.6
Dec. ..	23.3	9.6
Jan. 1942	25.8	9.2
Feb. ..	27.4	8.7
Mar. ..	29.1	7.4
April ..	31.1	7.0
May ..	30.9	7.1
June ..	33.8	6.5
July ..	32.6	6.3
Aug. ..	32.6	6.0
Sept. ..	29.6	8.6
Oct. ..	26.0	10.0

The greatest part of this material came from the 'north grounds', i.e. from the same fishing grounds as the livers studied by the writer, but figures for livers from the Agulhas Bank were also included in the monthly averages as they showed exactly the same range of values. The livers were extracted by alkali digestion followed by centrifuging and in all cases the yield of oil was 80-90% of the theoretical. The E value of the oil was determined by a Hilger vitameter A. Here again the oil content of the liver was at its lowest during the early summer months and at its highest during the winter; as before, the reverse was true for the vitamin A potency of the oil. The chief difference between these results and those obtained by the writer is that in 1942 the lowest values for the vitamin potency of the oil - and, conversely, the highest figures for the yield of oil - were recorded from June to August, i.e. rather later in the year than in 1940. Differences of this nature, however, are to be expected, for in no two years are the seasons exactly the same. This has been observed not only in the case of the stockfish but also in all the other species handled in the present work.

The results of Molteno and Rapson, on the other hand, are far more difficult to fit into this picture. Their data on the oil content of the liver are rather scattered and do not show any definite trend, but the vitamin A content of their oils shows a well-defined seasonal cycle. These authors, however, record the lowest values for the vitamin content of the oil during February and March, while the most potent oils were obtained from May to June. In other words, their experience was almost the reverse of that described above. This difference cannot be accounted for by any differences in the fishing grounds or in the age group of the fish as the livers examined also came from the north grounds and the fish were of the same size group as those handled by the writer. Neither is it likely that it is due to any sampling error as the size of their samples [5-80 kg. (188)] was, on the average, considerably greater than those examined in the present work. These results must, therefore, be taken to be representative of the behaviour of the stockfish on the west coast during 1938 and the early part of 1939.

No explanation of the seasonal changes in the liver oil of the stockfish can be offered at present. It does not appear likely that the sexual cycle plays an important part in these changes. According to Barnard (10) the stockfish spawn from July to October and the figures for the landings of ripe roe by the trawlers support this view. The oil content of the livers, however, reaches a minimum rather later in the year than this, so that it is difficult to explain the results entirely in terms of the effect of spawning on the fat reserves of the fish. Further, the results of Roux and of the writer indicate that there is an increase in the vitamin A content of the liver, as distinct from the liver oil, during this period; this is also difficult to reconcile with the effects of spawning. Nevertheless, it is possible that the sexual cycle does have some influence on the oil content and vitamin potency of the livers, but is overshadowed by some other factor.

It is also not possible to say how far the seasonal changes in the liver oil are related to the food cycle. The diet of the larger fish consists chiefly of rat tails (Macrurus spp.) while the young stockfish feed mainly on 'krill' (Euphausids). Nothing, however, is known of the seasonal variations in the abundance of the food supply, nor are any data on the oil content or vitamin potency of these species available. Further studies on the stockfish are thus necessary. Part of the information needed will be supplied within the next 3 or 4 years from the records of the oil content and vitamin potency of the livers processed by Messrs. Vitamin Oils. From these figures it should be possible to get a fair idea of the differences in the seasonal changes from year to year. At the same time work on the abundance, fat content, vitamin content, etc. of the food supply should be undertaken. Then when complete data on all the different aspects of the subject are available, it may be possible to build up a satisfactory picture of the influence of the various factors on the oil content and vitamin potency of stockfish liver and intestinal oils.

Influence of the Size of the Fish on the Vitamin A Reserves of the Liver.

The influence of the size of the fish on the weight of the livers and the yield of oil and vitamin A is given in Table 14. The fish examined here were all caught in the same haul. They were brought ashore ungutted and were weighed and cleaned at the University.

TABLE 14.

Size group.	No. of fish.	Av. wt. livers.	% Oil	$E^{1\%}_{1cm.}$	Vitamin A reserves of liver.
0 - 1 lb.	17	12 gm.	40.3	2.2	7 mg.
1 - 3 ..	29	24 ..	41.3	3.4	21 ..
3 - 5 ..	8	68 ..	38.4	5.1	84 ..
5 - 10 ..	9	93 ..	38.3	4.8	107 ..
10 - 16 ..	7	221 ..	37.3	7.2	372 ..

These results indicate that the size of the fish has little influence on the oil content of the liver. On the other hand, the vitamin A potency of the oil tends to increase with increase in the weight of the fish. This is in agreement with the findings of other workers, for example Lovern (167) and Pugsley (211) with the halibut, McPherson (182) with the Newfoundland cod and Pugsley (210) with the British Columbia greyfish. The increase in the vitamin A reserves of the livers of the bigger stockfish is very striking and tends to support the theory that the vitamin A in fish livers is the result of the gradual accumulation of the vitamin present in the food.

Commercial Importance of Stockfish Liver and Intestinal Oils.

The production of stockfish liver oil in South Africa is of very considerable commercial importance. As may be seen from Table 15, the livers constitute roughly 4% of the weight of the fish as landed, i.e. beheaded and gutted.

TABLE 15.

Percentage by Weight of the Visceral Organs of the Stockfish.

	On whole fish	On fish as landed
Head	16.7%	22.7%
Liver	3.2%	4.3%
Intestine	1.0%	1.4%
Stomach	2.4%	3.3%
Gonads	1.6%	2.2%

From these figures it may be calculated that nearly a million pounds of stockfish livers are available for extraction annually. The potential oil production is thus in the neighbourhood of 30,000 gallons a year. The oil, which is extracted by alkali digestion of the livers, is of good colour and has only a slightly fishy taste. In vitamin A potency it is far superior to the average cod liver oil, but its vitamin D content is relatively low. In the municipal clinics and welfare centres in this country, stockfish liver oil has replaced imported cod liver oil. The bulk of the oil produced, however, is exported and much of it goes to America where it is used for the manufacture of concentrates.

So far no attempt has been made to extract the intestinal oil. This contains, on the average, 250,000 I.U. of vitamin A per gram and is a very potent source of this vitamin; the vitamin D content, on the other hand, is negligible. Because of their low oil content, the extraction of the intestines is a matter of some difficulty. The best method of treatment would probably consist of alkali digestion followed by the addition of a solvent oil such as peanut oil. Alternatively, the livers and intestines could be worked up together, the liver oil acting as a solvent for the vitamin A of the intestines. By this means the vitamin A potency of the liver oil could be increased by at least 60% and its value more than doubled.

2. THE KINGKLIP : GENYPTERUS CAPENSIS (SMITH).

It is convenient to consider the kingklip next, for, not only does it resemble the stockfish in its habits, but the livers of the two species are also very similar in their oil content and in the vitamin A and D potency of the oil.

The kingklip belongs to the family Ophidiidae. According to Barnard (10), it is very similar to Genypterus blacodes (Bl. Schn.) which is found in Australia, New Zealand and Chile and a detailed comparison of the two species might prove them synonomous. The kingklip is one of the finest food fishes of the Cape and is of considerable commercial importance although the annual landings (Table 16) are far smaller than in the case of the stockfish. It is caught almost exclusively by the trawlers, particularly by those operating on the west coast.

TABLE 16.

Landings of Kingklip (1935 - 1938) (4).

Year	Quantity	Value
1935	1,025,268 lb.	£12,115
1936	1,117,353 lb.	£12,555
1937	1,069,794 lb.	£12,096
1938	1,182,109 lb.	£13,234

A brief survey of the seasonal changes in the yield and vitamin A potency of kingklip liver oil was made by Molteno and Rapson (190). This work has now been repeated and extended to include information on the vitamin D potency of the oils. A short study of the intestinal oils has also been made.

Experimental.

For sampling of the material and the extraction and analysis of the oil, the same procedure was followed as was described in the case of the stockfish.

Seasonal Studies of Kingklip Liver Oil.

As may be seen from Fig. 8 and from the data presented in Table 17, the liver oil of the kingklip shows no marked seasonal changes. The yield of oil varies very little from month to month and most of the values lie between 30 and 40%. The samples collected at the end of 1939 are exceptional, but these were extracted on a semi-commercial scale and it is possible that the recovery of the oil was not complete. The variations in the oil content are rather irregular and it is not possible to detect any seasonal trend from these results. On the other hand, the changes in the vitamin A potency of the oil are rather more marked and here there are indications of a definite seasonal trend. The highest values were obtained during the summer months while the lowest figures were recorded from June to August. The vitamin D content of the oil apparently remains constant throughout the year.

Unfortunately in the kingklip it has not been possible to follow up these results. No complete record of the oil content and vitamin A potency of the livers extracted by Messrs. Vitamin Oils is available as there was in the case of the stockfish. The figures, as far as they go, however, are on the whole in agreement with those discussed above. Again, the variations in the yield of oil were irregular. As before, the most potent oils were obtained in February, but the lowest values were recorded somewhat later in the year, namely during September and October.

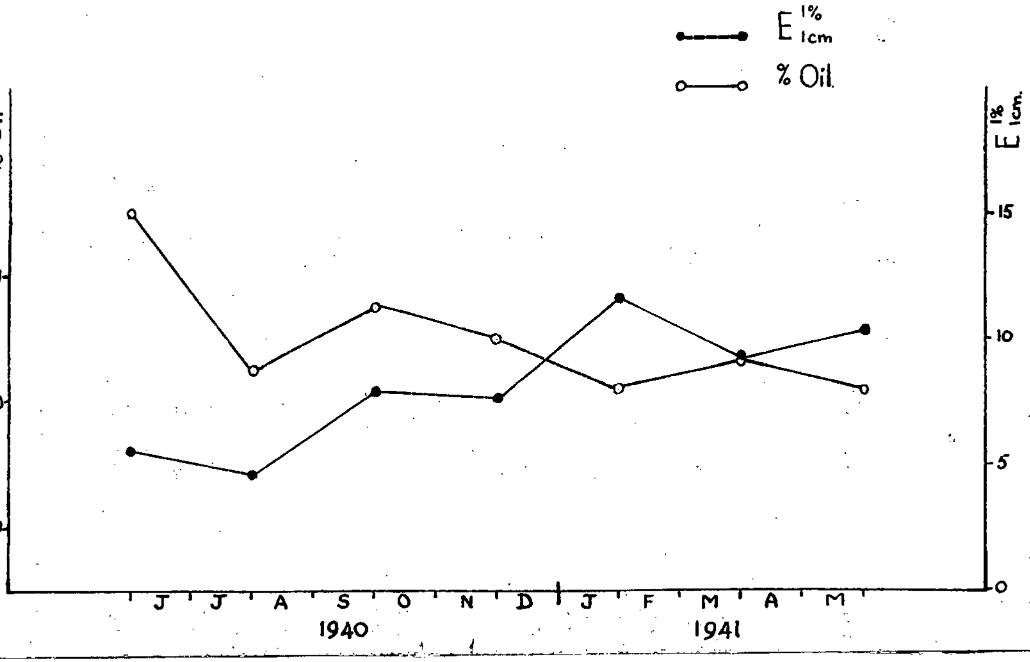


FIG. 8. Kingklip Liver Oils
Seasonal changes in the yield of oil and the vitamin A potency of bimonthly samples.

