CXXXIX. THYROID AND VITAMIN B₁¹

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(Received 1 June 1939)

FROM 1930 onwards Abelin and colleagues have drawn attention to the importance of diet in general in experimental hyperthyroidism [e.g. Abelin, 1930]. In more specific work Himwich *et al.* [1932] claimed that in experimental hyperthyroidism dogs require more than the normal amount of vitamin B₁, and Cowgill & Palmieri [1933] found that pigeons with hyperthyroidism needed additional amounts of vitamin B₁ if their body weight was to be maintained. Later Sure & Buchanan [1937], using young rats, and Drill & Sherwood [1938], using more mature animals, were able to counteract with vitamin B₁ the fall in weight produced by thyroid administration.

During the course of an investigation on the tissue respiration of thyroid-treated rats, we soon became aware that the kidneys and livers were oedematous. This turned our attention to the relation between thyroid feeding and vitamin B₁. In experiments commenced before the last-mentioned paper appeared we have been able to confirm fully the results of the above workers. In addition Drill [1938], using a yeast fermentation method of estimation which has not been free from criticism, has reported a diminution of the vitamin B₁ content of several rat tissues following thyroid feeding. From catatorulin tests and determinations of the cocarboxylase content of boiled extracts from various tissues we can however state definitely that a genuine vitamin B₁ deficiency exists in the tissues of rats with hyperthyroidism.

Reagents used

Cocarboxylase. The cocarboxylase used as a standard was a synthetic product kindly presented by Messrs Merck (U.S.A.). This was standardized by comparison with a pure specimen of cocarboxylase from Dr K. Lohmann and found to contain 65% of active material.

Vitamin B₁. Synthetic vitamin B₁ hydrochloride was kindly presented by Messrs Hoffmann La Roche and Messrs Bayer.

Sodium pyruvate. For the catatorulin experiments crystalline sodium pyruvate was prepared as previously described [Peters, 1938]. For the cocarboxylase experiments a preparation was made by Mr C. Long by the neutralization of pyruvic acid by sodium ethoxide in alcohol.

Desiccated thyroid and thyroxine. These were commercial products.

Baker's yeast. This was obtained from the Distillers Co., Ltd.

Our best thanks are due to Messrs Merck, Hoffmann La Roche and Bayer for their gifts.

¹ A preliminary report of these experiments has appeared in *J. Soc. chem. Ind., Lond.*, 58, 471, 1939.

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The rats used, all males, were taken from the usual laboratory stock. Throughout all the experiments the animals were fed on the following basal diet:

Rice starch	...	70
Casein	...	20
Salt mixture	...	5
Agar agar	...	2
Cod liver oil	...	3

To 100 g. of this diet were added 10 g. dry yeast.

The yeast and cod liver oil supplements provided the vitamin requirements. Mr H. W. Kinnersley has carried out curative pigeon tests and estimations by fluorescence of the vitamin B₁ in the yeast. He reports that after treatment with takadiastase the value obtained by the bird test (16 animals) was 4.8 µg./g., and by the thiochrome method 5.0 µg./g. He states (private communication) that, in the absence of takadiastase digestion, much less appeared to be present.

The average vitamin B₁ intake per day was about 7 µg. per animal. Experiments with over 40 animals have shown that there was no increase in growth rate if larger amounts of the vitamin were added to the diet. As far as vitamin B₁ is concerned the standard diet may be said to be adequate for normal growth and development.

(1) *Protection from loss of weight following thyroid administration by injection of vitamin B₁*

Three groups of 6 animals (av. wt. 180 ± 10 g.) were fed on the basal diet for 12 days. Group I was a control. Each animal in Group II was given daily 1 mg. thyroxine (dissolved in 0.5 ml. *M*/100 KOH) and 0.5 mg. synthetic vitamin B₁ hydrochloride (dissolved in 0.5 ml. 1% NaCl) by subcutaneous injection. Group III was given the thyroxine without the vitamin. It is seen in Fig. 1 that the animals in Group III (thyroxine only) rapidly lost weight, but that this fall was partially prevented if vitamin B₁ were given in addition (Group II). This completely confirms the results of Drill & Sherwood [1938].

