CXCVII. VARIATIONS IN VITAMIN A CONTENT OF FISH-LIVER OILS, WITH PARTICULAR REFERENCE TO SEASONAL FLUCTUATIONS IN THE POTENCY OF HALIBUT-LIVER OIL.

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The object of the present work was initially to gather data concerning the vitamin A potency of the liver oils obtained from various species of fish. It was soon found that the vitamin A content of halibut-liver oil was remarkably high in comparison with oils from other species, and the scope of the investigation [*cf.* Lovern, 1932] was widened to embrace the elucidation of the factors capable of influencing the vitamin A content.

Collection of material. Livers were obtained mainly from commercial line-fishing vessels operating from Aberdeen and fishing in waters near Iceland, the Faroes and N.W. Scotland. The average duration of a trip was 2–3 weeks, and in each case the livers from all catches were mixed and stored at 0° in tins holding about 22 lb. The livers were received in varying states of preservation; some were very fresh and firm, whilst in other cases the contents of a tin had become a semi-liquid mass from which individual livers could not be separated. Each batch of livers was minced, mixed well, dried with anhydrous sodium sulphate and extracted with ether, and a dark sticky oil was finally obtained.

The halibut livers varied in weight from 4 oz. to 7 lb., but the great majority were between the limits of 12–20 oz., so that 1 lb. represents the approximate average weight of the halibut livers obtained by us in Aberdeen.

Towards the end of 1932 it became impossible to purchase halibut livers directly, as the production had been acquired in advance for the commercial extraction of the liver oil, but through the courtesy of Messrs Isaac Spencer and Co., Ltd., and Messrs Allen and Hanburys, Ltd., quantities of liver (10–14 lb. at a time) were purchased periodically. During the earlier period when supplies were obtained direct from the trawlers, arrangements were made for the skippers to note the position of the fishing ground, the nature of the stomach contents and the size of the halibut represented by the catch. As a rule, the livers in a batch were of assorted sizes, but occasionally fairly homogeneous catches of large or small halibut were obtained and the livers were classified as "mainly large" or "mainly small." The position of fishing grounds was also ascertained for the livers purchased from commercial houses during the later period. Through the courtesy of Messrs Parke, Davis and Co., we were also able to examine representative samples of commercial halibut-liver oil prepared from the livers of fish caught in Pacific waters and in the Davis Straits (*vide infra*). Much of the data obtained is unsuitable for a detailed report, but the following main conclusions may be quoted.

(i) No very precise correlation between vitamin A potency and the position of the fishing ground can be established, although there are indications that higher maximum values are obtained in the liver oils from halibut caught in more northerly waters, probably reflecting the tendency of the larger fish to migrate northwards.

(ii) No significance can be attached to the stomach contents or the intensity of feeding [see Lovern and Sharp, 1933].

(iii) There is considerable evidence that the vitamin A potency of halibut-liver oils (in common with those of several other species) increases with increasing size of liver, and therefore age of fish.

(iv) Well marked seasonal variation was shown.

Vitamin A tests. Rough Carr-Price determinations were carried out at Aberdeen on the fresh oils, followed by spectrometric assay at Liverpool, using the Hilger-Nutting spectrophotometer for the antimony trichloride colour test, and Hilger E 3 spectrographs for the direct determination of vitamin A content on the basis of intensity of absorption in the ultra-violet shown by alcoholic solutions of liver oil.

Of all the constituents of fish-liver oils, vitamin A is by far the most variable in concentration. This is true not only in comparing oils from different species, but also as reflecting the differences between specimens of oil obtained from individuals of the same species. Our experience makes us unable to attach any precise significance to the idea of a fish-liver oil "typical" as regards vitamin A potency.

Variations in vitamin A content from species to species, arising possibly from different requirements and feeding habits, are perhaps only to be expected, but their magnitude is nevertheless surprising (see Table I). The percentages are calculated on the basis of the richest vitamin A distillates [*cf.* Carr and Jewell, 1933]:

$$E^{1\%}_{1\text{cm.}} \begin{cases} 617 m\mu,\ 5000 \\ 580 m\mu,\ 2600 \end{cases} \text{antimony trichloride colour test.}$$

$$328 m\mu,\ 1600 \text{ direct ultra-violet absorption test.}$$

78,000 Carr-Price units.