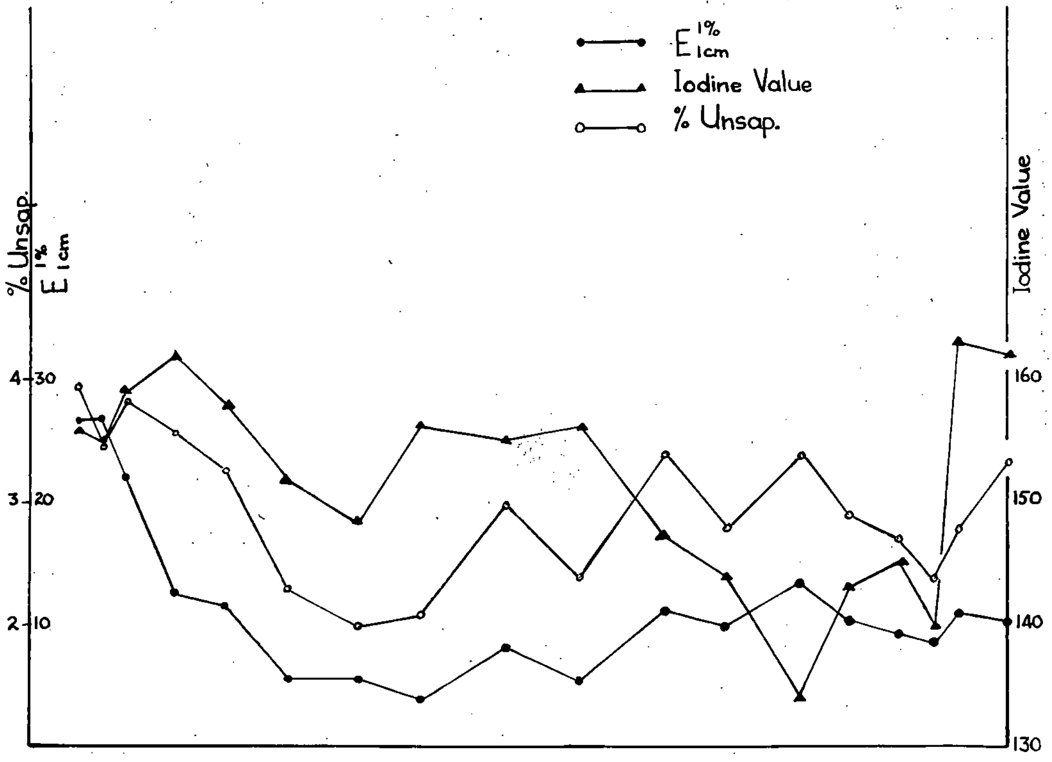


FIG. 9. Kingklip Liver Oils
Relation between the E value, iodine value and the unsaponifiable matter.

TABLE 17.

Seasonal Variations in the Oil and Vitamin Content of Kingklip Livers.

Date	Total wt. of sample (kg.)	Method of extraction	% Oil	Iodine value	Sap. value	Unsap. matter %	Vitamin A $E^{1\%}_{1cm.}$	Vitamin D I.U./gm.
20.11.39	Bulk sample	Alkali digestion	ca 30	155	184	2.91	8.5	
23.11.39	"	"	15-20	156	185	3.94	26.4	130
5.12.39	"	"	10-15	155	183	3.46	26.7	
20.12.39	"	"	10-15	159	182	3.82	21.9	
12. 1.40	"	"	10-12	162	185	3.66	12.5	
11. 2.40	"	"	ca 25	158	183	3.25	11.5	
6. 6.40	5.6	Ether/Na$_2$SO$_4$	45	152	177	2.29	6.6	110
15. 7.40	2.0	"	30	148	180	1.99	6.4	
12. 8.40	1.9	"	35	156	175	2.04	3.8	
26. 9.40	5.1	"	36	155	184	2.97	2.0	
14.10.40	5.9	Pepsin digestion	39	150	187	2.65	-	
4.11.40	8.2	"	40	156	183	2.58	6.3	
12.12.40	2.4	"	30	147	183	3.37	11.0	85
13. 1.41	8.1	"	36	144	183	2.77	9.9	
17. 2.41	7.5	"	26	134	182	3.37	13.4	
17. 3.41	5.5	"	36	143	182	2.87	10.0	
7. 4.41	5.6	"	34	145	197	2.70	9.2	
28. 4.41	Bulk sample	Alkali digestion	ca 30	140	200	2.36	8.5	
4. 5.41	"	"	31	163	184	2.78	10.7	
1. 6.41	"	"	-	162	183	2.82	10.1	

These results are very similar to those obtained in the stockfish. The yield of oil, the vitamin A and D potency and the analytical figures all fall within the same range in both species. Further, the two fish show the same general seasonal trend, i.e. in both cases the vitamin A content of the oil is at its lowest during the winter and reaches a maximum in the summer months. The fact that the maxima do not coincide exactly does not detract from the general similarity of the results as the two studies do not cover exactly the same period and, as has already been pointed out in the case of the stockfish, the seasonal changes vary slightly from year to year. This similarity between the liver oils of the stockfish and the kingklip is interesting because the two species are not closely related. The stockfish belongs to the family Gadidae of the division Anacanthini while the kingklip belongs to the division Percomorphi and is more closely related to the snoek, kabeljou, etc. than to the stockfish. On the other hand, the two species are very similar in their habits. Both are deep-sea fish and, in the case of the livers examined here, both were caught on the same grounds. This suggests that the changes in the vitamin A potency of the liver oil may be related to some change in the environment or the food supply which affects both species. Unfortunately nothing is known about these factors and all that can be done is to point to this as a possible line of approach for future research.

In this connection it is also interesting to note that in the case of the kingklip the results of Molteno and Rapson (loc. cit.) bear the same relation to those of the writer as they did in the case of the stockfish. These workers found that the vitamin A content of kingklip liver oil remained more or less constant throughout the year except for two samples examined during January where, contrary to the results of the present work, the potency fell sharply. The significance of these results is difficult to assess. On the om hand, the two samples in question are rather isolated and there is an interval of nearly 3 months before the next sample; this makes their significance rather doubtful. On the other hand, a similar fall in the vitamin A potency of stockfish liver oil was observed during that particular period. This lends further support to the suggestion that in both fish the vitamin A content of the liver oil is influenced by the same factor or factors.

It is also of interest to compare the liver oils of the kingklip with those of the closely related New Zealand ling (Genypterus blacodes) which has been studied by Shorland (56) (231) (232).

TABLE 18.

Comparison of the Liver Oils of the Kingklip (Genypterus capensis) with those of the N.Z. Ling (G. blacodes).

	% Oil.	I.V.	Unsap.	$E^{1\%}_{1cm.}$	Vitamin D.
Kingklip	30-40	140-160	2-4%	5-13	100 I.U./gm.
Ling (232)	35	137-153	2-4%	8-12	500 I.U./gm.

As may be seen from Table 18, the yield of oil, the analytical figures and vitamin A potency are similar in the two species. It is surprising therefore to find that the vitamin D content of kingklip liver oil is only one-fifth of that of ling liver oil. This, however, may be a general feature of the oils of the two countries; it will be discussed further under the stonebass. The ling, like the kingklip, shows no major seasonal changes in the vitamin A content of the liver oil. According to Shorland (231) the highest values are also recorded during January and February and the lowest during July and August. In the ling, the changes in the vitamin potency of the oil are apparently related to spawning when the vitamin A reserves are used up to a greater extent than the oil.

How far this is also true for the kingklip it is not possible to say as no information on the spawning habits of the latter are available.

The Intestinal Oils of the Kingklip.

A short study of the intestinal oils has been made. In the kingklip the intestine is long and muscular and the pyloric caeca which usually number 7 or 8 are small; as in the stockfish, the 'intestine' was taken to include both the intestine proper and the pyloric caeca. As may be seen from Table 19, the intestines are of little use either as a source of oil or of vitamin A. In view of the similarity of the liver oils, the contrast between the vitamin A content of the intestinal oils of the kingklip and those of the stockfish is very striking. Moreover, if it is shown that the vitamin A content of the liver oils is related to the food supply, this difference should be of considerable importance.

Table 19.

Kingklip Intestinal Oils.

Date	Wt. of sample	% Oil	$E^{1\%}_{1cm.}$	I.V.	Unsap.
3. 3.41	>2 kg.	1.1	8.5	176	18.6%
30. 6.41	3.6 ..	1.7	6.5	175	14.1%
13.10.41	1.3 ..	1.9	4.9	151	14.8%
16.12.41	1.3 ..	0.9	16.1	-	-

Influence of Age on the Vitamin A Reserves of the Kingklip.

The influence of the size of the fish on the vitamin A reserves of the liver and intestine is shown in Table 20. In the kingklip, as in the ling (231), the vitamin A content of the liver oil shows no relationship to the age of the fish.

This is rather unusual and has been attributed, in the case of the ling, to the high vitamin A content of the diet which makes very little accumulation of vitamin A necessary to produce a liver rich in the vitamin. On the other hand, if the yield of oil is taken into account, it is found that the concentration of vitamin A in the liver does increase with the size of the fish. Moreover, the percentage of liver in the fish also rises sharply. Consequently, in the kingklip, as in most other fish, the vitamin A reserves of the liver are proportionately far greater in the big fish than in the smaller ones. The same is true of the intestinal vitamin A.

TABLE 20.

Influence of Size of Fish on the Vitamin A Reserves.

Size group	No. of fish	Av. wt. fish	Av. wt. liver	% Liver in fish	Av. wt. intestine	% Int. in fish
< 5 lb.	17	$3^1/_2$ lb.	21 gm.	1.3	30 gm.	1.9
5-10 lb.	15	7 ..	87 ..	2.7	64 ..	2.2
10-20 ..	10	$14^1/_2$ ..	215 ..	3.3	125 ..	1.9

	Liver Oil.			Intestinal Oil.		
Size group	% Oil	$E^{1\%}_{1cm.}$	Vit. A in liver (mg./100 gm.)	% Oil	$E^{1\%}_{1cm.}$	Vit.A in intestine. (mg./100gm.
< 5 lb.	15.6	9.7	95	0.7	3.3	1.4
5-10 lb.	36.3	5.5	124	0.5	21.0	6.6
10-20 ..	34.6	7.9	172	0.9	16.0	8.6

Commercial Importance of Kingklip Liver Oil.

The liver oils of the kingklip are of fair commercial importance though the annual production (about 1500 gallons) is far less than in the case of the stockfish. Kingklip liver oil is very similar to stockfish liver oil in taste and appearance as well as in vitamin A and D content and may be used to supplement the supplies of the latter.

3. THE SNOEK : THYRSITES ATUN (EUPHR.).

The snoek belongs to the group Scombroidei which includes fish such as the mackerel, the bonito and the tuna which are noted for the high vitamin A and D potency of their liver oils.

The snoek is found throughout the Southern Hemisphere. The same species is known in Australia and New Zealand as the barracouta and in Chile as the sierra. The snoek is a truly oceanic fish and very little is known of its migratory habits, even along the South African coast. It first appears on the west coast, in the region of Walvis Bay, between October and January. It arrives at points further south along the coast later in the year. Table Bay is usually reached in April or June and the season here lasts till about the end of July. These dates very considerably from year to year and the writer was extremely fortunate that in both 1940 and 1941 the snoek appeared in the vicinity of Cape Town unusually early and it was possible to get data extending over the greater part of the year. The snoek exhibits a decided dislike for warm water and is therefore not caught further east than Mossel Bay or Port Elizabeth. Where the snoek goes to when it leaves these shores is unknown; the whole question of its migrations will be discussed later in greater detail.

The snoek is rarely taken in the trawl. It is caught almost entirely on hand lines and is the most important species handled by the inshore fisheries in South Africa. It is highly prized as a food fish, especially by the Cape Coloured community. The quantity and the value of the catch varies considerably from year to year depending on whether the season is good or bad. In Table 21 are given figures for the landings of snoek at Cape Town in the last few years.

In the snoek, the pyloric caeca are 8-10 in number and extend for about a third of the length of the intestine; altogether they make up rather more than half the total weight of the 'intestines'. The amount of mesenteric fat found associated with the intestines varied considerably, depending on the condition of the fish. This was removed as completely as possible before the oil was extracted.

The oil was extracted either by ether extraction or by pepsin digestion. Ether extraction was used throughout the 1940 season, whereas in 1941 pepsin digestion was used in all cases except in the penultimate sample which had been preserved in formalin and which was ether extracted.

Seasonal Studies of the Liver and Intestinal Oils.

It is well-known that the snoek vary greatly in their condition at different times of the year. In Table Bay and False Bay, the fish caught from April or June till the end of July are fat and in prime condition. Those taken at the end of the season, however, are very lean and are known amongst fishermen as the 'poor' snoek. As is to be expected, the liver and intestines show the same changes in oil content as does the fish as a whole.