This protection from fall in weight conferred by vitamin B₁ has also been shown in the following new experiment. Two further groups of animals (av. wt. 135 g.) were fed on the basal diet for 7 days, and at the end of this time a daily dose of 0.4 g. desiccated thyroid was given to each animal. During the 7 days on the basal diet only, Group V received daily by subcutaneous injection 250 µg. vitamin B₁ hydrochloride. Group IV received no such pretreatment. The pretreated animals (Fig. 2) fell in weight at a much slower rate than the untreated controls. Hence a pretreatment with vitamin B₁ also gives a protection against rapid fall in weight.

The weight changes in these feeding experiments are summarized in Table I. Application of Fisher's *t* test to the difference of means of Groups II and III gives *t*=4.33. For *P*=0.01, *t*=3.17. The difference between the means of Groups II and III is thus clearly significant.

The diet, although low in vitamin B₁, contains enough of the vitamin for normal growth as is evidenced by the control Group I. Apparently, however, it does not contain enough of the vitamin to give protection from the large falls in weight produced by thyroxine injections.

Fig. 1.

Fig. 1. Changes in weight of rats injected with thyroxine with and without vitamin B₁. Each curve=average for 6 rats. Curve I: control.* Curve II: thyroxine (1 mg. per day) plus vitamin B₁ (0.5 mg. per day). Curve III: thyroxine (1 mg. per day).

Fig. 2.

Fig. 2. Change in weight of rats fed on desiccated thyroid with and without pretreatment with vitamin B₁. Each curve=average for 8 rats. Curve IV: 0.4 g. desiccated thyroid per day (no pretreatment). Curve V: 0.4 g. desiccated thyroid per day (pretreated with 250 µg. vitamin B₁ per day for 7 days).

Table I. Mean change in weight of rats

Treatment	No. of animals	Mean change in weight (g.)	S.E.
I. Control	6	+20.2	±2.6
II. Thyroxine (1 mg. per day). Vitamin B ₁ (0.5 mg. per day)	6	+ 2.2	±2.1
III. Thyroxine (1 mg. per day)	6	-26.6	±7.8
IV. 0.4 g. desiccated thyroid per day (no pretreatment)	8	-38.9	±4.6
V. 0.4 g. desiccated thyroid per day (pretreated with 250 µg. vitamin B ₁ per day for 7 days)	6	- 1.0	±4.1

(2) *Catatorulin effects with brain tissue*

The O₂ uptake of minced brain tissue taken from normal rats and rats with hyperthyroidism was determined, both in the presence and absence of added vitamin B₁, by the usual Warburg manometric technique, using sodium pyruvate as substrate. Each experiment was run for 2 hr. after a 15 min. equilibration period.

* The average weight of the control group (curve I) is some 10 g. higher than that of groups II and III. This was due to an oversight; we have no reason to believe that animals in a slightly lower weight group would react differently.

Table II shows the catatorulin tests, the % increase being calculated, as is the usual practice, for the period 60–120 min. [Peters, 1938]. Both rats fed on thyroid and those injected with thyroxine gave positive effects which were not observed with control animals on the same diet. Two thyroxine-injected animals which were treated for 12 days instead of the usual 16–18, however, did not show the effect.

Table II. *O₂ uptake (μl./g. fresh tissue/hr.) of normal and hyperthyroid brain tissue, with and without addition of vitamin B₁ (5 μg.). Substrate, sodium pyruvate. Temperature, 38°. The O₂ uptake was measured over the period 60–120 min.*

Exp. no.	Treatment	Days of treatment	Pyruvate	Pyruvate plus vitamin	Difference	% catatorulin effect
35	Normal	—	1733	1795	+ 62	+ 3.5
36	"	—	1682	1643	- 39	- 2.5
37	"	—	1644	1570	- 74	- 4.5
41	"	—	1377	1342	- 35	- 2
39	"	—	1515	1488	- 27	- 2
Mean (±s.e.) = -1.5 (±1.3)						
33	Thyroid-fed	16	1891	2115	+ 224	+ 12
34	"	16	1843	2111	+ 268	+ 14.5
38	"	15	1710	1806	+ 96	+ 5.5
56	"	17	1475	1648	+ 173	+ 12
57	"	17	1524	1684	+ 160	+ 10
61	"	18	1535	1565	+ 30	+ 2
45	Thyroxine-injected	18	1753	1911	+ 158	+ 9
58	"	18	1315	1429	+ 114	+ 8.5
59	"	18	1236	1360	+ 124	+ 10
60	"	18	1465	1638	+ 173	+ 12
Mean (±s.e.) = 9.6 (±1.1)						