The samples of oil from some species represent only a relatively small number of livers, so that considerable variations from our figures may be possible in later tests. Nevertheless, experience up to the present points to the conclusion that the liver oils of haddock, whiting, skate of small or medium size, codling and immature or small fish generally are markedly inferior in vitamin A potency to average cod-liver oil; the oils from pollack, saithe, hake and ling (probably also torsk) are usually similar in potency to cod-liver oil and subject to roughly the same variations; salmon, turbot, sturgeon and halibut yield liver oils which are vastly richer in vitamin A than cod-liver oil. The richer the source the more widely does the potency appear to vary as between sample and sample.

According to the data of Bills [1927] the over-all range of antirachitic activity of liver oils is some 500:1, the majority of oils falling within a range of 30:1. Vitamin A has a wider range, at least 2500:1, and, so far as we can ascertain, no parallelism between vitamin A and vitamin D potencies can be substantiated. At present, we have no views to submit regarding the significance of a 2500:1 range in vitamin A content; we are more concerned to ascertain the facts relating to variations (within a given species) in batches of oil, each representing

Table I. *Vitamin A content of fish-liver oils.*

Liver oil	Estimated percentage of vitamin A in the samples examined
Catfish (*Anarrhichas lupus*)	0·06
Cod (*Gadus callarias*) (mean of 43 samples)	0·04
Codling (*G. callarias*) (smaller specimens)	0·004
Codling (*G. callarias*) (larger specimens)	0·008
Haddock (*G. aeglefinus*)	0·004
Pollack, lythe (*G. pollachius*)	0·04
Saithe, coalfish (*G. virens*)	0·09
Whiting (*G. merlangus*)	0·005
Conger eel (*Conger vulgaris*)	0·25
Dab (*Pleuronectes limanda*)	0·015
Lemon sole (*P. microcephalus*)	0·07
Plaice (*P. platessa*)	0·1
Hake (*Merluccius vulgaris*)	0·04
Halibut (*Hippoglossus vulgaris*) (highest recorded, 1932)	10·0
(*H. vulgaris*) (lowest recorded, 1932)	0·17
Ling (*Molva vulgaris*)	0·015
Monk (angler-fish) (*Lophius piscatorius*)	0·02
Newfoundland flat-fish (*Platysomatichthys hippoglossoides*)	0·04
Pike (*Esox luscius*)	0·08
Rat-fish (*Chimera mirabilis*)	0·008
Salmon (*Salmo salar*)	0·3
"Blue" skate (*Raia batis*)	0·007
Thornback skate (*R. clavata*) (small specimens)	0·01
(*R. clavata*) (large specimens)	0·05
Sturgeon (*Acipenser attilus*)	0·8
Torsk, tusk (*Brosmius brosme*)	0·08
Turbot (*Psetta* (*Rhombus*) *maximus*)	0·3

too many livers to have any meaning as regards individual fishes but large enough, it is hoped, to be significant as regards the major causes of fluctuations and variations in potency.

Two distinct kinds of variation are discernible, progressive and seasonal, the seasonal being superimposed on the progressive variation; and without excluding the possibility of less obvious factors, the following variables appear to be important:

(i) Size (or age) of fish.
(ii) Sexual condition.
(iii) Diet.

The progressive aspect of the vitamin A variability seems to depend on (i), whilst (ii) and (iii) appear largely to account for the seasonal changes.

Within a given species we have found that large livers yield as a general rule a more potent oil than small livers, and our qualitative observations are supported by the recent more quantitative work of MacPherson [1933], who has found a linear relationship between age of fish and the potency of cod- and American plaice- (*Hippoglossoides platessoides* Fabricius) liver oils. As the livers of the larger members of a species usually represent a greater proportion of the total body weight than is the case with smaller specimens, and as, further, the larger livers generally yield a higher percentage of oil, the total vitamin A reserve of the fish increases rapidly with size. This probably results from a purely mechanical storage of the excess of vitamin (ingested as such or synthesised *in vivo* from carotene) over the requirements of the fish. Be the explanation what it may, the age factor is of considerable importance. Thus in the cod, the ratio between vitamin A potencies for mature fish and codling is about 10:1, corresponding to about 20 or 30:1 in the actual weight of vitamin A per unit weight of fish. Qualitatively confirmatory evidence is available for skate and halibut.