The seasonal changes in the liver and intestinal oils have been summarized in Table 22. The liver oils were studied for two seasons. The changes in oil content and vitamin A potency are expressed graphically in Fig. 10; these show the same tendency in both years. The intestines were only studied in 1941 but the results obtained fit into the general picture already drawn for the liver oils. As can be seen from Fig. 10, the earliest snoek of the year have livers which contain rather less oil than those caught later on during the height of the season. The vitamin A and D potency of this oil is higher than that of the later samples but there is apparently little change in the total vitamin

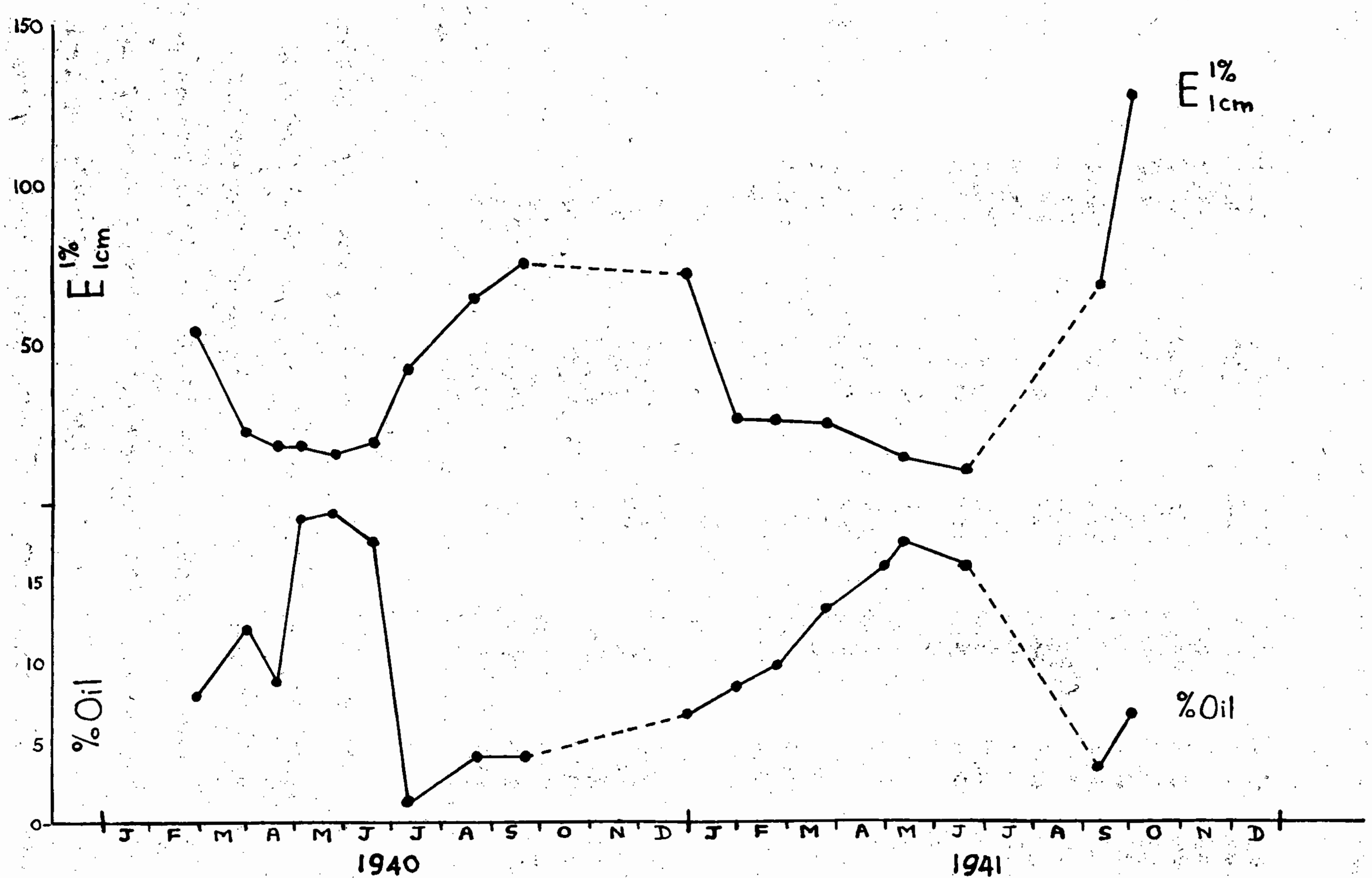


FIG. 10. Snoek Liver Oils
Seasonal changes in the yield of oil and
the vitamin A potency of monthly samples.

TABLE 22.

Seasonal Changes in Snoek Liver and Intestinal Oils.

Date	Wt. of sample (kg.)	% Oil	Iodine value	% Unsap. matter	Vitamin A $E^{1\%}_{1cm.}$	Vitamin D I.U./gm.
			Liver Oils.			
1. 3.40	0.3	8.0	120	5.58	52.8	
28. 3.40	1.1	12.1	168	3.38	22.4	
17. 4.40	-	8.8	-	-	17.6	
4. 5.40	0.8	19.1	164	3.33	17.6	500
25. 5.40	1.4	19.4	171	2.73	15.0	
17. 6.40	0.7	17.7	137	2.31	-	
20. 6.40	2.6	17.3	154	2.82	19.0	
12. 7.40	1.9	1.3	119	-	41.6	
20. 8.40	0.7	4.1	126	10.1	64	6000
19. 9.40	0.5	4.1	-	-	75.2	
3. 1.41	3.6	6.9	165	14.05	72.0	1700
3. 2.41	2.7	8.5	136	5.67	26.1	
24. 2.41	1.5	9.8	140	5.81	25.4	
24. 3.41	1.7	13.3	155	4.17	24.6	
28. 4.41	7.9	16.2	161	2.66	-	860
8. 5.41	3.6	17.6	159	2.50	13.3	
16. 6.41	1.2	15.9	148	1.25	10.4	
11. 9.41	0.4	3.7	-	-	69.2	
2.10.41	0.2	6.9	176	18.7	128	
			Intestinal Oils.			
24. 3.41	0.7	8.0	158	6.77	49	80
8. 5.41	>1.8	8.1	168	4.86	29	
19. 5.41	1.1	13.1	162	4.53	27	
16. 6.41	0.9	8.9	167	6.85	34	
11. 9.41	0.5	3.2	-	-	135	
2.10.41	0.3	4.8	185	32.3	294	
2. 6.42	1.5	15.1	163	4.07	14	

content of the liver. As the season advances, the oil content increases steadily until the livers contain 16-20% of oil. At the end of the season, when the poor snoek are caught, there is a dramatic change. The livers are lean and dark red in colour in contrast with the lemon coloured livers of the preceeding months. The oil content drops suddenly to 1-4%. The vitamin A potency of the oil increases, slowly at first and then more rapidly. There is also an increase in the vitamin D potency. The intestines follow exactly the same cycle as the livers.

It is interesting to note that the liver oil shows a marked seasonal variation in vitamin D potency. The snoek thus differs from the stockfish where, as we have already noticed, the vitamin D content of the liver oil remains more or less constant through the year even though there is a definite rise and fall in the vitamin A potency. It has unfortunately not been possible to calculate whether there is any gain or loss of vitamin D in the liver of the snoek during the year. It is possible that the vitamin D content of the liver as a whole remains unchanged, but that infiltration of fat during the season results in dilution of the liver oil during this period. On the other hand, as will be shown later, there is a definite loss of vitamin A in the poor snoek and the same may be true for vitamin D. No seasonal study of the vitamin D potency of the intestinal oils was made. All the 1941 samples were blended together; the vitamin D content of the pooled oils (80 I.U. per gm.) is considerably lower than that of the livers, but the difference is not as great as in the stockfish.

Before it is possible to discuss these results further, it is necessary to consider the migration of the snoek along the South African coast in greater detail. This matter has been dealt with fairly fully by Biden (16), and the following discussion is taken largely from this source. The snoek which visit the west coast are all mature fish. Young snoek less than 24 inches (the legal limit) are comparatively rare visitors to these shores. Baby snoek, 4-6 inches long, are sometimes found in the stomachs of the fully grown fish caught in the Walvis Bay region, but the young of this size remain for the most part more than 100 miles out to sea and are very seldom observed within a mile of the shore. It is popularly believed that the snoek which reach Walvis Bay between November and January later travel southwards along the coast following the shoals of sardines which are their chief food. According to this theory, the early or late arrival of the snoek at the Cape is largely influenced by irregular shore currents between Walvis Bay and Table Bay and by alternating winds - factors which are responsible for the course of the sardine.

An alternative explanation for the fish's different seasons on the west coast is that different shoals strike inshore from the South Atlantic, arriving at different points along the coast at different times of the year, i.e. the fish caught in Table Bay and False Bay do not belong to the same shoal as those caught at Walvis Bay. The gradual withdrawal of the shoals from the different coastal areas may, according to Biden (loc. cit.) 'correspond with an annual change of current to a south-westerly direction and incidentally synchronize with current influences controlling the migration of the sardine'. At present it is not possible to say which of these two theories is correct. Tagging of snoek has been carried out, but of the thousands of fish which were marked only 2 or 3 were recovered and these did not throw any light on the problem. (26). In this connection a seasonal study of the snoek caught in Walvis Bay would be of great interest and might supply an important clue.

Equally little is known about the course of the snoek when they leave these shores. It is popularly believed by the fishermen that they turn somewhere between Mossel Bay and Port Elizabeth and strike out to sea in a south-easterly direction. This, however, is pure speculation. From a broader view of the migration and taking into account the fact that the fish visits the coasts of Chile, Australia and New Zealand, it has been suggested that the true migratory course is circular - round the globe from west to east in low latitudes. Here again there is no evidence to support such a theory. It would, however, be interesting to compare the liver oils of fish caught in different parts of the world from this point of view. Unfortunately the only other figures available are those obtained by Davies and Field (55) and Jowett and Davies (133) for the Australian barracouta. These are given below (Table 23). The fish examined were 35-40 inches in length, i.e. the same size as the snoek caught in South African waters. They were all lean and most of them carried well-developed gonads. They resembled the poor snoek in the size of the liver and the yield of oil, but the vitamin A potency of the oils was lower than the values obtained in the present studies. This would indicate that the Australian fish have lower vitamin A reserves than those caught in South Africa (compare also Table 24).

TABLE 22.

Barracouta Liver Oils - Jowett and Davies (133).

Date	Wt. of liver	% Oil	$E^{1\%}_{1\ cm.}$	Total vitamin A in liver
28. 4.37	24 gm.	10.4	5.3	8.3 mg.
"	26 gm.	3.5	24.2	13.7 "
"	6 gm.	5.0	20.2	3.8 "
"	16.5 gm.	6.7	17.7	12.3 "
20.11.37	41 gm.	2.4	65.6	40.5 "
"	46 gm.	3.0	12.0	10.4 "
"	51 gm.	10.2	8.0	26.0 "
"	39 gm.	4.4	44.8	48.0 "

So far nothing has been said about the spawning habits of the snoek. Barnard (10) states that the snoek spawn in September and in the present studies it was observed that the poor snoek all had well-developed gonads. The condition of these fish is therefore attributed to the strain imposed on them by the demands of the maturing eggs and sperm.

The seasonal changes in the liver and visceral oils are thus related to the sexual cycle. That changes occur during the spawning period which profoundly affect the vitamin A content of the liver oil has long been realized. The cod, for example, eats little or no food at such times and the drain on the fat reserves incidental to fasting is increased by the utilization of large quantities of liver oil in the development of the genital products. A certain amount of vitamin A is transferred with this oil to the gonads (267) and the total vitamin reserves of the liver are depleted. The snoek differs from the cod in that the liver is not the main storage depot; the head, for example, may contain up to 20% of oil, i.e. 4 or 6 times the total oil content of the liver. Nevertheless the effect of spawning on the liver is apparently very similar to that observed in the cod. The figures obtained (Table 24) indicate that there is a loss of vitamin A from the liver, but owing to the fact that the fat reserves are depleted to a greater extent than those of the vitamin, the residual oil has a higher content of both vitamins A and D than the oil obtained when the fish are fat.

TABLE 24.

Influence of the sexual cycle on the vitamin A reserves of the liver.

Date	Average wt. of liver.	% Oil	$E^{1\%}_{1\ cm.}$	Total vitamin A in liver.
4. 5.40	80 gm.	19.1	17.6	168 mg.
12. 7.40	50 "	1.3	41.6	28 "
25. 8.40	60 "	4.1	64	84 "
19. 9.40	ca40 "	4.1	75.2	78 "
24. 3.41	65 "	13.3	24.6	134 "

It was unfortunately not possible to repeat these observations in 1941 or to extend them to include the 'visceral' oils as no samples could be obtained in July or August owing to weather conditions. The fish examined in September and October were not caught in False Bay. Sample No. 19 (11.9.41) consisted of fish caught at Gansbaai, some 60 miles further east along the coast, while the last sample (2.10.41) was taken by the trawlers - a very unusual occurrance. These fish are, therefore, not strictly comparable with those caught earlier in the season. The figures obtained by Molteno (188), however, confirm these results. The snoek he examined were all caught in False Bay. The vitamin A reserves of the livers (Table 25) have been calculated from his data.