Since O'Brien & Peters [1935] found that even with avitaminous rat cerebrum the catatorulin effects were small, the observed increases with hyperthyroid tissue are of interest.

Cohen & Gerard [1937], using the delayed substrate technique of Quastel & Wheatley [1932], find that for most substrates there is a higher O₂ uptake with hyperthyroid brain tissue than with normal. This does not apply to sodium pyruvate. Their technique however is not free from criticism, as small traces of substrate present may stabilize the enzyme systems [Peters, 1936]. Our observations indicate that with glucose brain tissue from hyperthyroid animals has a higher O₂ uptake than that from normal ones, and also confirm Cohen & Gerard's claim, qualitatively but not quantitatively. That the absence of effect found by them in the case of sodium pyruvate might be due to a vitamin B₁ deficiency is suggested by Table III. With this substrate, though there is no significant difference between the O₂ uptakes of normal and hyperthyroid tissue, with vitamin B₁ added the difference becomes significant.

Table III. *Average O₂ uptake (μl./g. fresh tissue/hr.) of brain tissue from normal and hyperthyroid rats, and of tissue from hyperthyroid rats after the in vitro addition of 5 μg. vitamin B₁. Substrate, sodium pyruvate. The O₂ uptake was measured over the period 60–120 min. Temperature, 38°.*

Treatment	No. of observations	Mean O ₂ uptake	S.E.
Normal	9	1580	±50
Hyperthyroid	12	1530	±65
Hyperthyroid + vitamin B ₁	14	1800	±72

(3) *Catatorulin effects with kidney tissue*

In early experiments Thompson [1934] reported catatorulin effects with kidney tissue from avitaminous animals, using sodium lactate. Table IV (sliced tissue) and Table V (minced tissue) show that with sodium pyruvate hyper-

Table IV. O_2 uptake ($\mu\text{l./g. fresh tissue/hr.}$) of normal and hyperthyroid kidney tissue (tissue slices) with and without the addition of vitamin B_1 ($5 \mu\text{g.}$). Substrate, sodium pyruvate. Temperature, 38° . The O_2 uptake was measured over period 60–120 min.

Exp. no.	Treatment	Pyruvate	Pyruvate plus vitamin	Difference	% catatorulin effect
16	Normal	3768	4979	+1210	+32
17	"	4257	5265	+1009	+24
18	"	3780	4842	+1062	+28
					Mean = +28
15	Thyroid-fed	4050	4838	+784	+19
25	Thyroxine-injected	3818	5148	+1330	+35
					Mean = +28
14	Thyroid-fed. Vitamin B_1 injected (1 mg. per day for 3 days)	4790	4565	-225	-4.5

Table V. O_2 uptake ($\mu\text{l./g. fresh tissue/hr.}$) of normal and hyperthyroid kidney tissue (tissue mince) with and without the addition of vitamin B_1 ($5 \mu\text{g.}$). Substrate, sodium pyruvate. Temperature, 38° . The O_2 uptake was measured over period 60–120 min.

Exp. no.	Treatment	Pyruvate	Pyruvate plus vitamin	Difference	% catatorulin effect
20	Normal	1725	2018	+293	+17
26	"	1640	1795	+155	+9.5
27	"	985	1238	+253	+26
					Mean = +17
19	Thyroid-fed	2540	2857	+317	+12.5
22	Thyroxine-injected	1909	2100	+191	+10
23	"	1651	1853	+202	+12
24	"	1995	2380	+385	+19.5
25	"	2440	2933	+494	+20
					Mean = +15
21	Thyroid-fed. Vitamin B_1 injected (1 mg. per day for 3 days)	2888	2785	-103	-3.5

thyroid kidney tissue gives large catatorulin effects; but tissue from normal animals gives effects of the same order which are abolished by previous daily injection of vitamin B_1 . It is of interest to note that the O_2 uptakes of kidney slices in pyruvate were very large during the first half hour period. Values as high as $8000 \mu\text{l./g. fresh tissue/hr.}$, representing a Q_{O_2} of 32, were recorded.