It has long been recognised that during the spawning period changes occur which may profoundly affect the vitamin content of the liver oil. The cod, for example, eats little or no food at such times, and the drain on fat-reserves incidental to fasting is increased by the utilisation of large quantities of liver oil in the development of the genital products [*cf.* Hjort, 1914]. A certain amount of vitamin A is transferred with this oil to the gonads [Zilva *et al.*, 1924], and the total vitamin reserves are to that extent depleted; but the residual liver oil is so reduced in quantity that the concentration of vitamin A in the oil is higher than when the fish is feeding normally. It appears, in fact, that at the spawning period both vitamin A and D potencies vary approximately inversely as the oil content of the liver [Drummond and Hilditch, 1930]. This utilisation of liver oil at spawning would of itself give rise to a peak in the potency curve immediately after spawning. Resumption of feeding results in gradual dilution of the liver oil as fat is again stored, the drop in potency being mitigated by replenishment of vitamin A reserves. The average percentage of oil in fish-livers varies from species to species (*e.g.* halibut 18–20 % (average); monk or angler fish, 40 %; certain gadoid fishes, up to 70 %) as also does the range over which the percentage of oil varies for the pre- and post-spawning periods, but the range rarely exceeds 2 or 3 to 1 for the spawning effect. The males of the various species are much less affected than the females.

Diet may next be considered as a contributory cause of fluctuations in potency. In a sense, diet is fundamental, since there is no evidence of direct synthesis of vitamin A by fishes as appears to be possible with vitamin D in some species [*cf.* Bills, 1927], and in any event the richer the diet, the greater are the vitamin reserves likely to be. Drummond *et al.* [1922], Jameson *et al.* [1922] and Hjort [1922] traced the fish-liver vitamin A through a series of intermediate stages to the diatoms (unicellular vegetable organisms in the plankton), which are able like land plants to produce carotene as a result of photosynthesis. There is as yet no evidence that diatoms or the minute planktonic animal organisms (copepods, larval forms of various marine creatures, *etc.*) which subsist on diatoms, contain vitamin A as such, or vitamin D. The published biological assays of the growth-promoting effects of planktonic organisms exhibit curiously good agreement in some cases, and very bad agreement in others, so that seasonal variations may perhaps be suspected here also. In any case, the plankton exhibits very well-marked seasonal fluctuations in abundance, an effect due primarily in the case of diatoms to seasonal changes in the intensity of sunlight and less obviously to fluctuations in silica, nitrogenous matter and carbon dioxide in the sea water. The curves in Fig. 1 show the mean annual changes in the abundance of planktonic flora and fauna generally in Port Erin Bay for the period 1907–1920, based on data given by Johnstone *et al.* [1924], and are fairly representative for the whole of the northern hemisphere, with the reservation that in 1932 the spring maximum of the diatoms may have been a few weeks earlier than usual (in Plymouth Sound this occurred in March). It would appear from the curves that the greater part (probably 80 %) of the total amount of carotene annually produced by the diatoms is synthesised in the spring or early summer, with a comparative scarcity, so far as initial synthesis is concerned, during the rest of the year. A time-lag of uncertain duration must exist before this carotene reappears in the form of vitamin A in the liver oils of large fishes, owing to the number of links in the food-chain. The spring maximum must first of all affect the copepods and larvae living on diatoms; next, immature fish and small mature fish (*e.g.* herring, caplin), certain molluscs and other organisms which subsist on copepods; thence, either directly or through one or two further stages, the larger fish. The effect is, moreover, liable to be wholly or partly obscured by other factors such as the presence of large reserves of liver oil and changes in oil content due to spawning. Indeed, the last factor gives rise to the only seasonal fluctuation which can be discerned at all in the majority of species. The halibut, apparently, is an exception.