TABLE 25.

Date	No. of fish	Av. wt. of liver	% Oil	$E^{1\%}_{1cm.}$	Total vitamin A in liver.
26. 2.38	6	130 gm.	7.1	48	227 mg.
28. 7.38	2	76 "	3.5	61	101 "
28. 7.38	1	71 "	2.8	19	24 "
9. 8.38	4	51 "	2.5	46	37 "
20. 8.38	2	40 "	3.8	180	170 "
24. 8.38	3	68 "	4.9	34	78 "
10. 3.39	4	95 "	17.2	32	163 "
4. 4.39	3	105 "	21.7	7	103 "

Again it is observed that the livers of the poor snoek contain less vitamin A than those which are not subjected to the strain of spawning. There are certain exceptions but, as the number of fish in each sample is rather small, these are probably due to differences in the vitamin A reserves of the individual fish.

The utilization of the liver oil in this way during spawning should give rise to a peak in the potency curve immediately after spawning. It was not possible to observe this in the case of the snoek as the fish are not caught during this period. After spawning, the fish may be expected to start to build up their fat reserves again. The oil content of the liver increases but the drop in the potency of the oil should be mitigated by the fact that the fish is also building up its vitamin reserves. Something of this trend is seen in fish caught in the early months of 1941. Here we get a steady rise in the oil content from 7% in January to 16% by the end of April and at the same time the vitamin A potency of the oil falls from an E value of about 20 to an E value of 13.

It is thus possible to explain the seasonal changes in the liver and intestinal oil observed in these studies in terms of the sexual cycle. It should, however, be mentioned that many of the fishermen consider that the poor snoek are quite distinct from the prime fish and only appear after the fat snoek have left these shores. Biden (loc. cit.) discusses this theory and advances the suggestion that the emaciated condition of these fish is due to parasitic infection and not to the effects of spawning. How far this is true is difficult to say for, while it has been shown that the snoek may become infected with a unicelluar parasite, Chloromyxum thyrsites, which attacks the muscle fibres and causes the fish to become 'pap' within 24 hours after catching (87), proof that all the poor snoek are so infected is lacking. The present studies, however, provide some support for this theory. The very sudden drop in the oil content of the liver observed between June and July fits in better with this explanation than with the idea of the gradual development of the gonads. It may also be that the truth lies half-way between these two theories, i.e. the condition of the poor snoek may actually be due to the effects of spawning, but the fish may belong to a different shoal which visits False Bay after the earlier shoal has left. What the true explanation is remains for future research to decide.

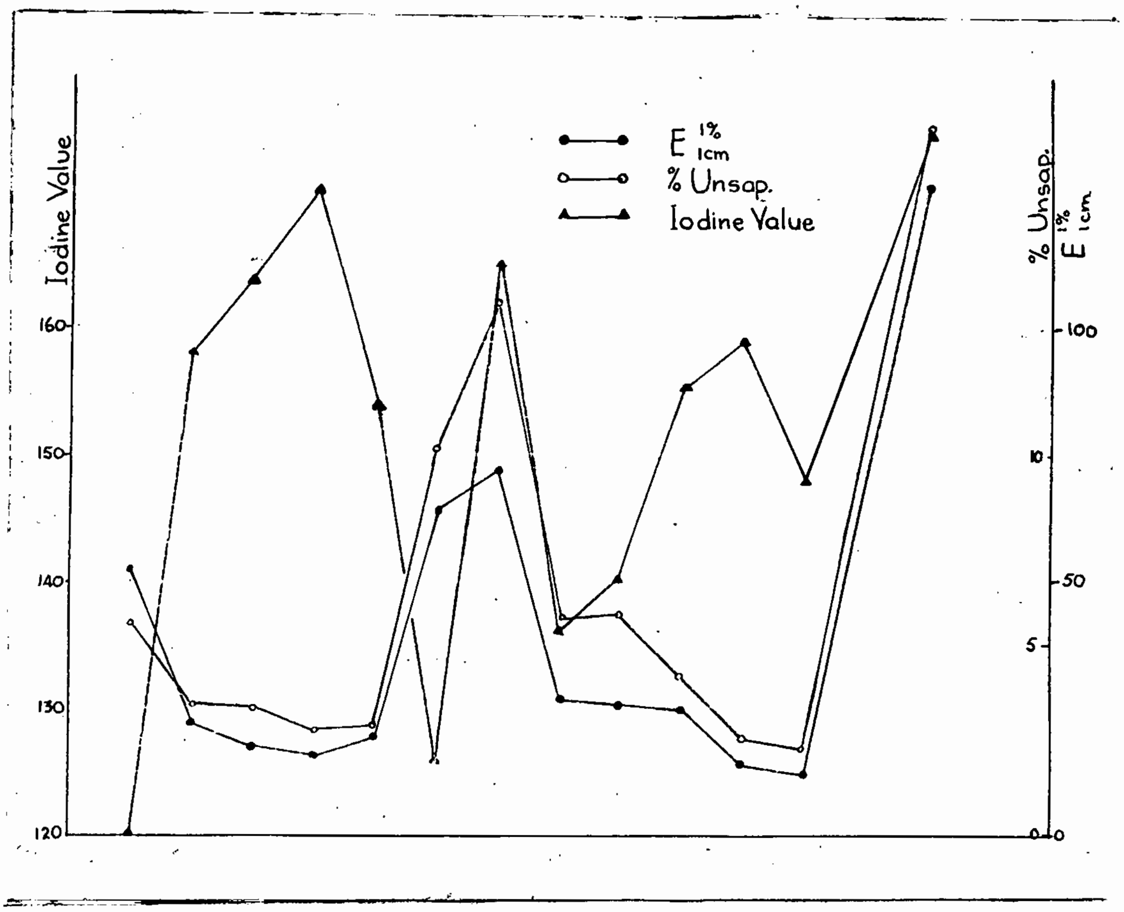


FIG. 11. Snoek Liver Oils
Relation between E value, iodine value
and percentage unsaponifiable matter.

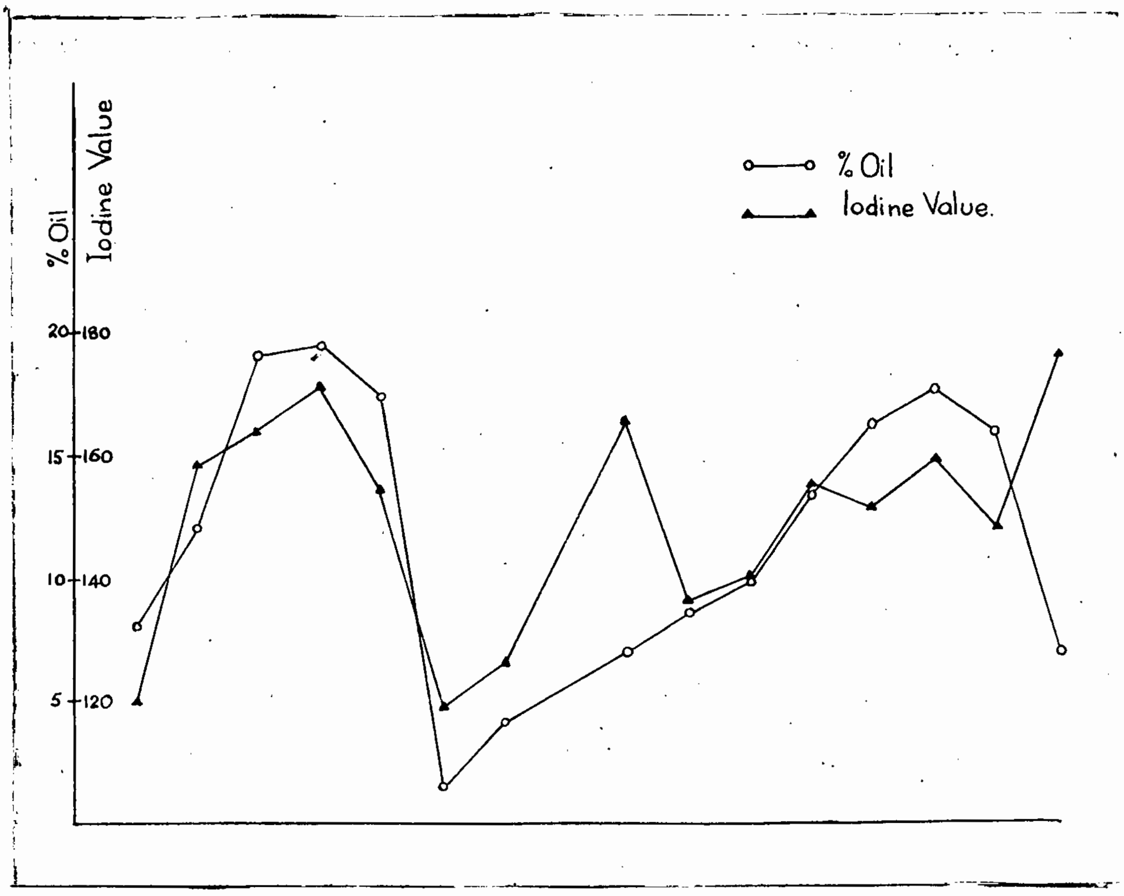


FIG. 12. Snoek Liver Oils
Relation between yield of oil
and iodine value.

In the study of the seasonal changes of the liver oil of the snoek the relationship between the vitamin A potency, iodine value and percentage unsaponifiable matter of the various samples is of considerable interest. This is shown in Fig. 11. As usual, there is a close relationship between the vitamin A potency of the oil and the percentage unsaponifiable matter. The iodine value is for the most part inversely proportional to the vitamin A content and is directly related to the percentage oil in the liver. This is clearly shown in Fig. 12. The exceptionally high iodine value of some of the high potency oils may possibly be explained by the high iodine value of the unsaponifiable matter (compare the stonebass liver oils). This relationship between the oil content of the liver and the iodine value fits into the general picture already drawn for the seasonal changes in the liver and intestinal oils. During their stay in False Bay the fish are feeding actively on a very fatty diet which consists chiefly of sardines (Sardinia spp.) but which also includes maasbankers (Trachurus trachurus), mackerel (Scomber colias) and any other small fish which cross their path. Sardines analysed by other members of this Department contained 7.2% of oil. This oil, moreover, was highly unsaturated, the iodine value being 210. The rise in iodine value of the snoek liver oil between February and May is thus a reflection of the highly unsaturated nature of the diet. The composition of the depot fats of any fish is normally characteristic of that species but, as has been shown in the case of the herring (165), when the fish are feeding actively, the selective mechanism may be temporarily unable to cope with the situation and the composition of the depot fat tends to approximate to that of the diet.

In the poor snoek, the fat reserves are used up and the iodine value of the liver oil falls. A similar tendency has also been observed in the head oils (Table 26) (216).

TABLE 26.

Snoek Head Oils.

Date	% Oil.	Iodine value.
12.5.42	15.2	158.5
16.6.42	19.6	162.1
7.8.42	10.8	148.1

The same has also been reported in the case of the herring (165) and the geelbek (189). These results all indicate that, when a fish is fasting or for any other reason drawing on its fat reserves, the more highly unsaturated constituents of the fat are preferentially utilized.

In the studies on the geelbek mentioned above, Molteno and Rapson found that vitamin A was transferred from the liver to the 'viscera' during the period of intensive feeding. They concluded that these results are in accord with the view that vitamin A is associated with the process of fat assimilation in this fish. In the case of the snoek it is not possible to make a definite statement on this point as only a few analyses of the intestines were made. The results, as far as they go, do not, however show any significant increase of the total vitamin A of the intestines with an increase in the oil content. It is possible that in the snoek any such transfer of vitamin A from the liver to the intestines would be completely masked by the changes in vitamin A content due to the spawning cycle.

The Commercial Importance of Snoek Liver and Intestinal Oils.

The liver and intestinal oils of the snoek form a valuable source of vitamin A and vitamin D. The collection and extraction of the livers was begun in 1941, and in 1942 operations were extended to include the intestines as well. The full utilization of all the material available, however, has not yet been achieved.

