CCLXIII. THE UTILIZATION BY THE RAT OF VITAMIN A AND CAROTENE ADMINISTERED IN DIFFERENT MEDIA.

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BEFORE the adoption of the International Standard of reference for vitamin A, a large amount of work was carried out by several workers to determine the best solvent for its administration to experimental animals. A full account of this work has been given in the Medical Research Council's Special Report, No. 202 [1935]. The general conclusion was that the Standard (a particular sample of β -carotene) was more effective when administered in coconut oil or arachis oil (particular samples only) than when administered in olive oil, hardened cotton-seed oil, ethyl laurate or liquid paraffin. There was some evidence from colorimetric determinations that the carotene was less stable in the latter solvents than in the samples of coconut oil used. Baumann & Steenbock [1933, 2] found by colorimetric determination less destruction of carotene in a refined cotton seed oil than in any other solvent. The possibility that carotene might be utilized differently in different solvents was mentioned in the Medical Research Council's Report.

It seems admissible to test this possibility by the following consideration. If the carotene in a particular solution is imperfectly utilized by the animal, the extent to which different animals utilize it would, presumably, vary. That is, if less than 100 % of the carotene were utilized, it could not be expected that all animals would utilize exactly the same proportion. On this view, therefore, the variation in response of animals given carotene in these "poor" solvents would be greater than that of animals given carotene in the "best" solvent if the "best" solvent owes its superiority to the better utilization of the carotene in it. A measure of this variation can be made by the generally accepted method of estimating the standard deviation (σ) of the test.

Since there are not enough figures available to make a reliable comparison between the variation in response of animals given carotene in coconut oil and that of animals given carotene in other oils, other comparisons of a similar nature have been made from the large amount of material available from the carrying out of tests in this laboratory. Comparisons which at once suggested themselves as useful for this purpose and for which there was sufficient material, were (a) cod liver oil and plant tissues; (b) butter and margarines containing a vitamin concentrate. The vitamin A of cod liver oil is regarded as true vitamin A whilst that of plant tissues is probably entirely in the form of carotene. The vitamin A of butter is probably largely true vitamin A (about 15 % of it was found to be carotene by Baumann & Steenbock [1933, 1]), whilst that of the margarines is also true vitamin A but is contained in different solvents, mostly vegetable oils. The examination of the standard deviations of the responses to these different sources of vitamin A has failed to show any real differences, and it therefore seems probable that the differences in results obtained by the solution of carotene in different oils cannot be due to differences in utilization of the carotene or vitamin A contained in them.

The general estimate of the standard deviation of a single determination (σ) for the response of rats to a dose of vitamin A.

A general estimate of the standard deviation of a single determination (σ) for the response of rats to a dose of vitamin A given daily for 5 weeks after they had ceased to grow on a diet deficient in that factor was published by Coward [1932] from results obtained during the period November 1929 to December 1931. The method of calculating σ was fully described in that paper. From these same experiments the standard deviation of the response of rats during the first three weeks of the test has been calculated (Table I). The tests have since been

Table I. *Variation in response to doses of vitamin A.*

(The whole of the tests from November 1929 to July 1936.)

Serial No. of tests	Dates (approx.)	No. of rats	No. of groups	Σd^2	σ^2	σ
Bucks						
1-108*	Nov. 1929-Dec. 1931	677	112	70,579	126.69	11.26
109-189	Jan.-Dec. 1932	337	72	48,185	182.19	13.50
190-303	Jan. 1933-Sept. 1934	449	111	72,985	216.52	14.71
304-402	Oct. 1934-Oct. 1935	409	92	39,404	124.30	11.15
403-514	Nov. 1935-July 1936	377	95	28,931	102.58	10.13
1-514	Nov. 1929-July 1936	2249	482	260,084	147.19	12.13
Does						
1-108*	Nov. 1929-Dec. 1931	719	112	43,990	72.47	8.51
109-189	Jan.-Dec. 1932	468	82	51,626	133.69	11.56
190-303	Jan. 1933-Sept. 1934	610	116	48,917	99.02	9.95
304-402	Oct. 1934-Oct. 1935	529	97	35,240	81.57	9.03
403-514	Nov. 1935-July 1936	486	108	31,864	84.30	9.18
1-514	Nov. 1929-July 1936	2812	515	211,637	92.14	9.60

* Including 4 inserted afterwards.

carried on with no avoidable differences, and from time to time the standard deviation of the three weeks' test has been determined from the whole of the results available. These are collected in Table I. No explanation of the rise in the value of σ in 1932 and 1933 is suggested, or of the subsequent fall in 1934, 1935 and 1936. The values are given to show that the variation in the response of animals to apparently the same treatment may itself vary from time to time. The average value for σ is given as a general estimate of its value for tests carried out in this laboratory.

In addition a calculation has been made of the standard deviation of the response in all tests made on cod liver oil Z since it was adopted as a subsidiary standard of reference in this laboratory in November 1932. These results are included in the general estimate of the standard deviation, of which they form only about 16 %. The value of σ for cod liver oil is, according to both male and female rats, equal to the average for all the tests (Table II).

Table II. *Variation in response to vitamin A in cod liver oil Z.*

(November 1932 to July 1936.)