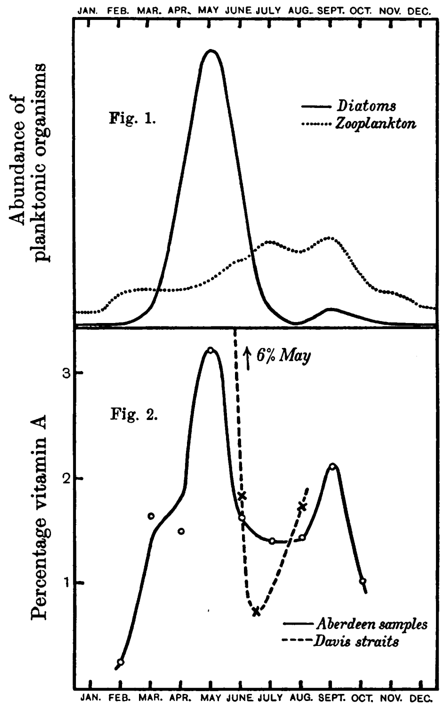

Fig. 1. Mean annual plankton variations, 1907–20, Port Erin Bay; adapted from Johnstone *et al.* [1924].

The diatom curve is roughly quantitative. The zooplankton curve, which is more diagrammatic, reflects the July and September maxima due to copepods, the main curve being modified by larvae (early spring and November) and protozoa (June–July).

Fig. 2. Seasonal variations in vitamin A content of halibut-liver oils (1932).

Insufficient samples were obtained during March to justify drawing the curve with a further subsidiary maximum in the early spring.

Tables II and III summarise data which have been obtained during 1932 on halibut-liver oils. Some comment must be made on the statistical significance of the results in the last columns of Tables II and III, from which the curves in Fig. 2 were drawn. The halibut livers from Aberdeen are representative samples of catches made during the greater part of the year, and not less than 1500 livers were worked up on a laboratory scale over the whole period. The material from the Davis Straits was obtained from some 100,000 livers (4500

Table II. *Halibut-liver oils (samples from Torry Research Station).*

Mean date of catch	Approx. total wt. of livers per batch in lbs.	Carr-Price B.U.	$E^{1\%}_{1\,\text{cm.}}$ 617 $m\mu$	$E^{1\%}_{1\,\text{cm.}}$ 580 $m\mu$	$E^{1\%}_{1\,\text{cm.}}$ 328 $m\mu$	Estimated % vitamin A	Aberdeen samples % vitamin A for month (weighted mean)
12. ii. 32	30	—	10·8	7·7	5·5	0·30	
15. ii. 32	30	—	12·1	8·7	6·0	0·34	0·26
22. ii. 32	30	60	7·5	6·0	4·25	0·23	
23. ii. 32	30	60	6·2	4·8	3·5	0·17	
7. iii. 32	30	—	70	47	30	1·9	
10. iii. 32	30	1600	139	80	42	2·8	1·65
16. iii. 32	30	71	10	6·5	5·0	0·25	
5. iv. 32	30	66	8·2	5·5	—	0·2	
18. iv. 32	30	1030	—	—	—	*ca.* 2	
18. iv. 32	30	1100	—	—	—	*ca.* 2	
24. iv. 32	33	820	67	44	26	1·6	1·5
27. iv. 32	16	540	42	29	19	1·1	
28. iv. 32	33	1900	132	89	50	3·1	
30. iv. 32	44	210	17·3	14·6	—	0·5	
3. v. 32	44	205	25	14·5	80	0·5	
16. v. 32	30	2770	176	98	57·5	3·6	
17. v. 32	33	3030	241	194	80	5·0	3·23
18. v. 32	22	7630	430	260	164	10·0	
20. v. 32	30	780	72·7	44·5	24	1·5	
21. v. 32	30	865	90	49	27·5	1·7	
4. vi. 32	30	285	18	14	8·5	0·53	
10. vi. 32	30	890	113	75	39·3	2·5	1·63
12. vi. 32	30	625	45	29·5	15·4	1·0	
14. vi. 32	30	1240	121	74	36·5	2·5	
1. vii. 32	30	290	20·8	12·4	6·8	0·42	
3. vii. 32	30	1170	124	73	35	2·45	
16. vii. 32	30	1800	175	101	47·5	3·4	1·41
18. vii. 32	77	775	85	52	23	1·7	
30. vii. 32	200	500	52	33·5	15	1·0	
3. viii. 32	—	270	27	17	8·6	0·55	
3. viii. 32	—	1365	148	90	—	3·1	
3. viii. 32	33	480	58	35	19	1·2	
9. viii. 32	14	125	16·4	9·7	6·4	0·38	
9. viii. 32	14	1870	188	108	58	3·75	
10. viii. 32	—	1340	165	98	45	3·3	
10. viii. 32	—	385	34	22	11	0·75	1·44
12. viii. 32	44	210	17·5	12·5	8·75	0·53	
14. viii. 32	—	3000?	134	81	42	2·8	
15. viii. 32	132	950	77	44·5	24·4	1·6	
19. viii. 32	14	240	20·2	14	8·7	0·5	
24. viii. 32	14	525	58	34	19·2	1·25	
30. viii. 32	110	355	37	20	12·3	0·8	
7. ix. 32	14	1080	83	50	25·5	1·7	
9. ix. 32	30	1510	159	87	50	3·2	
9. ix. 32	14	685	78	44	24	1·6	
12. ix. 32	14	640	61·5	34	21·7	1·3	
13. ix. 32	14	1640	126	72	41·2	2·7	
22. ix. 32	14	610	54	34	16·6	1·1	
24. ix. 32	14?	165	13	8·5	5·5	0·33	2·12
30. ix. 32	44	1750	166	94·5	48·7	3·2	
30. ix. 32	166	1000	98	55	30	2·0	
30. ix. 32	—	1740?	102	60	32	2·2	
30. ix. 32	—	975	71	44·5	24	1·55	
30. ix. 32	—	895	59	34·6	19	1·25	
30. ix. 32	—	800	52·4	30·4	17·1	1·1	
30. ix. 32	—	1470	86	52	29·5	1·8	
7. x. 32	14	385	34	24	15·3	0·95	
13. x. 32	14	420	40	24	14·5	0·90	1·02
21. x. 32	14	160	13·9	8·8	5·4	0·32	
26. x. 32	—	1530	90	50	31	1·9	