Both the livers and the intestines may readily be extracted by the alkali digestion process and the oils obtained in this way are deep yellow in colour and have only a slight fishy taste. The vitamin A and D potency of the liver oil is of the same order as an average halibut liver oil. There should be a considerable demand for it for medicinal purposes and it should be able to replace halibut liver oil in the South African market. Because of its relatively low vitamin D content, snoek intestinal oil is not as suitable for medicinal purposes as is the liver oil. It is however a rich source of vitamin A and as such is very valuable, especially at the present time when the demand is chiefly for high vitamin A potency oils.

The amount of oil which could be produced annually is considerable. The percentage by weight of the different visceral organs in a number of fish caught on 24.3.41 is given in Table 27. The values for the liver and intestines are somewhat lower in the poor snoek, but these are relatively unimportant from the commercial point of view as by far the greatest proportion of the fish are caught during the 'fat snoek' season.

TABLE 27.

Percentage by Weight of the Visceral Organs of the Snoek.

Liver	1.6%
'Viscera'	1.6%
Stomach	2.1%
Gonads	1.3%.

4. THE KABELJOU : SCIAENA HOLOLEPIDOTA (LACEP.).

The kabeljou is listed as Sciaena hololepidota (Lacep.) by Barnard (10) and as Johnius hololepidotus (Lacep.) by Marchand (172); the latter nomenclature is used in the annual reports of the Division of Fisheries but the former, more conservative one, is preferred by the writer.

The kabeljou has a world-wide distribution. It is found in the North and the South Atlantic, in the Mediterranean, off Madagascar and Mauritius and off the east coast of Australia. It is known in Europe as the maigre or meagre and in Australia as the jewfish. It is everywhere regarded as a valuable food fish though large examples tend to become coarse. Unfortunately no data on the liver oils of kabeljou caught in other parts of the world are available for comparative purposes. The kabeljou belongs to the family Sciaenidae which is also represented in South Africa by the geelbek (Atractoscion aequidens) and the baardman (Umbrina capensis), both of which are of fair commercial importance. The Californian white sea bass (Cynoscion nobilis) is also a member of this family.

After the stockfish and the snoek, the kabeljou is the most important species handled by the commercial fishermen in South Africa. It is caught by both the inshore boats and the trawlers and, as may be seen from Table 29, up to 1938, the annual landings were considerable. Later figures are not available but it is reported (223) that recently the landings of trawl caught fish have fallen off very considerably. This, however, may be only a temporary decrease. The kabeljou is found all around the coast from Walvis Bay on the west to Natal on the east, but it is caught chiefly in the waters between False Bay and East London. It grows up to 6 ft. in length and 160 lb. in weight; the fish handled by the commercial fishermen, however, are usually less than 30 lb.

TABLE 29.

Annual Landings of Kabeljou (1935 - 1938) (4).

Year	Quantity	Value
1935	2,351,955 lb.	£25,027
1936	3,021,905 lb.	£34,483
1937	2,872,755 lb.	£33,478
1938	3,326,591 lb.	£37,264

Experimental.

Sampling of Material.

The livers studied were from fish weighing about 4 lb. caught by the trawlers operating on the Agulhas Bank. The fish were gutted on board the trawler and the livers brought ashore in tins in the usual way.

Extraction of Oil.

The earlier samples (up till October 1940) were ether extracted; all subsequent samples were pepsin digested.

Seasonal Studies of Kabeljou Liver Oil.

From the Cape to East London, the season for kabeljou is from October to April. The trawlers, however, land fish more or less continuously throughout the year and the writer was able to get regular monthly samples for a period of 16 months.

As may be seen from Fig. 13 and from the data presented in Table 30, there is a well-defined seasonal variation in both the yield and the vitamin potency of kabeljou liver oil. The oil content of the livers is lowest during the off season. In October when the fish become more abundant, the yield begins to rise and reaches a maximum round about January,

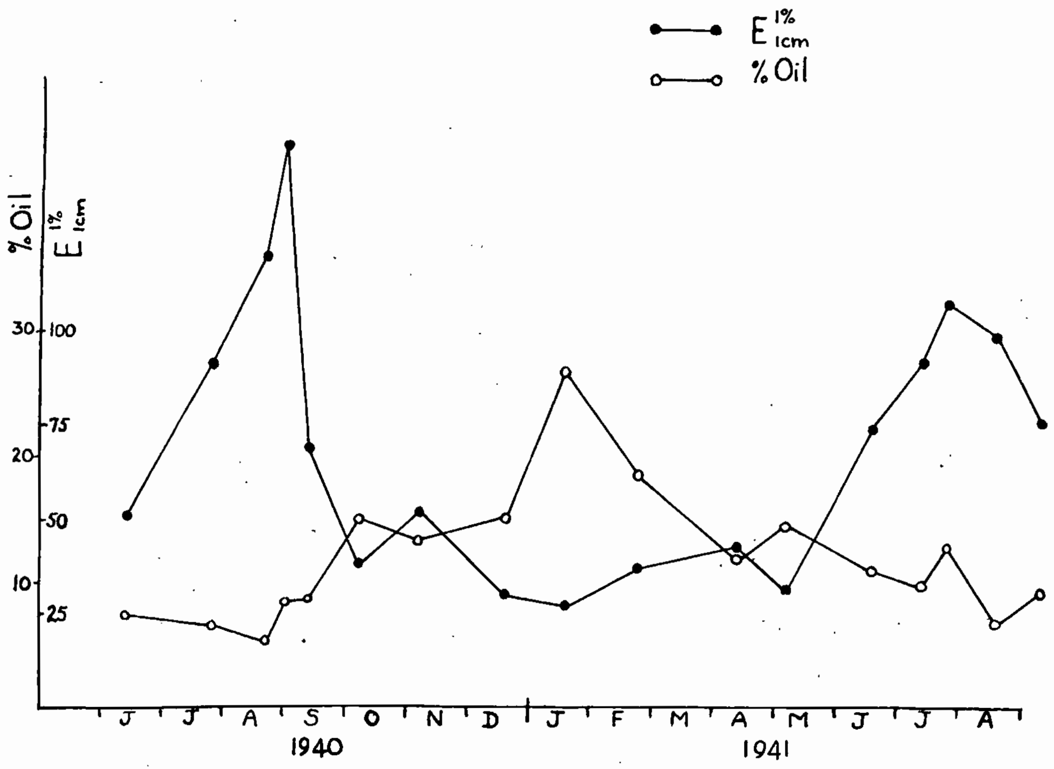


FIG. 13. Kabeljou Liver Oils
Seasonal changes in the yield of oil and the vitamin A potency of monthly samples.

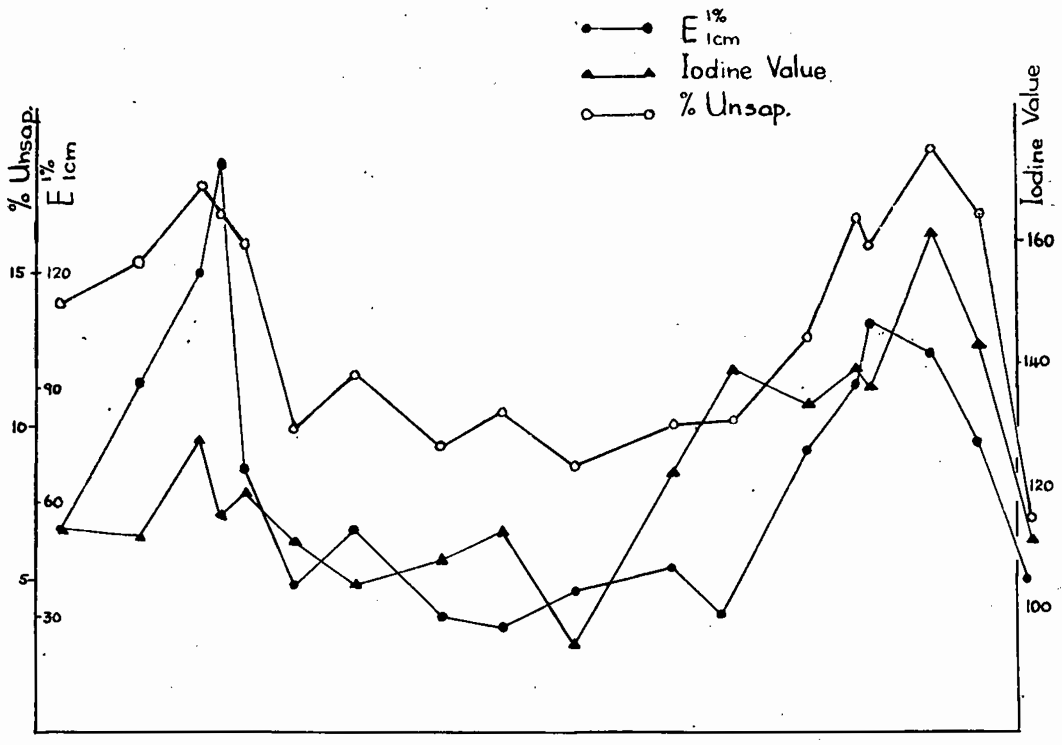


FIG. 14. Kabeljou Liver Oils
Relation between E value, iodine value and percentage unsaponifiable matter.

after which it falls off again. The vitamin A content of the oil is roughly inversely proportional to the yield. It remains at a low level from October to May or June and then rises sharply in July, August and September. The vitamin D content of the oil behaves in the same way.

TABLE 30.

Seasonal Variations in the Oil and Vitamin Content of Kabeljou Livers.

Date	Wt. of sample (kg.)	% Oil	Iodine value	% Unsap. matter	Vit. A $E^{1\%}_{1cm.}$	Vit. D (I.U./gm.)
4. 3.40	1.7	7.9	-	8.1	22	
11. 3.40	0.3	12.9	125	9.6	66	
12. 6.40	3.6	7.4	113	13.9	51	4000
22. 7.40	3.0	6.7	112	15.3	91	
21. 8.40	1.8	5.4	128	17.8	120	
2. 9.40	1.6	8.5	115	16.9	149	
12. 9.40	7.7	8.9	119	15.9	69	
8.10.40	3.2	15.2	111	9.9	38	1700
7.11.40	5.4	13.3	104	11.7	59	
19.12.40	1.0	15.2	108	9.3	30	
16. 1.41	4.5	26.9	113	10.4	27	
24. 2.41	2.7	18.6	94	8.7	37	
10. 4.41	0.5	11.9	122	10.0	43	
8. 5.41	1.1	14.4	139	10.1	31	
16. 6.41	0.7	10.9	133	12.9	74	6000
14. 7.41	0.2	9.8	139	16.7	92	
21. 7.41	1.0	12.9	136	15.8	107	
18. 8.41	1.5	6.7	161	18.9	99	
11. 9.41	1.1	9.2	143	16.9	76	
6.10.41	1.6	26.5	105	7.0	43	

These seasonal changes do not appear to be related to the sexual cycle. According to Barnard (10), the kabeljou spawn from October to December. It is just at this period, however, that the oil content of the liver begins to rise. The vitamin A content of the oil falls but that of the liver remains more or less constant. The drain on the fat reserves of the fish incidental to spawning must, therefore, be small, and, as in the case of the halibut (167), it is apparently completely masked by the influence of other factors. At present, the nature of these is not known.

Molteno (188) has reported the results of a few analyses of the livers of kabeljou caught by the inshore boats in False Bay. It is interesting to compare these figures with those given above. The fish he handled were, on the whole, bigger than those studied by the writer, but the range of values encountered (Table 31) is essentially the same as that for fish caught on the Agulhas Bank.

TABLE 31.

Kabeljou from False Bay (Molteno).

Livers.

Date	No. of fish	Av. wt. of fish	% Oil	$E^{1\%}_{1cm.}$
2.2.38	2	10 lb.	6.5	19
3.3.38	2	24 lb.	8.0	26
26.6.38	4	15 lb.	9.5	69
24.1.39	4	31 lb.	7.8	88
3.2.39	5	$1^1/_2$ lb.	11.4	3.5

Commercial Importance of Kabeljou Liver Oil.

With the exception of kabeljou liver oil, South African fish liver oils contain relatively little vitamin D. Kabeljou liver oil is, therefore, particularly valuable as a source of this vitamin. Its vitamin A content is also appreciable and is superior to that of an average halibut liver oil. The kabeljou is closely related to the white sea bass (Cynoscion nobilis), the liver oil of which has the highest chick D efficiency ratio yet recorded (265 - 314) (23). So far, nothing is known of the rat:chick ratios of the vitamin D of South African fish oils, but if the kabeljou is found to resemble the white sea bass in this respect, its value as a

5. THE STONEBASS : POLYPRION AMERICANUS (BL. SCHN.).

The Stonebass is a very large deepsea fish caught exclusively by the trawlers. It grows to 7 feet in length and weighs up to 160 lbs. The fish usually caught, however, weigh between 30 and 50 lb. It is of fair commercial importance in South Africa although it is never landed in large quantities. According to the annual reports of the Division of Fisheries, the landings of stonebass before the outbreak of the war were as given in Table 32.