Catatorulin effects were also observed in both normal and hyperthyroid tissues with glucose, but they were never as large as with pyruvate. As usual, liver slices showed no vitamin effect in the presence of glucose or of butyrate, hexanoate or octanoate.

(4) *Coccarboxylase contents of boiled extracts of heart, kidney, liver and brain*

Method. The coccarboxylase contents of the boiled extracts of the various organs were determined by the method of Ochoa & Peters [1938]. The CO_2 produced by the enzymic decarboxylation of pyruvic acid in the presence of

excess free vitamin B₁ and Mg⁺⁺, was measured manometrically. Alkaline washed baker's yeast was used as a source of apocarboxylase and the CO₂ produced by a measured volume of boiled extract was compared with that produced by known amounts of the standard cocarboxylase.

The animals were killed by decapitation, and after bleeding the tissue was weighed in a weighing bottle, minced finely with forceps and scissors and ground in a small porcelain mortar with 2 vol. distilled water. The suspension was then plunged into a boiling water bath for 3–5 min., and after cooling was centrifuged; 0.3 ml. was used for each determination.

Each manometric vessel contained the following:

In main chamber

- (1) 1 ml. yeast suspension (1 g. dry baker's yeast suspended in 10 ml. *M*/15 phosphate buffer pH 6.2. The yeast was washed 3 times with 45 ml. *M*/10 Na₂HPO₄ and once with 45 ml. distilled water.
- (2) 0.1 ml. MgCl₂ solution (containing 10 µg. Mg).
- (3) 0.1 ml. vitamin B₁ solution (containing 10 µg. vitamin B₁ hydrochloride).
- (4) 0.3 ml. boiled extract or standard cocarboxylase solution.

In side chamber

0.2 ml. sodium pyruvate solution (90 mg. crystalline sodium pyruvate dissolved in 3 ml. *M*/15 phosphate buffer pH 6.2).

Nitrogen was used in the gas space but in some of the later experiments air was used. The difference was found to be negligible. The substrate was tipped after a preliminary 15 min. equilibration period, and the CO₂ evolved in 30 min. measured. The temperature was 28°.

Results

Animals not treated with vitamin B₁

Table VI gives the average value of the cocarboxylase in the boiled extracts from:

- I. Animals fed on basal diet.
- II. Animals fed on basal diet plus 0.4 g. desiccated thyroid per day.
- III. Animals fed on basal diet but with the yeast supplement replaced by autoclaved marmite. This was a B₁-avitaminous diet and the animals were used when they showed typical symptoms of deficiency.

Table VI. *Average values for cocarboxylase contents of rat tissues (µg./g. fresh tissue)*

Group	Treatment	Tissue	No. of obser- vations	Mean	2 × S.E. of mean
I	Basal diet	Heart	8	1.95	±0.22
		Kidney	8	0.93	±0.14
		Liver	7	1.32	±0.28
		Brain	5	1.87	±0.44
II	Basal diet plus 0.4 g. desiccated thyroid per day	Heart	8	1.31	±0.24
		Kidney	8	0.63	±0.16
		Liver	8	0.65	±0.20
		Brain	5	1.39	±0.40
III	Avitaminous diet (symptoms)	Heart	6	0.41	±0.12
		Kidney	4	0.30	±0.10
		Liver	6	0.43	±0.08
		Brain	6	0.79	±0.14

The individual values are given in the appendix (Tables IX, X and XI) and are calculated on the assumption that the tissues contain 70% water. The

water content was found to vary from animal to animal, especially in the case of liver and kidney tissue. On the whole these tissues from hyperthyroid animals contained a slightly higher percentage of water than corresponding tissues from normal animals. But as the variation was never greater than 5%, and as dry weights were not determined in every case, the results were calculated on a wet weight basis on the above assumption.