Table III. *Halibut-liver oils from the Davis Straits.*

Fish caught (1932)	Carr-Price B.U.	$E^{1\%}_{1\,cm.}$ $617\,m\mu$	Vitamin A %	$E^{1\%}_{1\,cm.}$ $580\,m\mu$	Vitamin A %	$E^{1\%}_{1\,cm.}$ $328\,m\mu$	Vitamin A %	Mean % vitamin A
May	4700	300	6·0	170	6·54	90	5·62	6
June	1400	90	1·80	49	1·88	28·7	1·80	1·83
June–July	630	37	0·74	19	0·73	12	0·75	0·74
August	1150	85·4	1·71	47	1·81	27·5	1·72	1·74

tons of fish) and the livers worked up on a technical scale. The results of the small scale and large scale treatments are consistent. The monthly average vitamin A potencies of the samples obtained from Aberdeen exhibit a very sharp maximum in May and a subsidiary maximum in September, and the curve for the Davis Straits samples is qualitatively similar over the period for which the data are available.

Discussion.

The interpretation of Fig. 2 is a matter of some difficulty. The observed fluctuations in monthly average potency cannot be attributed entirely to variations in oil content due to spawning. In the first place, the range of potency covered by the Davis Straits samples is about 8:1, whereas all the oils examined have represented between 12 and 35 % of the liver weight (*i.e.* a range of 3:1), the great majority lying between 15 and 25 %. Secondly, the seasonal fluctuation in potency is just as marked and shows the same tendency to the same two maxima whether spawning occurs in February–May (N.W. Scotland) or June–August (northern waters). For this reason all the data for the Aberdeen oils have been combined in one curve, whether the fish were caught off Iceland, the Faroes, or Scotland, and the "statistical" halibut represented by this curve may be regarded as spawning over the whole period from February to August.

Unless there is some more obscure process operating on or within the halibut, the only remaining likely cause of seasonal fluctuations in potency is the variation in available carotene, and it seems significant that the only measure we have of this factor, namely the abundance of diatoms, should show, qualitatively at all events, such a strikingly similar curve (Fig. 1). We hesitate to stress the parallelism unduly without further confirmatory evidence, and a completely convincing correlation is naturally very difficult to achieve since the halibut does not subsist directly on diatoms. Nevertheless, it seems to us highly probable that when carotene (in the plankton) is abundant, vitamin A reserves are being replenished by all fish, and that when these supplies are small the reserves are being drawn upon. The effect on liver oil potency will not be very marked in most fish, but the halibut appears to reflect this ebb and flow in so marked a way as to obscure even the spawning factor. The fact that the vegetable plankton is richer in colder waters and that the halibut-liver oils from the Davis Straits reached in May an average potency nearly twice as high as the Aberdeen samples, is not inconsistent with this view, though the larger size of fish may also account for the phenomenon. If the latter view is correct, it would appear that the larger the fish, the greater the fluctuation, as is shown by the low minimum reached by the Davis Straits curve.