TABLE 32.

Landings of Stonebass (1934 - 1938) (4).

Year	Quantity	Value
1934	27,041 lb.	£253
1935	28,000 lb.	£266
1936	26,916 lb.	£230
1937	22,215 lb.	£185
1938	41,876 lb.	£364

The stonebass belongs to the sea bass family Serranidae. It is thought to be identical with the New Zealand bass (Polyprion americanus) (10) and is closely related to the groper (P. oxygenios) which is caught in considerable quantities in the Cook Straits (53). The liver oils of the latter have been studied in some detail by Shorland and figures reported are very similar to those obtained in the present work. The stonebass is also allied to the black sea bass (Stereolepis gigas) of California and the ishinagi (S. ishinagi) of Japan. The members of this family are noted for the high vitamin A content of their liver oils. Bills et al. (23), for example, have reported the vitamin A potency of the liver oil of the black sea bass as 520,000 I.U. per gram. The different species of Polyprion are not much less potent. According to Shorland (232) groper liver oil and bass liver oil may contain up to 5% of vitamin A (125,000 I.U. per gm.) and two samples of stonebass liver oil examined by Molteno (188) contained 11.4 and 13.5% respectively. It was, therefore, felt that, in spite of its minor commercial importance, the liver oil of the stonebass would repay further study. This has indeed been the case, for, not only are the stonebass liver oils the richest source of vitamin A yet encountered in South Africa, but they also present several other features of interest.

Experimental.

Sampling of Material.

As can be seen from Table 33, the number of livers in each sample is rather small. This was unavoidable as a trawler seldom catches more than 3 or 4 stonebass in one trip except at certain times of the year. The fish were gutted on board the trawler and at first the livers were brought ashore in a tin in the usual way, but from March 1941 onwards a different method of collection was adopted. It was found that the bulk of the stonebass livers landed at Cape Town were received by Messrs. Vitamin Oils Ltd. included among the stockfish and kingklip livers. They were separated from the other livers as the latter were passed through the mincing machine. By the co-operation of the firm, representative slices of all the stonebass livers obtained in this way were set aside and allowed to accumulate for 2-3 weeks until a reasonable sample could be obtained. During this period they were hard frozen in the company's cold storage plant so that even after 3 weeks they were still firm and in good condition.

Extraction of Oil.

Except where otherwise stated, the material was extracted by pepsin digestion. In the case of the livers, the method

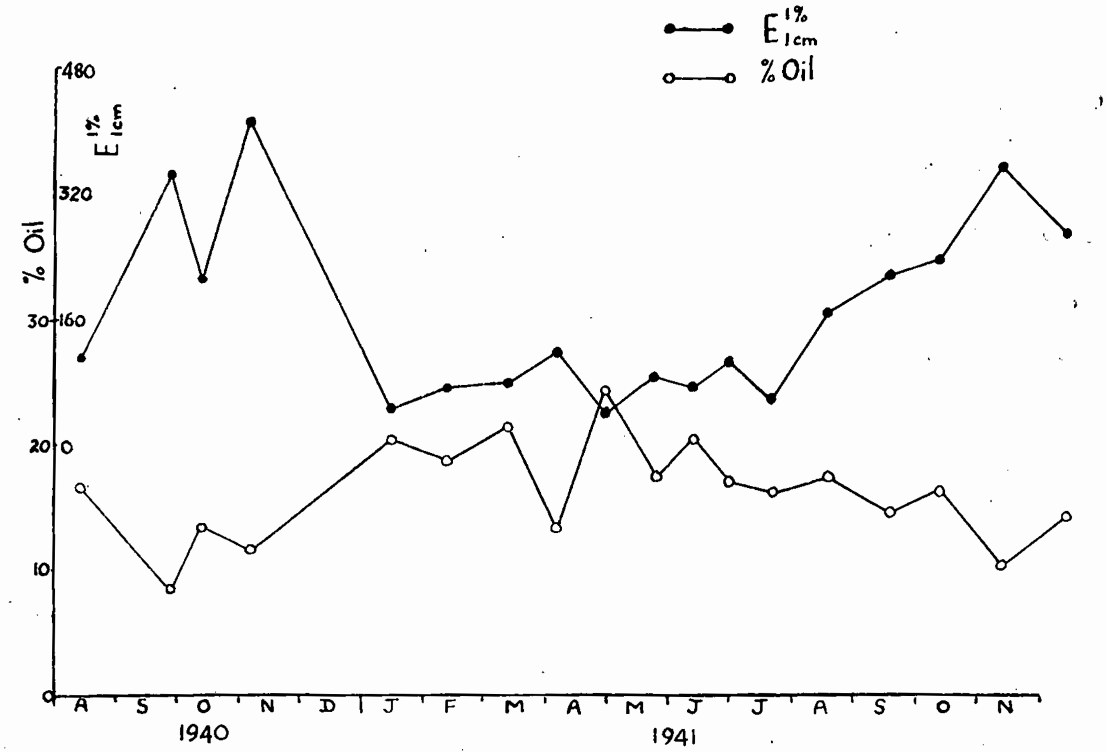


FIG. 15. Stonebass Liver Oils
Seasonal changes in the yield of oil and the vitamin A potency of monthly samples.

was checked against the ether extraction technique; the results in the two cases were exactly the same, indicating that the high values for the vitamin A potency are genuine and are not due to a partial concentration of the vitamin by the removal of the fatty acids, etc.

Seasonal Studies of Stonebass Liver Oils.

The stonebass liver oils were studied over a period of 15 months. The variations during this period in the yield of oil, its vitamin A and D potency and its chemical characteristics are summarised in Table 23; the seasonal changes in the oil content of the liver and its relation to the vitamin A potency of the oil are expressed graphically in Fig. 15.

In spite of the limited size of the samples, a definite seasonal trend is obvious from these results. The vitamin A potency is at its highest during the spring and early summer and the oils obtained during these months are a very rich source of the vitamin. In January the potency of the oil falls off fairly rapidly and it remains at a low level during the autumn and winter months, but even during this period it is far superior to an average halibut liver oil. In August the potency begins to rise again to reach a maximum in about November and the whole cycle is repeated. The oil content of the livers shows a similar variation, but in the opposite direction, in other words, it is at a minimum from October to December and it rises steadily during the first 4 months of the year to reach a maximum in about May, after which it falls off again. It should be noticed that the seasonal variation in the oil content is far less pronounced than the changes in the vitamin A potency, so that there is a considerable increase in the concentration of vitamin A in the livers during the spring and early summer months. It is not possible at present to relate these changes in the liver oil to either the influence of spawning or to the food cycle, as no information about these subjects is available. Again a plea must be made for further work on the life histories and feeding habits of at least the more important species of South African fish.

TABLE 33.

Seasonal Changes in Stonebass Liver Oil.

Date	Approx. no. of livers	% Oil	Iodine value	% Unsap. matter	Vit. A $E^{1\%}_{1cm.}$	Vit. D. (I.U./gm.)
25.11.39	Bulk sample	ca10 ø	184	21.4	238	
11.12.39	"	ca25 ø	183	21.6	227	
12. 8.40	4	16.5 x	127	10.5	109	
26. 9.40	7	8.5	170	36.0	344	1300
14.10.40	20	13.3	136	22.4	206	
7.11.40	15	11.7	187	38.5	415	
16. 1.41	2	20.3	98	6.5	42	700
13. 2.41	2	18.7	103	8.7	71	
13. 3.41	12	21.4	121	6.7	80	
7. 4.41	4	13.3	123	11.8	116	
28. 4.41	>13	24.2	132	6.1	38	
26. 5.41	10	17.3	116	8.6	84	
12. 6.41	30	20.4	111	6.8	67	
30. 6.41	12	17.0	123	11.4	103	
21. 7.41	11	16.1	112	6.8	85	
18. 8.41	10	17.4	142	15.5	165	950
18. 9.41	15	14.5	159	21.7	214	
13.10.41	10	16.3	160	22.0	234	
10.11.41	9	10.2	181	34.9	354	
15.12.41	12	14.0	175	29.6	265	

ø Alkali digestion
x Ether extraction

As may be seen from Table 33, some indication of a seasonal variation in the vitamin D potency of the oils was obtained but this was far less marked than in the case of vitamin A. From these figures it seems probable that the vitamin D content of the liver as a whole remains fairly constant throughout the year, but that during the Autumn and Winter months the infiltration of fat results in a dilution of the liver oil obtained during this period. The relatively low vitamin D content of the stonebass liver

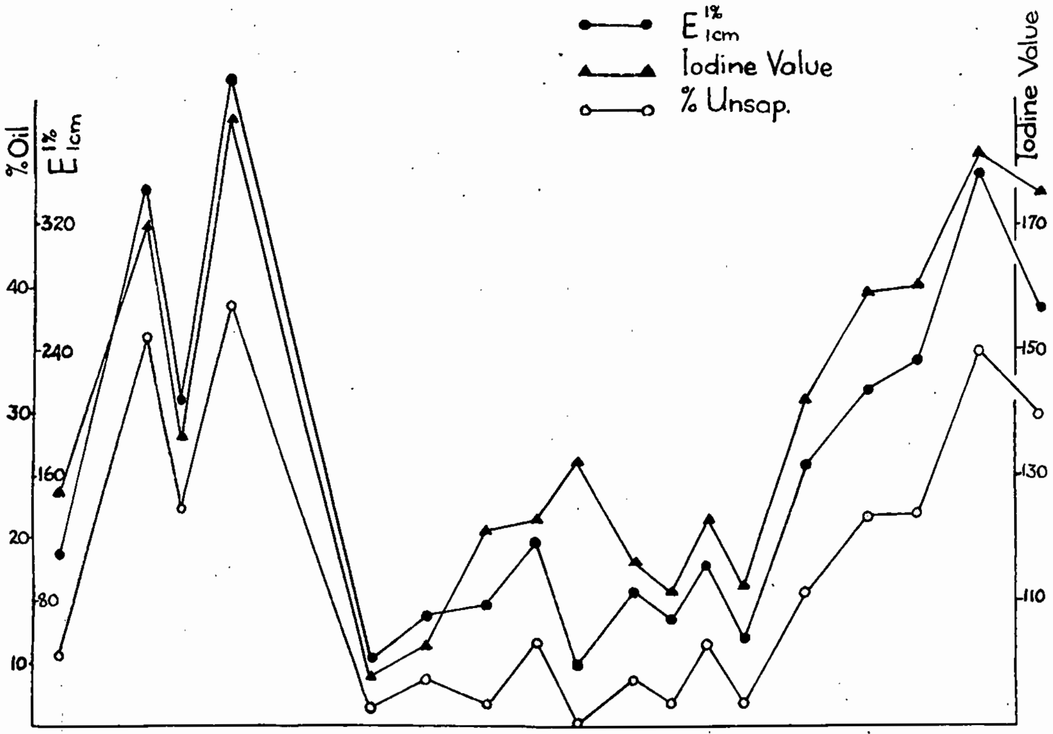


FIG. 16. Stonebass Liver Oils
Relation between E value, iodine value
and percentage unsaponifiable matter.

