decarboxylase from *Strep. faecalis* (Epps, 1944). The specific protein of the tyrosine decarboxylase was then precipitated and freed from codecarboxylase (Epps, 1944). The activity of this specific protein was then tested towards tyrosine alone and in the presence of codecarboxylase (preparation 8 *a*); a boiled preparation of tyrosine decarboxylase (8 units), and a boiled preparation of lysine decarboxylase (15 units). The values in Table 7 show that the apo-enzyme can be reactivated towards its substrate by the presence of the codecarboxylase preparation, or of the boiled inactive preparations of either enzyme. Thus the lysine decarboxylase preparation contains a substance which will act as the codecarboxylase of the tyrosine decarboxylase.

SUMMARY

1. Codecarboxylase is widely distributed amongst animal tissues, plant tissues, yeasts and bacteria.

2. Codecarboxylase can be extracted from dried brewer's yeast by mildly alkaline solutions.

3. A method is described for the purification of codecarboxylase extracted from dried brewer's yeast. An over-all purification of 15,000 times can be achieved.

3. The resulting preparation is colourless or faintly yellow, contains C, H, N but no P or S, and has a single absorption band in the ultra-violet at 265mμ.

4. The coenzyme activity is stable to alkaline hydrolysis but is rapidly destroyed by N/10 H_2SO_4 at 100°.

5. Evidence is presented that *l*(+)-lysine decarboxylase and *l*(−)-tyrosine decarboxylase have the same codecarboxylase.

We wish to express our gratitude to Mr B. Slater, Mr J. Selwyn and Miss S. Taylor for assistance with preparative work; to Dr F. Bergel and Roche Products Ltd. for assistance with large-scale preparations; to Prof. A. C. Chibnall and Dr M. Dixon for helpful criticism; to the Medical Research Council for a grant towards expenses and a full-time grant to one of us (H. M. R. E.), and to the Beit Memorial Fellowship Trustees and the Foundation of St John's College, Cambridge, for full-time grants to the other (E. F. G.).

REFERENCES

Epps, H. M. R. (1944). *Biochem. J.* **38**, 242.
Gale, E. F. (1940). *Biochem. J.* **34**, 392, 846, 853.
—— (1942). *Biochem. J.* **36**, 64.
Gale, E. F. & Epps, H. M. R. (1944). *Biochem. J.* **38**, 232.
Van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.

A Liver Storage Test for the Assessment of Vitamin A

By K. GUGGENHEIM and W. KOCH, *Department of Hygiene and Bacteriology, Hebrew University, Jerusalem*

(*Received* 8 *January* 1944)

The introduction of chemical methods for the determination of vitamins led most investigators to prefer these methods to biological assays. The chemical methods are simpler, cheaper and require less time. The physiologist, however, is not only interested in the quantity of vitamin given, but in its utilization by the organism. This question can be answered by the biological assay. Often, especially in the investigation of liver oils, there are considerable differences among the chemical, physical and biological results of vitamin A determinations (Coward, Dyer, Morton & Gaddum, 1931; Edisbury, Gillam, Heilbron & Morton, 1932; Morgan, Edisbury & Morton, 1935; Pritchard, Wilkinson, Edisbury & Morton, 1937; Robinson, 1938; Embree, 1939; Lunde & Kringstad, 1940). These differences have brought about such uncertainty regarding the value of the vitamin A potency of some materials that in these cases it is now agreed to consider only the results of bioassay as reliable. Nevertheless, great disadvantages of the methods of bioassay for vitamin A lie in the time and effort needed for their performance; and this is especially true of the curative growth test, the method most commonly used.

The difficulties in the determination of the vitamin A content of biological materials are even greater, if these materials contain both vitamin A and carotene, or even carotene alone. The usual chemical method for carotene determination, that of Guilbert (1934), is not exact enough, because α-, β-, and γ-carotene and kryptoxanthin are all determined together, and although all are precursors of vitamin A they differ in their biological potency. Because of the different amounts of the various carotenes present in different plant foods, this kind of determination cannot give the real vitamin A

potency of the substance tested. At present no adequate means exist which allow quantitative separation of the individual carotenoids. Although admirable in the qualitative separation of carotenoids, the technique of chromatography has yet to prove of value in their quantitative determination (Peterson, 1941).