Fig. 3. Cocarboxylase ($\mu\text{g./g. fresh tissue}$) of rat tissue. N, normal. H, hyperthyroid. A, avitaminous. Ordinate: mean $\pm 2 \times \text{S.E. of mean}$.

As with the pigeon, when the rat is showing avitaminous symptoms the cocarboxylase content of its tissues is much lower than normal. The values for the hyperthyroid animals (Fig. 3) are intermediate between those for the normal and the avitaminous ones.

Animals injected with vitamin B₁ daily

Table VII represents the values for animals which were given 250 $\mu\text{g.}$ vitamin B₁ daily by subcutaneous injection.

I. Animals fed on basal diet.

II. Animals fed on basal diet plus 0.4 g. desiccated thyroid per day.

Table VII. *Cocarboxylase ($\mu\text{g./g. fresh tissue}$) in tissues of rats injected daily with 250 $\mu\text{g. vitamin B}_1$.*

Exp. no.	Heart	Kidney	Liver	Brain
I. Normal animals				
79	7.51	3.82	6.38	2.53
80	9.01	4.24	9.81	2.80
87	7.10	3.91	7.24	2.21
II. Hyperthyroid animals				
41	7.82	—	6.71	—
75	8.01	3.02	4.41	—
76	8.63	3.10	3.70	—
82	7.76	3.25	6.21	1.89
83	9.04	3.88	4.01	2.51

The cocarboxylase values are much higher here than in those animals which were not injected with vitamin B₁. There was little difference between the values in normal and hyperthyroid animals except perhaps in the case of the liver. Thus it would seem that in the presence of excess vitamin B₁ thyroid feeding produces little diminution in the cocarboxylase content of the tissues.

Animals pretreated with vitamin B₁

Table VIII gives the average values for animals that had been pretreated with 250 μ g. vitamin B₁ per day (subcutaneous injections) for 7 days.

I. Pretreatment for 7 days (basal diet plus 250 μ g. vitamin B₁), then basal diet only.

II. Pretreatment for 7 days (basal diet plus 250 μ g. vitamin B₁), then basal diet plus 0.4 g. desiccated thyroid per day.

Table VIII. *Average values for cocarboxylase content of tissues from rats pretreated with 250 μ g. vitamin B₁ per day for 7 days (μ g./g. fresh tissue).*

Group	Treatment	Tissue	No. of observations	Mean	2 $\times$ S.E. of mean
I	Pretreatment 7 days. Basal diet only	Heart	6	1.85	± 0.18
		Kidney	6	0.90	± 0.10
		Liver	6	1.35	± 0.24
		Brain	6	1.92	± 0.08
II	Pretreatment 7 days. Basal diet plus 0.4 g. desiccated thyroid per day	Heart	6	1.61	± 0.20
		Kidney	6	0.71	± 0.16
		Liver	6	0.95	± 0.18
		Brain	6	1.47	± 0.16

The individual results are given in the appendix (Tables XII and XIII).

The idea of the pretreatment was to increase the cocarboxylase of the tissues and then to trace the relative rate at which this level fell. It is seen that, especially in the case of liver and brain, the cocarboxylase values fell more rapidly in the hyperthyroid than in the normal animals. This difference is not so significant in the case of heart and kidney. Comparison with the figures of Table VII shows that even in the case of untreated animals the cocarboxylase values fall on a diet adequate but low in vitamin B₁. It is also seen that the figures of Table VIII are not greatly different from those of Table VI. The animals whose values are represented in Table VIII, however, were under treatment for a longer time.

DISCUSSION

The high levels of cocarboxylase found in various rat tissues after excess of vitamin B₁ had been provided in the diet confirms the results of Ochoa & Peters [1938]. A smaller supply of vitamin reduces the amount of cocarboxylase found, but not to a degree incompatible with normal health; this would be consistent with the view that the extra amounts were merely stores. We hesitate to use the word "saturation" which has acquired a special meaning, in connexion for example with vitamin C, but the analogy is close. It would seem that maximum possible levels of cocarboxylase are not necessary for full health.