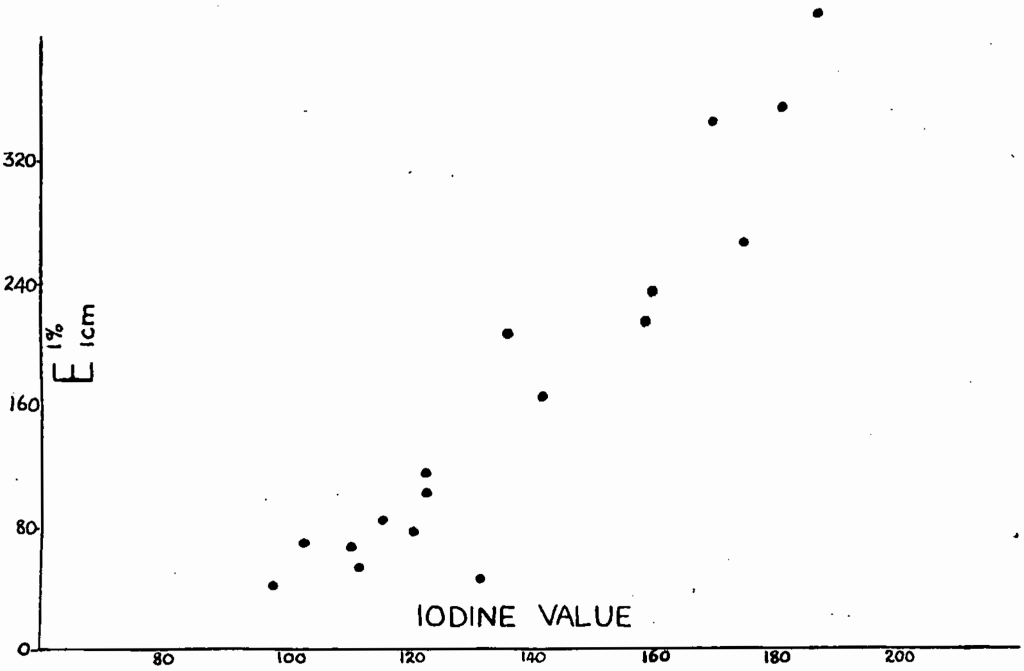


FIG. 17. Stonebass Liver Oils
Relation between iodine value and E value.

oils is noteworthy. For example, it is only half as potent as the liver oil of the groper which, according to Cunningham (53), assays 2300 I.U. per gram. In this respect the stonebass resembles the kingklip which is also far less valuable as a source of vitamin D than its New Zealand counterpart. When further studies have been carried out in both South Africa and New Zealand, it will be of interest to compare the figures for the vitamin D potency of related species and to see to what extent this is generally true of the oils of the two countries and if possible to trace its relationship to other factors such as differences in the vitamin D content of the plankton etc.

Chemical Studies of Stonebass Liver Oil.

Stonebass liver oils present many features of interest and a further study of their composition would be desirable. As may be seen from Fig. 16, there is a very close parallelism between the vitamin A potency of the oil and the iodine value and the percentage unsaponifiable matter. A similar relationship is also found in the stockfish intestinal oils and the kabeljou liver oils (Figs. 7 and 14 respectively) but in neither of these cases is it as marked as in the stonebass liver oil. The parallelism between the vitamin A content of the oil and the percentage unsaponifiable matter is to be expected and there is no need to comment on it except to point out that the proportion of vitamin A in the unsaponifiable matter of stonebass liver oil is very high. On the average, the vitamin constitutes 60% of this fraction which would, therefore, be a very suitable starting material for further studies on the pure vitamin.

The almost linear relationship between the iodine value of the oil and its vitamin A content (Fig. 17) is more interesting. A similar relationship has been reported by

Evers Jones and Smith (80) and Haines and Drummond (98) for halibut liver oils. The latter authors (96) have attempted to explain their results as follows: 'Our wide experience of oils derived from fish caught in waters off Iceland, Greenland and Labrador leads us to think that in any one large area the composition of the liver oil as regards glycerides is reasonably uniform. Therefore, because of the relatively large proportion present, variations in the vitamin A content of the liver are reflected directly in the analytical figures for the oil itself'. To test this hypothesis in the case of the stonebass liver oils, an attempt has been made to calculate the extent to which vitamin A (iodine value 356) is responsible for the observed iodine value of the oils.

TABLE 34.

Influence of the Vitamin A Content of Stonebass Liver Oil on the Iodine Value.

Sample No.	% Vitamin A	% Unsap.	Iodine Value of Oil.	Iodine Value of non-vitamin A fraction
3	6.8	10.5	127	110
4	21.5	36.0	170	120
5	12.9	22.4	136	103
6	25.9	38.5	187	128
7	2.66	6.5	98	91
8	4.44	8.7	103	91
9	4.87	6.7	121	110
10	7.26	11.8	123	105
11	2.39	5.1	132	127
12	6.27	8.6	116	102
13	4.20	6.8	111	100
14	6.45	11.4	123	107
15	3.45	6.8	112	104
16	10.3	15.5	142	117
17	13.4	21.7	169	128
18	14.6	22.0	160	127
19	22.1	34.9	181	132
20	16.6	29.6	175	139

The results are rather significant. As may be seen in Table 34, the iodine value of the non-vitamin A fraction of the oil shows far less variation than that of the oil as a whole.

Moreover the observed variations, in particular the higher degree of unsaturation of some of the more potent oils, may be accounted for to a certain extent by variations in the percentage of unsaponifiable matter other than vitamin A. In this connection it is of interest to note that in the case of the kabeljou (Fig. 11) there is a closer parallelism between the iodine value of the liver oil and the percentage unsaponifiable matter than between the iodine value and the E value. This is easy to understand since kabeljou liver oil contains a high proportion of unsaponifiable matter of which only 30% is vitamin A. In both these species studies of the iodine value of the unsaponifiable matter, on the lines of the work of Evers and Smith (loc. cit.), would probably yield valuable results.

It is also interesting to compare the stonebass liver oils with those of the groper. Here, too, the iodine value of the oil is related to the vitamin A content. For example, the spring oils which contain 0.5% of vitamin A have an iodine value of 82 while the winter oils contain 5% of the vitamin and have an iodine value of 122 (232). These figures are very similar to those for stonebass liver oils of a similar potency, a point which is particularly interesting since Shorland and Hilditch (233) have examined the component fatty acids of groper liver oil and found that they differ in many respects from those of a typical marine teleostean. One of the abnormal features of groper liver oil is the high content of saturated acids (24.4 - 28.8%) as compared with the usual values of 15 - 20%. (164). In addition, the glycerides are characterized by a low proportion of C_{20} and C_{22} poly-ethenoid acids and an unusually high content of palmitic and hexadecenoic acids. These features, especially the first two are reflected in the low iodine value of the spring oils. Since the iodine values of stonebass liver oils are similar to those of the groper oils, it is probable that they will also show these departures from the normal range of composition.

Stonebass Head and Visceral Oils.

In the stonebass the liver is not the main fat depot. Most of the oil is stored in the head, chiefly in the bone cavities. The pyloric caeca and intestines also contain a certain amount of oil, but these depots are less important than the liver. A better appreciation of the relative importance of the different fat depots is obtained if their size is taken into consideration. In Table 35, therefore, are given the average figures for the proportions of the different organs in 9 fish which were brought ashore ungutted on the 10.11.41.

TABLE 35.

Weight of the Visceral Organs of the Stonebass.

	Actual wt.	As percentage of wt. of whole fish.
Whole fish	18.2 kg.	-
Head	5.0 ''	27.5
Liver	288 gm.	1.59
Pyloric caeca	259 ''	1.43
Intestine	250 ''	1.38
Stomach	585 ''	3.22
Gonads	206 ''	1.13

Very little work was done on the head oils. A semi-quantitative extraction of one of the smallest heads of the fish examined in Table 35 yielded approximately 7% of oil, a figure which is in good agreement with the value 6% reported by Shorland and Hilditch (233) in the case of the groper. This oil did not give a blue colour with the Carr-Price reagent and it was not studied further. The results, however, do show the relative importance of the liver and the head as fat depots since the livers of these fish only contained 10% of oil, i.e. the amount of oil stored in the head was more than 10 times that stored in the liver.

A few 'spot' analyses were made on the oils from the pyloric caeca and the intestine. These are given in Table 36. The results indicate that the intestine is of very little interest either as a source of oil or vitamin A. The pyloric caeca, on the other hand, contain quite an appreciable amount of vitamin A and their oil content during the winter months is high enough to make their extraction worth while. They would appear, therefore, to be worth further study.

TABLE 36.

Stonebass Visceral Oils.

Date	Material	No. of fish.	% Oil.	$E^{1\%}_{1cm.}$	% Unsap. matter	I.V.
12. 6.41	pyloric caeca	2	9.1	28.6	6.76	157
10.11.41	"	9	3.3	78.8	16.63	160
10.11.41	intestine	9	1.8	10.8	16.2	151

The Commercial Importance of the Stonebass Oils.

Stonebass livers have been collected and extracted since the middle of 1941. They contain 10-20% of oil and so may be readily treated by alkali or ammonia digestion. The oils obtained in this way are a deep orange colour and are very viscous. The annual production of stonebass liver oil is very small, the potential output, as calculated from the figures in Tables 32 and 35, being less than 20 gallons a year. The oil, however, is very valuable, especially during the spring and summer months when a natural vitamin A concentrate is obtained. Even during the winter months the oils have a higher vitamin A potency than halibut liver oil, though the vitamin D content is relatively lower.

The pyloric caecal oils, too, are probably worth exploiting but further work is required before the utilization of this material can be put on a sound basis.

PART II.

The studies of the liver and visceral oils of the fish dealt with in this part of the thesis are less complete than those in Part I. The results, however, are given briefly so that some idea may be obtained of the importance of these species as sources of vitamin A. In addition, the results of 'spot analyses' of the liver oils of a number of different species are given. This work was largely of an exploratory nature and serves to indicate those species which may be worth further study.

6. THE YELLOWTAIL OR ALBACORE : SERIOLA LALANDII (C. AND V.

The yellowtail belongs to the Jack family Carangidae. It is very similar to the yellowtail of Australia and New Zealand (Seriola grandis) and also to the Californian yellowtail or amberjack (S. dorsalis). According to Barnard (10), 'it seems probable that many of the nominal species described by various authors will be united when a revision of the genus is undertaken.'

In South Africa, the yellowtail is more or less confined to the warm waters of the Agulhas current. The largest catches are made in the False Bay area where the fish appear from October to April. It is of minor commercial importance and the total annual landings do not exceed 300,000 lb. (172).

Experimental.

The livers examined were all from fish caught in False Bay. The oil was extracted either by ether extraction (1940) or by pepsin digestion (1941).

TABLE 37

Yellowtail Liver and Intestinal Oils.

Liver Oils.

Date	No. of fish	Av. wt. of fish (lb.)	Av. wt. liver (gm.)	% Oil	$E^{1\%}_{1cm.}$	% Unsap. matter	I.V.
2. 5.38	6	9	87	27.5	13	-	-
12.10.38	3	20-25	120	7.0	118	-	-
22.10.38	3	$7^{1}/_{2}$	68	6.8	70	-	143
23. 2.40	4	6	45	7.0	-	6.2	157
1. 3.40	2	20	87	3.3	259	19.3	175
15. 3.40	18	$8^{1}/_{2}$	38	5.3	62	11.1	-
1. 4.40	3	20	97	9.9	230	21.8	187
25. 4.40	5	6	-	12.7	15	4.4	144
31. 5.40	18	5-10	60	12.3	20	3.6	155
27. 6.40	11	7-15	88	9.5	113	9.0	167
31. 3.41	ca 100	-	-	11.4	38	6.9	168
31. 3.41	4	8	43	6.3	40	9.5	153
31. 3.41	8	16	78	7.3	67	11.1	167
31. 3.41	4	>20	102	13.1	78	9.7	160
Intestinal Oils.							
31. 3.41	12	13	156	6.6	5.0	4.5	163

In order to make the data in Table 37 more complete, the figures obtained by Molteno in 1938 (188) have been included. From this brief survey, the liver oil of the yellowtail appears to merit further study. The variations encountered in the present study in the vitamin A content of the liver oil are large and do not show any seasonal trend. This may be due to the small size of most of the samples. On the other hand, it seems that the vitamin content of the liver oil is influenced to a very considerable extent by the size of the fish. As has already been pointed out, this is observed in most species. In further work on the yellowtail, therefore, the various size groups should be studied separately.

The yield and the vitamin A potency of the liver oil of the South African yellowtail are similar to the values reported by Wood and Kuchel (264) for the Australian species, viz.:

Yield of oil	10%
Vitamin A	42,000 I.U. per gm. ($E^{1\%}_{1cm.}$ = 26)
Vitamin D	9,000 I.U. per gm.

The livers of the yellowtail are worth collecting and extracting. The yield of oil is low, but the vitamin A content of the liver oil, especially in the case of the bigger fish, is appreciable. The intestines and pyloric caeca, on the other hand, do not appear very promising. The quantity of yellowtail liver oil which can be produced in South Africa is small. At certain times of the year, however, large quantities of the fish are caught and in the future it is probable that much of it will be canned. Yellowtail liver oil may, therefore, become one of the bye-products of the canning industry.