These considerations led us to seek a shorter and simpler biological method than those in use. Our starting point was the fact that the liver is the most important reservoir of the body's vitamin A (Moore, 1931; Baumann, Riising & Steenbock, 1934). Consequently we considered the possibility of determining the available amount of vitamin A in materials by feeding them to rats and then determining chemically the amount of vitamin A stored in the liver. The results of such a method would depend upon the utilization of vitamin A by the organism and would be obtained within a few days. Before accepting such a method of bioassay it would be necessary to decide whether there exists a constant relation between the amount of vitamin A given to the rats and the amount stored in their livers.

METHODS

Rats weighing 35–50 g. were used. The food of the mothers consisted of sprouted wheat, barley, bran, the seasonal vegetables and milk. Two weeks before their progeny were weaned no vegetables were included in their food, the amount of vitamin A thus being reduced. The livers of the young rats then contained only 3–10 i.u. vitamin A. This amount of vitamin A disappeared completely within 2–6 days, when the young rats were given a vitamin A-free diet consisting of 65% rice flour, 13% casein (ethanol extracted), 10% olive oil, 8% dried yeast, 4% salt mixture and vitamin D, 100 i.u./kg. food (mixed with the oil).

This treatment produced rats with no signs of avitaminosis, which were suitable for our experiments.

Vitamin A ('Prepalin', Glaxo Laboratories Ltd.) was administered *per os* in 0·1 ml. olive oil. On the first and second days the rats were given a known amount of vitamin A/10 g. body weight. On the fourth day the rats were guillotined, the livers prepared according to Lindquist (1938), and their content of vitamin A determined according to the method of Carr & Price. The estimation was made by means of a block-comparator. The comparison colour was a solution containing 6·5% $CuCl_2$ and 0·3% $CoNO_3$ (Kuhn & Brockmann, 1931). For the control colorimetric determination vitamin A ('Prepalin') was used as a standard.

Some authors prefer the stufenphotometer or the photoelectric colorimeter, because they give a more exact determination of the rapidly fading blue colour. We found, however, that with a little experience with the block comparator we could obtain a high degree of precision. Control estimations showed that the experimental error never exceeded 10%. Pett & Le Page (1940) also report a satisfactory degree of accuracy with a simple colorimeter.

Altogether the livers of 312 rats were examined, 156 males and 156 females. The rats were divided into five groups, each rat of each group receiving on the first and second day of experiment 10, 15, 20, 30 or 35 i.u. of vitamin A/10 g. body weight respectively. Each group contained the same number of males and females. For the examination two livers from rats of the same group and the same sex were pooled.

RESULTS AND DISCUSSION

After the administration of various amounts of vitamin A to rats depleted under conditions as specified above, the amounts of vitamin A shown in Table 1 were recovered from the liver.

Table 1. *Amounts of vitamin A recovered from rat liver after its administration*

No. of pairs of rats*	Vitamin A administered (i.u./10 g. body weight†)	Total vitamin A recovered (i.u.)	
		Mean	S.D.‡
42	10	4·3	0·905
24	15	10·8	2·18
56	20	16·4	4·02
24	30	24·5	3·23
10	35	29·5	2·69

* Equal numbers of males and females were used.
† The same dose was given daily for 2 days.
‡ S.D. = standard deviation.

A plot of the amounts administered on a logarithmic scale and the amounts recovered on a linear scale, as is done in Coward's reference line for the

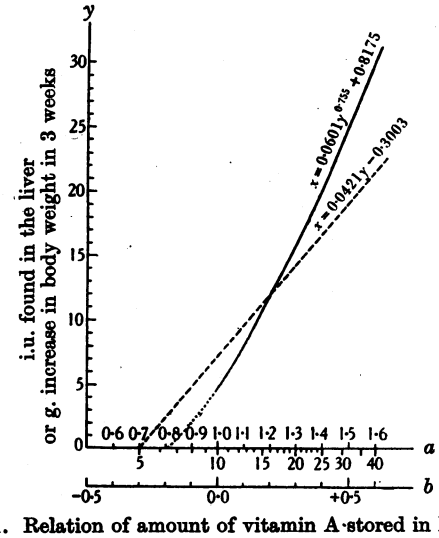

Fig. 1. Relation of amount of vitamin A stored in liver of rats to the dose administered. —— Liver storage reference curve. ----- Growth response reference curve of Coward.

(*a*) log units / i.u. } given/10 g. body weight.