Hyperthyroidism caused a fall in tissue cocarboxylase which was proved not to be due to a failure in phosphorylating capacity. The finding of low levels of brain cocarboxylase associated with catatorulin effects is consistent with previous work. It would be interesting to speculate upon the reasons for the diminution in tissue vitamin (combined or free), but in the absence of information as to why vitamin B₁ disappears in the course of metabolism, such speculations seem to be unprofitable; that the disappearance is due to excess metabolism in a wide sense seems likely. Such an explanation would be consistent with the reported changes in the concentrations of other vitamins in the tissues. We may instance the changes in the vitamin A stores (liver) following thyroid administration described by Abelin [1933] and Mlinko [1938], and in the vitamin C

stores (liver, adrenal cortex etc.) by Svirbely [1935], Nespor [1936], Mosonyi [1936], Thaddea [1938] and Mlinko [1938] using guinea pigs, and by Sure & Theis [1938; 1939] using rats. Protection from loss of weight in experimental hyperthyroidism with vitamin A has been described by Euler & Klussman [1932] and Logaras & Drummond [1938], and with vitamin C by Svirbely [1935], Demole & Ippen [1935] and Scafer [1936]. Drill & Sherwood [1938] also claim that other substances in yeast extracts lessen the fall in weight produced after maximal vitamin B₁.

The important point to realize is that the giving of thyroid or thyroxine to rats changes their normal requirements for vitamin B₁ in the sense that they need larger amounts, in proportion to the food eaten, to maintain proper tissue levels of vitamin. In the past [Kinnersley *et al.* 1928; Peters, 1930]¹ experimental evidence has been discussed for the existence of idiosyncrasies in the needs of similar animals for vitamin B factors. The suggestion arising now from the work of others (as well as ourselves) upon vitamin B₁ that such variations have an endocrine basis rests upon secure experiment for the first time. Further exploration is wanted, because a recognition of such individual variation in human requirements for various food elements might prove very significant.

SUMMARY

1. In confirmation of the work of others subcutaneous injection of vitamin B₁ gives protection from the loss in weight produced in rats by thyroxine injections. A pretreatment with vitamin B₁ also lessens the rate at which thyroid fed rats fall in weight.

2. Catatorulin effects are obtained with brain tissue from hyperthyroid animals but not from normal animals on the same diet.

3. Catatorulin effects, abolished by previous injection of the animal with vitamin B₁, are obtained with kidney tissue from both hyperthyroid and normal animals fed on a basal diet containing small but adequate amounts of vitamin B₁.

4. The cocarboxylase content of boiled tissue extracts from hyperthyroid animals is intermediate in value between those from normal animals and those from animals showing symptoms of vitamin B₁ deficiency.

5. Injection of vitamin B₁ increases the cocarboxylase content of the tissues of both normal and hyperthyroid animals.

6. After 7 days of pretreatment with vitamin B₁ the cocarboxylase content of the tissues falls more rapidly in hyperthyroid than in normal animals.

We are indebted to the Rockefeller Foundation for grants in aid of this work. Our thanks are due to Mr R. W. Wakelin for valuable technical assistance and to Miss Kempson for help with the rats.

¹ *Inter alia* they found that individual pigeons placed upon polished rice diet tended to develop symptoms in a remarkably constant time; they called this the "day constant".

REFERENCES

- Abelin (1930). *Biochem. Z.* **228**, 165.
 — (1933). *Hoppe-Seyl. Z.* **217**, 109.
 Cohen & Gerard (1937). *J. cell. comp. Physiol.* **10**, 223.
 Cowgill & Palmieri (1933). *Amer. J. Physiol.* **105**, 146.
 Demole & Ippen (1935). *Hoppe-Seyl. Z.* **235**, 226.
 Drill (1938). *Amer. J. Physiol.* **486**, 122.
 — & Sherwood (1938). *Amer. J. Physiol.* **124**, 683.