	No. of rats	No. of groups	Σd^2	σ^2	σ
Bucks	347	82	38,330	144.64	12.03
Does	452	85	32,498	88.55	9.41

Comparison between the variation in response to doses of vitamin A (a) in cod liver oil and (b) in plant tissues.

Since there is evidently some influence affecting the variation in response of the whole stock of animals from time to time, a comparison of the variation of two groups of animals can only be made safely with those animals which are on test simultaneously, so that whatever is affecting the variation must affect both groups equally. During the last twelve months we have been estimating the vitamin A contents of a large number of vegetable tissues (fruits, vegetables, pulses etc., some of them dried but most of them fresh) by comparison with our cod liver oil Z. These, together with a few more tests carried out previously, have given us sufficient material for this comparison. Our procedure for such comparisons is to prepare large numbers of rats by giving them the vitamin A-free diet, starting several litters two or three times a week, so that we have a continuous supply of rats becoming steady in weight on a shortage of vitamin A. As the rats become ready for test, they are divided into groups, one to be given a dose of the standard, another to be given a dose of the test substance. We do not regard it as essential that litter-mates should be used for comparisons, for we have determined (unpublished results) that the variation in response of litter-mates to the same dose of vitamin A, given when the rats have become steady in weight, is not less than that of rats from different litters. We are fully aware that the members of different litters have different reserves of vitamin A, but we consider that our procedure of just exhausting these reserves reduces the rats from different litters to a fairly similar condition. Naturally, in prophylactic tests or in partially prophylactic tests, litter-mate comparison is essential.

In assigning the rats which are ready for test to the different groups we think it is highly important that as far as possible equal numbers of rats should be assigned to the different groups each day. Whatever may be the various causes of the variation in response, those which may be felt from day to day (such as changes in temperature shown by Tourtellotte & Bacon [1935] to have an effect in vitamin D experiments) must be allowed to have equal influence on the different groups. If our supply of rats ready for test is good, we may compare two or even three test substances with the standard, putting the rats into the groups for (a) standard, (b) test A, (c) test B, and (d) test C respectively in regular sequence.

With the comparison controlled in this way, we have found that, according to the male rats of the tests, the variation in response to the vitamin A of cod liver oil is the same as to that of plant tissues, whilst according to the female rats, the variation in the response to the vitamin A of cod liver oil is a little larger than to that of the plant tissues but not significantly so (Table III).

Table III. *Comparison between cod liver oil and vegetables tested at the same time with regard to the variation in response to vitamin A.*

(May 1933 to July 1936.)

		No. of rats	No. of groups	Σd^2	σ^2	σ
Cod liver oil	Bucks	163	36	14,245	112.17	10.59
	Does	193	38	14,727	95.01	9.75
Vegetables	Bucks	227	60	18,793	111.20	10.54
	Does	294	72	16,531	74.46	8.63

*Comparison between the variation in response to doses of vitamin A in
(a) butter, and (b) margarine containing a vitamin concentrate.*

For some years we have been making a comparison almost every month between a sample of a "vitamin margarine" and a sample of dairy butter, both bought at the same time and in the open market. Thus we have ample material from which to judge the variation in response to doses of vitamin A (*a*) as contained in butter and (*b*) as contained in this margarine. The comparisons were always made as described in the last section of this paper, i.e. they were made by simultaneous tests of the two products. The result may be seen in Table IV.

Table IV. *Comparison between butters and vitaminized margarines tested at the same time, with regard to variation in response to vitamin A.*

(January 1930 to July 1936.)

		No. of rats	No. of groups	Σd^2	σ^2	σ
Butters	Bucks	257	54	33,007	162.60	12.75
	Does	315	56	24,483	94.53	9.72
Margarines	Bucks	281	67	28,042	131.04	11.45
	Does	371	69	30,300	100.66	10.03

The standard deviation (σ) of a single determination according to the male rats was rather higher for the butters than for the margarines, but according to the female rats it was rather higher for the margarines than for the butters. Thus it must be concluded that there is no greater or less variation in the utilization of the vitamin A of butters than in that of the margarines containing the vitamin A concentrate.

SUMMARY.

Carotene has been found by several workers to be most effective as vitamin A on experimental animals when administered in coconut oil. This may be due to partial destruction in the "poorer" oils which has been demonstrated colorimetrically, but it has also been suggested that it may be partially due to a less complete utilization from the poorer oils.

If this is so, then it is to be expected that the utilization, being less than complete, would vary between different animals. This would bring about a greater variation in the response to a given dose of carotene than that which is observed normally when, presumably, utilization is complete. Comparisons of the variations in response to doses of carotene or vitamin A in different solvents or from different sources should, therefore, if carried out under strictly controlled conditions, indicate differences in utilization of the vitamin.

Comparisons of the variation in response to (*a*) the vitamin A in cod-liver oil and that contained, probably wholly as carotene, in plant tissues (for which 877 rats were used), and (*b*) the vitamin A (partly carotene) of butter and the vitamin A of margarines containing a vitamin concentrate (for which 1224 rats were used) show no significant differences.

It is therefore concluded that there is no difference in the powers of animals to utilize vitamin A or carotene either as supplied to them in cod liver oil or in

plant tissues, or as supplied to them in butters or as concentrates in various vegetable oils. The causes of the difference in effectiveness of carotene dissolved in different oils must be sought elsewhere.

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