(*b*) log units given (growth test).

growth test (Coward, 1939, p. 34), gave not a straight line but a parabola (Fig. 1). The equation of this curve was (Toft & McKay, 1940, p. 279):

$$x = 0{\cdot}0601y^{0{\cdot}755} + 0{\cdot}8175,$$

where x = logarithm of vitamin A given expressed as i.u./10 g. body weight, and y = the total units recovered from the liver. This reference curve holds good up to 35 i.u. administered. The lowest amount actually administered was 10 i.u./10 g. rat; by inserting zero for y, it seems probable that 6·5 i.u./10 g. body weight will not cause any liver storage at all.

To compare the reliability of our method with that of Coward, three sets of growth tests were performed. As described by Coward (1939), young rats were kept on a vitamin A-free diet until no further increase in weight occurred. Then each of them was given 1 i.u. vitamin A/day for 3 weeks. Since the livers of two animals had to be pooled to obtain clear readings in the Carr-Price reaction in low concentrations, the mean increase in weight of two animals, paired at random, had to be taken for comparison. The results of these tests are given in Table 2.

Table 2. *Three weeks' curative growth test*

(Each rat was given 1 i.u. vitamin A daily for 3 weeks.)

Date	No. of pairs of		Weight increase (g./pair)	
	Males	Females	Mean	S.D.
July–Aug. 1941	3	3	9·0	3·64
Nov.–Dec. 1941	3	3	18·2	4·21
Oct.–Nov. 1942	6	6	15·2	4·88

To facilitate comparison, Coward's growth response line (3 weeks' test, equal numbers of males and females) has been superimposed on our curve in Fig. 1. The formula of this line is:

$$y = 23{\cdot}77x + 7{\cdot}14 \quad \text{or} \quad x = 0{\cdot}0421y - 0{\cdot}3003,$$

where x = logarithm of dose, and y = body-weight increase in g. Three methods were adopted to assess and compare the accuracy of the growth test with the liver storage test.

(1) According to Gaddum (1931, quoted by Coward, 1939, p. 165), a measure of the accuracy of the growth test is given by the S.D. (standard deviation) divided by the slope of the curve of response. This ratio, called the S.D. of the logarithms of the result, is denoted by λ. Since Coward's logarithmic reference line for the growth test is a straight line, the slope is at all points the same. In Fig. 1 the slope of the Coward line is 1·187. The S.D. of weight increase in our three sets of growth tests (24 pairs of animals) was found to be 4·27, whence λ equals 3·59.

Our data are not precisely comparable with those of Coward because, as already mentioned, we considered the S.D. of the weight increase of pairs, while Coward calculated it for single animals. In the 3 weeks' growth test Coward (1939, p. 171) found a S.D. of the weight increase for males ($\sigma\,♂$) of 11·86 g. and for females ($\sigma\,♀$) of 9·74 g. Therefore the S.D. of the weight increase for an equal number of males and females is:

$$\sqrt{\frac{\sigma♂^2 + \sigma♀^2}{2}} = 10{\cdot}85\,\text{g.}$$

The S.D. of the weight increase for an equal number of pairs of males and females can be derived without great error, if the S.D. of the weight increase of single animals is divided by $\sqrt{2}$:

$$\frac{10{\cdot}85}{\sqrt{2}} = 7{\cdot}68\,\text{g.};$$

therefore

$$\lambda = \frac{7{\cdot}68}{1{\cdot}187} = 6{\cdot}47.$$

Our reference curve is a parabola. The slope, therefore, has to be determined by differentiation. Below are listed the values of λ for varying amounts of vitamin A administered/10 g. of rat:

I.u. administered	λ
10	0·573
15	1·105
20	1·860
30	1·357

All the values of λ are better than 3·59, which was obtained with the growth tests. The ratio of the values obtained from liver storage and growth tests is 1·86 : 3·59, even when we take the '20' level, which has the highest value of λ. Furthermore, from the standard errors of the two tests it may be calculated that 3½ times as many rats would have to be used in the growth test to obtain the accuracy of the liver storage test.

(2) The probable error of a growth test is estimated to be 20 % in the 3 weeks' growth test, and about 16 % in the 5 weeks' growth test, when ten male rats are used. In our three growth tests, based on 24 pairs of animals only, the probable error was 12 %.

In the liver storage test, even if we take the less favourable results obtained at a level of 20 units administered, the probable error was only 6·5 %, with five pairs of animals. This agrees with the results of calculations given above; for if one takes 3·7 times as many pairs of animals, i.e. 17 pairs instead of 5, the probable error becomes 6·5 % instead of 12 %.