- Euler & Klussman (1932). *Hoppe-Seyl. Z.* **213**, 21.
 Himwich, Goldfarb & Cowgill (1932). *Amer. J. Physiol.* **99**, 689.
 Kinnersley, Peters & Reader (1928). *Biochem. J.* **22**, 276.
 Logaras & Drummond (1938). *Biochem. J.* **32**, 964.
 Mlinko (1938). *Hoppe-Seyl. Z.* **256**, 42.
 Mosonyi (1936). *Klin. Wschr.* **15**, 24.
 Nespor (1936). *C.R. Soc. Biol., Paris*, **122**, 427.
 O'Brien & Peters (1935). *J. Physiol.* **85**, 454.
 Ochoa & Peters (1938). *Biochem. J.* **32**, 1501.
 Peters (1930). *J. State Med.* **38**.
 — (1936). *Biochem. J.* **30**, 2206.
 — (1938). *Biochem. J.* **32**, 2031.
 Quastel & Wheatley (1932). *Biochem. J.* **26**, 725.
 Scafer (1936). *Klin. Wschr.* **15**, 406.
 Sure & Buchanan (1937). *J. Nutrit.* **13**, 513.
 — & Theis (1938). *Proc. Soc. exp. Biol., N.Y.*, **37**, 646.
 — (1939). *Endocrinology*, **24**, 672.
 Svrbely (1935). *J. biol. Chem.* **111**, 147.
 Thaddea (1938). *Arch. exp. Path. Pharmacol.* **190**, 227.
 Thompson (1934). *Biochem. J.* **28**, 909.

APPENDIX

Table IX. *Coccarboxylase (μg./g. fresh tissue) in tissues of animals fed on basal diet*

Exp. no.	Heart	Kidney	Liver	Brain
68	1.80	0.81	0.90	—
69	2.16	1.17	1.26	—
72	2.16	0.95	1.08	—
73	2.06	0.99	1.12	1.85
85	1.49	0.63	—	1.35
86	1.49	0.68	1.22	1.53
92	2.03	1.08	2.07	2.21
95	2.39	1.12	1.62	2.43

Table X. *Coccarboxylase (μg./g. fresh tissue) in tissues of animals fed on basal diet plus 0.4 g. desiccated thyroid per day*

Exp. no.	Heart	Kidney	Liver	Brain
56	1.08	0.45	0.58	—
57	1.57	0.95	0.77	—
61	1.12	0.50	0.23	—
84	1.08	0.50	0.41	0.81
88	1.13	0.41	0.59	1.22
89	1.26	0.50	0.54	1.40
93	1.21	0.89	1.08	1.62
94	2.03	0.81	0.99	1.89

Table XI. *Coccarboxylase (μg./g. fresh tissue) in tissues of animals fed on avitaminous diet (animals showing symptoms)*

Exp. no.	Heart	Kidney	Liver	Brain
77	0.36	0.23	0.41	0.81
77a	0.52	—	0.53	0.68
77b	0.31	—	0.36	0.53
92	0.22	0.36	0.31	0.77
93	0.45	0.40	0.49	0.89
94	0.61	0.23	0.50	1.04

Table XII. *Coccarboxylase ($\mu\text{g./g. fresh tissue}$) in tissues of animals pretreated for 7 days (basal diet plus 250 $\mu\text{g. vitamin B}_1$) followed by basal diet only*

Exp. no.	Heart	Kidney	Liver	Brain
90	2.12	1.04	1.66	1.84
91	1.80	1.04	1.40	2.11
91 α	1.71	0.95	1.49	1.98
100	2.07	0.86	1.40	1.84
101	1.57	0.81	1.37	1.84
102	1.85	0.70	0.80	1.90

Table XIII. *Coccarboxylase ($\mu\text{g./g. fresh tissue}$) in tissues of animals pretreated for 7 days (basal diet plus 250 $\mu\text{g. vitamin B}_1$) followed by basal diet plus 0.4 g. desiccated thyroid per day*

Exp. no.	Heart	Kidney	Liver	Brain
90	1.30	0.54	0.88	1.57
91	1.53	0.53	1.04	1.56
91 α	1.93	1.04	1.22	1.71
100	1.57	0.76	0.67	1.44
101	1.49	0.63	0.76	1.21
102	1.85	0.75	1.10	1.30