7. THE MAASBANKER : TRACHURUS TRACHURUS (LINN.)

The maasbanker belongs to the same family, Carangidae, as the yellowtail. It is a small fish and the biggest examples do not exceed 4 lbs. in weight. It is of fair commercial importance and from 2 to 5 million pounds of fish are caught annually. It is caught chiefly by means of trekseine nets operated from the beach or from boats. In addition, a small number of the larger fish, up to 3 lb. in weight, are taken by the trawlers when trawling for more desirable species.

Experimental.

Sampling of Material.

All the fish examined were caught by trawlers operating on the west coast. The fish were brought ashore ungutted and they were weighed and the viscera were removed at the University. The 'intestine' was taken to include both the intestine and the pyloric caeca which are numerous and extend for about one-third of the length of the intestine. The 'total viscera' included the liver, the intestine and the pyloric caeca, the stomach and the gonads; it did not include the heart or gills.

Extraction of Oil.

The oils were all extracted by pepsin digestion.

Discussion of Results.

A short study has been made of the liver, intestinal and total visceral oils of the maasbanker. As may be seen from Table 38, the liver oils, especially in the case of the larger fish, are very rich in vitamin A. The potency of the intestinal oils is also high though it is less than that of the liver oils. Both the livers and the intestines, however, are small and, except during the late summer months, the yield of oil is low. There is little likelihood, therefore, that it will be possible in industrial practice to separate these organs from the rest of the viscera and process them separately.

Consequently, from the commercial point of view, the total visceral oils are of more interest.

TABLE 38.

Maasbanker Liver Oils.

Date	No. of fish	Av. wt. fish	Av. wt. liver	% Oil	$E^{1\%}_{1cm.}$	% Unsap. matter	I.V
20. 3.41	6	900 gm.	25 gm.	14.8	43	5.1	108
29. 5.41	13	1150 "	15 "	6.8	242	24.0	172
3. 7.41	30	900 "	7 "	6.7	320	32.9	159
11. 9.41	14	469 "	5 "	6.9	72	-	171
11. 9.41	16	656 "	7 "	7.7	111	13.1	185
29. 9.41	19	538 "	5 "	5.6	142	-	-
24.11.41	12	902 "	15 "	6.2	180	20.6	171
12. 1.42	33	1025 "	-	11.9	175	18.6	166
2. 3.42	22	1080 "	-	13.1	63	7.4	123

Intestinal Oils.

Date	No. of fish	Av. wt. fish	Av. wt. intestine	% Oil	$E^{1\%}_{1cm.}$	% Unsap. matter	I.V
20. 3.41	6	900 gm.	26 gm.	15.0	10.6	3.6	150
29. 5.41	13	1150 "	24 "	2.3	40.0	17.9	178
3. 7.41	30	900 "	14 "	2.9	61.6	16.0	161
11. 9.41	14	469 "	6 "	5.0	47.7	-	136
11. 9.41	16	656 "	8 "	4.0	53.9	-	134
29. 9.41	19	538 "	7 "	2.4	96.8	-	174
24.11.41	12	902 "	18 "	2.6	48.0	21.2	187
12. 1.42	33	1025 "	-	2.3	23.2	6.5	150
2. 3.42	22	1080 "	-	11.2	17.1	5.4	[illegible]

Table 39 /.....

TABLE 39.

Total Visceral Oils.

Date	No. of fish	Av. wt. fish	Av. wt. viscera	% Oil	$E^{1\%}_{1cm.}$	% Unsap. matter	I.V.
9. 6.41	6	900 gm.	56 gm.	4.6	49.3	14.6	166
26. 7.41	20	481 "	28 "	21.2	10.6	2.0	168
14. 8.41	7	472 "	31 "	8.8	9.9	5.3	139
14. 8.41	7	668 "	67 "	7.1	15.7	6.2	142
14. 8.41	19	1016 "	85 "	4.3	61.6	19.4	164
29. 9.41	7	639 "	46 "	2.8	30.6	16.0	160
29. 9.41	6	743 "	59 "	3.6	40.8	22.3	179
27.10.41	26	753 "	61 "	2.9	70.3	26.9	183
21.11.41	23	598 "	44 "	3.5	37.4	18.4	180

A preliminary study of these was made by the writer. As may be seen from Table 39, the vitamin A content of the total visceral oil is less than that of the liver or intestinal oils. This is to be expected as the stomach and gonads contain little or no vitamin A [cf. Pugsley (209)]. The fish were studied only during the winter months. During this period the oil content of the viscera was low. In the case of the bigger fish (over 600 gms.) this was apparently related to spawning which, judging from the appearance of the gonads, occurred from about September to November. During the late summer and autumn months, the fish are in good condition and the oil content of the total viscera may be as high as 25% (134).

The work described above was part of a collaborative study of the maasbanker. An examination of the head and body oils was also made. This work is still in progress and a detailed study of both the small 'trekked' fish and the larger trawled fish is planned.

8. THE VAALHAAI OR TOPE : GALEORHINUS CANIS (BOND.).

The vaalhaai is a species of small shark which is common on the South African coast. It grows up to 7 ft. in length and usually weighs 30 - 50 lb. Until recently it was of no commercial importance and was regarded as a nuisance by both the commercial and the sport fishermen.

The vaalhaai is very closely related to the soupfin shark (Galeorhinus zeopterus) of the Mexican and Californian waters. In the last 5 or 6 years the latter has been caught in very large numbers chiefly for its liver oil. At the present time, owing to the demand for vitamin A, soupfin shark livers are commanding fabulous prices as no other species of fish is known from which so large a yield of natural high potency oil can be recovered. It is estimated that to-day soupfin shark livers are supplying approximately half the vitamin A produced in the United States (205). An examination of the liver oil of the vaalhaai was undertaken with a view to the development of a similar industry in South Africa, and the figures reported here are the result of a preliminary study of this species.

Experimental.

The fish examined were all caught at Gansbaai by the inshore boats. The livers, usually single specimens, were brought to the University and the oil was obtained by ether extraction. In addition, a number of oil samples were analysed which had been extracted by Mr. C.N. Taylor at Gansbaai by steaming.

Discussion of Results.

As may be seen from Table 40, the vaalhaai, like the soupfin shark, is a valuable source of vitamin A. The livers constitute 7 - 10% of the total weight of the fish and contain from 25 to nearly 80% of oil, the potency of which ranges from 12,000 to 150,000 I.U. per gm. The proportion of liver and the range of oil content are the same in both the vaalhaai and the soupfin. The vitamin A potency of the former, however, is somewhat lower than that of the American species which yields an oil containing on the average 70,000 - 100,000 I.U. per gm. (205).

TABLE 40.

Vaalhaai Liver Oils.

Date	No. of fish	Method of extraction	% Oil	$E^{1\%}_{1cm.}$	% Unsap. matter	Iodine value.
20. 6.40	1	ether/Na$_2$SO$_4$	53	38.4	4.66	166
21. 6.40	5-10	steamed	62	41.6	4.38	178
8. 8.40	"	"	-	8.0	1.58	196
8. 8.40	"	"	-	14.6	2.49	193
8. 8.40	"	"	-	7.8	1.88	196
4. 9.40	1	ether/Na$_2$SO$_4$	28	61.8	5.66	139
4. 9.40	3	"	37	21.9	3.07	191
21. 9.40	1	"	60	8.6	1.94	188
1. 12.40	ca 80	steamed	-	13.6	3.09	189
1. 12.40	1	"	-	91.2	-	-
2. 2. 42	1	ether/Na$_2$SO$_4$	78	16.5	2.58	182

The results of this preliminary study were sufficient to indicate that the production of vaalhaai liver oil would be a very profitable enterprise. A study of the quantities of fish available and the best method of catching them was then undertaken by the fishing interests with the result that since 1941 3 companies have been operating to produce vaalhaai liver oil. No figures are available for the exact quantities of oil produced at present, but it is in the neighbourhood of 10,000 gallons a year. Further data on the fluctuations in the yield and the vitamin A potency of vaalhaai liver oil are being collected and the seasonal variations at the different points along the coast are being studied.

9. MISCELLANEOUS SPECIES.

Under this heading are grouped together a number of species which are of minor commercial importance though some of them, for example the dogfish and the common skate, are caught in fair numbers when trawling for more desirable species. Only one or two analyses of the liver oil of each species have been made, but the values are recorded in order to provide more data on the vitamin A content of South African fish oils in general and also to indicate which species are worth further study.

Experimental.

Extraction of Oils.

Either ether extraction, pepsin digestion or steaming was used.

TABLE 41.

Species	Date	No. of fish	Method of extraction	% Oil	$E^{1\%}_{1cm.}$	% Unsap. matter	I.V.
Hound	2.12.40	2	steaming	ca 50	1.4	2.00	188
Dogfish	4. 1.40	-	"	ca 60	5.3	9.31	144
"	2. 8.40	ca 30	ether	42	3.2	7.13	163
Common skate	12.8.40	30	"	38	negligible	2.49	180
Giant skate	12.8.40	1	"	39	1.9	3.77	177
" "	17.11.41	1	"	55	0.8	2.24	184
Blue shark	20. 1.40	3	steaming	ca 40	11.2	5.38	165
Butterfish	28. 7.41	12	pepsin	6.0	38.4	7.77	142
"	28.10.41	12	"	7.0	61.0	12.83	158
Jacopever	11. 9.41	ca 15	"	22.4	14.4	13.80	160
Red Steenbras	8.5.40	1	ether	7.7	132	19.7	113
White "	-.3.40	1	"	9.5	43.2	10.08	149

Discussion of Results.

The hounds (Mustellus spp.) are very similar to the vaalhaai in general appearance and may easily be mistaken for the latter. A casual examination of the teeth, however, serves as a rapid and certain means of distinguishing the two genera. This is a point of some importance. The hounds are often caught when fishing for vaalhaai and as the liver oil of the former contains very little vitamin A (Table 41), it is desirable that they shall not be included amongst the vaalhaai with the resulting lowering of the vitamin A content of the latter.

The Cape dogfish (Squalus acutipinnis, Regan.) is very similar to the Pacific greyfish (S. Sucklii, Girard), and the yield of oil and the vitamin A potency of the samples in Table 41 fall within the same range as the values reported by Pugsley (210) for the greyfish. The dogfish is caught in fair numbers when trawling for more desirable species. It is usually disposed of by dumping. Recently, however, the demand for vitamin A has led to the collection and extraction of the livers on a small scale. It is to be hoped that the production of dogfish liver oil will be continued after the war; greyfish liver oil is used to a large extent as a source of vitamin A for poultry feeds and there is no reason why dogfish liver oil should not be used for the same purpose.

Two species of skate have been examined. These are the common skate or thornyback (Raja clavata, Rond.) and the so-called 'giant skate'. The actual classification of the latter is not certain as only the liver was brought ashore. It is probably Raja marginata (Lacep.) which, according to Marchand (172), is the largest species of skate commonly caught in Cape waters. Both species are taken incidentally in the trawl, the former in fair numbers. Like the dogfish they are usually dumped. Neither of them appears of much interest as a source of vitamin A.

The blue shark (Carcharinus glaucus, Rond.) belongs to the same family as the vaalhaai. It is occasionally caught by the line boats when fishing for vaalhaai or other species. The liver oil is quite a good source of vitamin A, though it is less potent than that of the vaalhaai. Since 1941, limited quantities of blue shark liver oil have been produced.

The butterfish (Stromateus fiatola, Linn.) is caught occasionally by the trawlers. It is considered an excellent food fish. The vitamin A content of the liver oil of the samples examined was high, but the yield of oil was low and the livers were small (average weight 18 gm.) so that it is doubtful whether the extraction of the liver oil would be a commercial proposition.

The jacopever (Sebastichthys capensis, Gmelin) is used extensively on the west coast as a crawfish bait. It is of insignificant commercial importance. From the sample examined here, it appeared that it would merit further study and a detailed examination of the liver and visceral oils is in progress.

The red and white steenbras belong to the family Sparidae which is one of the largest and most important families in South African waters. Both fish are of fair commercial importance. The red steenbras (Dentex repestris, Valen.) is caught chiefly on the Agulhas Bank by the line boats. The specimen examined here, however, was caught in False Bay. It weighed approximately 100 lbs. and the liver weighed 926 gm. The white steenbras (Pagellus lithognathus, Cuv.) is found all around the coast and is caught irregularly by the trawlers. The specimen examined weighed 25 lb.; it was apparently just about to spawn. It was also caught in False Bay. The liver oil of the red steenbras especially was very rich in vitamin A and both species would appear to be worth further study if regular samples could be obtained.

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