The curves further suggest an enormous consumption of reserve vitamin A by the halibut during the summer and late autumn, and the possibility cannot at present be excluded, as an alternative explanation, that the potency fluctuation may arise from a rhythmic transference of vitamin A between the liver and some other part of the body, influenced by some unknown factor which varies seasonally in the same way as the diatoms. All this, of course, is pure speculation, but whatever their origin the fluctuations themselves seem real enough. It remains to be seen whether the halibut-liver oil curves published here are reproducible from year to year, subject, of course, to minor variations in time and intensity. We have, however, noticed the same general tendency for two successive years, and there is no reason to believe that they were exceptional.

The over-all range in variation of vitamin A in halibut-liver oils is seen from Table II to be certainly not less than 60:1 (10 to 0·17 %); more probably, since the samples each consisted of mixed oils from a number of livers, nearer 100:1, a much larger range than has been observed in any other species. Seasonal fluctuations account for only a small proportion of this range, and variations in the age of the fish caught (which, we are informed, would be from about 12 to 40 years) may here again be the dominant factor, as it appears to be in the case of cod [MacPherson, 1932; 1933].

We are, in short, led to infer that the oil obtained from any given halibut liver increases in mean annual potency as the fish grows older, and in addition fluctuates from season to season over a gradually increasing range. Further, the extraordinarily high proportion of vitamin A in some of the samples, together with these wide fluctuations in concentration, suggests that, at least in the halibut metabolism, vitamin A may play an additional rôle to that usually associated with the term "vitamin."

Summary.

1. The vitamin A content of fish-liver oils covers a range of at least 2500 to 1. No parallelism can be traced between the vitamin A and vitamin D potencies.

2. In certain species, the vitamin A content of the liver oil has been found to increase with the age and/or size of the fish, the total vitamin A reserve increasing more rapidly than the oil potency.

3. Attention is drawn to the fact that the greater part of the carotene annually produced by the diatoms, and thus available for conversion into vitamin A by marine animal life, is initially synthesised during a comparatively short period in the spring or early summer.

4. Halibut-liver oil is by far the richest known natural source of vitamin A available in quantity, but it has been found to vary in potency over a wider range than any other source. Oils containing from 0·17 to 10 % of vitamin A have been examined and these apparently do not represent extreme limits. No correlation has emerged between the immediate diet of the halibut and the oil potency [Lovern and Sharp, 1933].

5. Halibut-liver oils have been found to exhibit well-marked seasonal fluctuations in vitamin A concentration which cannot be attributed to changes in the oil content of the liver occasioned by spawning. The explanations offered are necessarily tentative, but the fluctuations themselves are quite definite.

6. The best oils from the standpoint of vitamin A content are most likely to be obtained from large halibut caught in northern waters in the late spring or early summer, and in the autumn. Very rich oils at other times of the year are exceptional. (*Mutatis mutandis* this may also apply to the southern hemisphere.)

REFERENCES.

Bills (1927). *J. Biol. Chem.* **72**, 751.
Carr and Jewell (1933). *Nature*, **131**, 92.
Drummond and Hilditch (1930). *Empire Marketing Board Report*, No. 35.
—— Zilva and Coward (1922). *Biochem. J.* **16**, 518.
Hjort (1914). *Rapports et Procès Verbaux du Conseil Internat.* **20**.
—— (1922). *Proc. Roy. Soc. Lond.* B **93**, 440.
Jameson, Drummond and Coward (1922). *Biochem. J.* **16**, 482.
Johnstone, Scott and Chadwick (1924). The marine plankton. (Univ. Press, Liverpool.)
Lovern (1932). *Nature*, **129**, 726.
—— and Sharp (1933). *Biochem. J.* **27**, 1470.
MacPherson (1932). *Ann. Rep. Newfoundland Fishery Research Commission.*
—— (1933). *Nature*, **132**, 26; *J. Soc. Chem. Ind.* (in press).
Zilva, Drummond and Graham (1924). *Biochem. J.* **18**, 178.