(3) As a check on the calculations, a further series of tests were made. Groups of rats, each consisting of two pairs, were given a varying number of units of vitamin A. In the first series, different groups were given 10, 20 and 35 i.u./10 g. body weight. The liver storage of vitamin A in the first group amounted to 2·75 i.u., in the second 10·25 i.u., and in the third 21·0 i.u. (Table 3). From our reference curve 2·75 i.u. recovered corresponds to 8·8 i.u. given; in other words the 10 i.u. administered acted only as 8·8 i.u., or the factor of potency of the

material used in relation to our curve is 8·8 : 10 = 0·88. Similarly for the dose of 20 units a factor of potency of 0·74 was obtained, and for 35 units, 0·76. Like Coward's reference line for the growth test, our curve does not give absolute but only relative values. Hence if we take the 20 units level, which lies roughly in the middle of our range, to be 100 %, the 10 i.u., in comparison with the action of 20 i.u., will be as 0·88 : 0·74 = 119 %, and the 35 i.u. as 0·76 : 0·74 = 103 %. The relative deviation would thus be 19 % in the first and 3 % in the second case. Further sets of tests are listed in Table 3.

Table 3. *Relative deviations of the amounts of vitamin A recovered from rat livers after administration of varying doses*

No. of pairs of rats	Vitamin A (i.u.) Given	Vitamin A (i.u.) Recovered from liver	Value of amount given* (i.u.)	Factor of potency†	Value of amount given (% of 20 i.u.)	Deviation from 20 i.u. value (%)
2	10	2·75	8·8	0·88	119	19
2	20	10·25	14·7	0·74	100	—
2	35	21·00	26·6	0·76	103	3
2	10	3·35	9·3	0·93	102	2
2	20	14·00	18·2	0·91	100	—
2	35	24·35	31·7	0·91	100	0
2	10	3·70	9·5	0·95	117	17
2	20	11·75	16·2	0·81	100	—
2	35	25·00	32·8	0·94	116	16
2	10	2·70	8·8	0·88	109	9
2	20	11·80	16·2	0·81	100	—
2	10	3·80	9·6	0·96	99	1
2	20	15·00	19·3	0·97	100	—
2	10	3·70	9·5	0·95	83	17
2	15	11·10	15·6	1·04	90	10
2	20	18·50	23·0	1·15	100	—
2	30	27·00	35·7	1·19	103	3
2	10	4·10	9·8	0·98	82	18
2	15	13·70	18·0	1·20	100	0
2	20	19·10	24·0	1·20	100	—
2	30	27·00	43·0	1·43	119	19
2	8	0	>6·5	>0·8	—	—
2	12	0·90	7·4	0·62	103	3
2	16	2·10	8·3	0·52	87	13
2	20	7·00	11·9	0·60	100	—
2	24	9·20	14·0	0·58	97	3
2	28	13·60	17·9	0·64	107	7
2	32	11·00	15·7	0·49	82	18

* Obtained from liver storage reference curve.

† Factor of potency is: $\frac{\text{Value of amount given}}{\text{Actual amount given}}$.

In the results tabulated, which admittedly represent rather few observations, the mean percentage error is 9·5, when rats receiving 20 i.u. are used as a base and other suitable amounts are given to other pairs. In these tests the S.D. of the experimental errors is 11·8 %, and the probable error is 7·8 %. Assuming a difference of twice the S.D. as probably not being due to chance, a deviation of more than 24 % from the base would be significant; none of the experiments showed a deviation of more than 19 %.

On the basis of the reasons given, it seems to us that the liver storage test has an accuracy at least of the same order as the 3 weeks' curative growth test, and has the advantage of being much shorter. Its limitations lie in the fact that the materials to be tested must contain an amount of vitamin A which will cause appreciable storage of vitamin A in the liver, when administered on two successive days after depletion of the normal stores in the liver.

SUMMARY

1. A method for the bioassay of vitamin A is described, which is based on the determination of the amount of vitamin A stored in the livers of rats previously depleted of this vitamin, when the material under test is fed on two successive days.

2. This test seems to be no less accurate than the 3 weeks' curative growth test, and requires only 4 days to perform.

REFERENCES

Baumann, C. A., Riising, B. M. & Steenbock, H. (1934). *J. biol. Chem.* **107**, 705.
Coward, K. H. (1939). *The Biological Standardisation of the Vitamins*. London: Ballière, Tindall and Cox.
—— Dyer, F. J., Morton, R. A. & Gaddum, J. H. (1931). *Biochem. J.* **25**, 1102.
Edisbury, J. R., Gillam, A. E., Heilbron, I. M. & Morton, R. A. (1932). *Biochem. J.* **26**, 1164.
Embree, N. D. (1939). *J. biol. Chem.* **128**, 187.
Guilbert, H. R. (1934). *Industr. Engng Chem.* (Anal. ed.), **6**, 452.
Kuhn, R. & Brockmann, H. (1931). *Ber. dtsch. chem. Ges.* **64**, 1862.
Lindquist, T. (1938). *Acta med. scand.* Suppl. **97**, 1.
Lunde, G. & Kringstad, H. (1940). *Tidskr. Kemi Bergv.* **20**, 14 (quoted in *Nutr. Abstr. Rev.* **10**, 46, 1940).
Moore, T. (1931). *Biochem. J.* **25**, 275.
Morgan, R. S., Edisbury, J. R. & Morton, R. A. (1935). *Biochem. J.* **29**, 1645.
Peterson, W. J. (1941). *Industr. Engng Chem.* (Anal. ed.), **13**, 212.
Pett, R. & Le Page, G. A. (1940). *J. biol. Chem.* **132**, 585.
Pritchard, H., Wilkinson, H., Edisbury, J. R. & Morton, R. A. (1937). *Biochem. J.* **31**, 258.
Robinson, F. A. (1938). *Biochem. J.* **32**, 807.
Toft, L. & McKay, A. D. D. (1940). *Practical Mathematics*. London: Pitman.

The Biological Value of Carotene from Various Sources and the Effect of Vitamin E on the Utilization of Carotene and of Vitamin A

By K. GUGGENHEIM, *Department of Hygiene and Bacteriology, Hebrew University, Jerusalem*

(*Received* 9 *May* 1944)

In 1934 the Second International Conference on Vitamin Standardization defined the international unit of vitamin A as the vitamin A potency of 0·0006 mg. of β-carotene. In the last decade, however, it has become clear that in many cases a simple relationship does not agree with the findings. In experiments with human beings (Booher & Callison, 1939; Booher, Callison & Hewston, 1939) and with animals (Guilbert, Howell & Hart, 1940; Smith & Otis, 1941; Treichler, Kemmerer & Fraps, 1942) it was found that vitamin A was utilized better than carotene. Moreover, several authors (Dyer, Key & Coward, 1934; Kraybill & Shrewsbury, 1936; Lease, Lease, Steenbock & Baumann, 1939; Sherman, 1941; Treichler *et al.* 1942) pointed out that the utilization of carotene by rats was influenced by the nature of the oil used as solvent. It appears, therefore, that the resorption and transformation of carotene into vitamin A depends on factors of which the nature and mechanism are as yet unknown.

It seems, therefore, that existing evidence is contrary to the assumption that 0·0006 mg. β-carotene always has the biological potency of 1 i.u. vitamin A. Its efficiency as pro-vitamin A depends, apparently, on many factors intimately connected with the material in which it is contained (various oils, plant materials). A more suitable descriptive term of the quantitative biological potency of carotene would be the *biological value of carotene*, which may be defined as:

$$\frac{\text{Actual vitamin A potency of the material, expressed in terms of i.u.}}{\text{Carotene content of the material, determined chemically, expressed in terms of i.u.}} \times 100.$$

The liver storage test, described in a previous communication (Guggenheim & Koch, 1944), served as a useful means for assessing vitamin A potency. Using this method, we have determined the biological value of carotene of various human and animal foods.

METHODS

For each examination, 8 or 10 test rats and an equal number of control rats were used, each group comprising an equal number of males and females. The preparation of the rats for the experiments, the vitamin A-free diet, the method of vitamin A determination in the rat livers, and the experimental procedure have been described in a previous paper (Guggenheim & Koch, 1944). The test rats and the control rats were given on two successive days the material to be tested or a measured amount of vitamin A respectively. On the fourth day the rats were killed and their livers examined. In the present study 'Prepalin' (Glaxo Laboratories Ltd.) was also used as a vitamin A standard preparation. The respective amounts of carotene and vitamin A of the materials given to the test rats, and that of vitamin A given to the control animals, were chosen according to their body weight. Carotene was determined according to the