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ON THE NATURE OF THE IODINE-CONTAINING COMPLEX IN THYREOGLOBULIN

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ON THE NATURE OF THE IODINE-CONTAINING COMPLEX IN THYREOGLOBULIN.

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In this paper are given the results of an attempt to determine the nature of the active complex in the iodine-containing active principle of the thyroid gland. Although the nature of this group was not determined, the quantitative physiological results here reported serve to establish certain predicted and other unexpected facts and to eliminate certain hitherto considered probabilities.

The problem was taken up both by analytical and by synthetic methods. In the former method the physiological activity and iodine content of the dried thyroid tissue, the globulin therefrom and various products of hydrolysis from this globulin were determined quantitatively. In the second method two iodized amino-acid derivatives, not previously tested by quantitative methods, were prepared synthetically and their physiological activity studied quantitatively.

In thus tracing the active complex a number of important assumptions were made. First, that the activity of unaltered thyroid tissue depends quantitatively on its iodine content. Second, that the best method known for measuring this activity directly and quantitatively is the Reid Hunt acetonitrile test.[1] Third, that in case the iodine is present in the products of hydrolysis in the same combination as in the globulin then, per unit of iodine, these will still possess an activity comparable with the original globulin. Fourth, that in case the iodine complex is an iodized amino-acid and that in case this is decomposed in the process of hydrolysis then the synthetic preparation of various iodized amino-acids or derivatives thereof and the quantitative testing of

[1] This *Journal*, i, p. 33, 1905.

these per unit of iodine may determine the probable nature of the iodine complex. In other words, the actual quantitative physiological activity per unit of iodine as measured by the Reid Hunt method was taken as the crucial test for the presence or absence of the unaltered iodine complex.

The historical development of the relation of thyroid activity to iodine content need not be considered at this time, especially in view of the thorough reviews and extensive confirmatory experiments made by Reid Hunt and A. Seidell,[2] as well as the comparative histological and chemical studies by Marine in coöperation with Lenhardt and Williams.[3] A careful study of these papers justifies the first assumption. The second assumption is also well taken provided the proper precautions are observed as shown by Reid Hunt and A. Seidell.[4] Other methods for testing the physiological activity of thyroid substance, based on changes in blood pressure,[5] on increasing the irritability of the depressor nerve,[6] on changes in nitrogen metabolism[7] and on curative effects in cretinism[8] have been employed, but are not applicable in a quantitative study, nor are they as specific reactions.

Of the third and fourth assumptions we had no definite proof. The studies of Oswald[9] and others show that during hydrolysis of thyreoglobulin only 30 per cent or less of the iodine remains in organic combination. The iodine thus combined is in the various fractions and qualitatively it has been determined[10] that probably the greater activity remains in the more complex products

[2] Bulletins 47 (1908) and 69 (1910) of the Hygienic Laboratory, U. S. Public Health and Marine Hospital Service.

[3] *Johns Hopkins Hospital Bull.*, xviii, p. 359, 1907; *Journ. Inf. Dis.*, iv, p. 417, 1907; *Archives of Internal Med.*, i, p. 349, 1908; *Ibid.*, iii, p. 66, 1909; *ibid.*, iv. p. 440, 1909; *ibid.*, vii, p. 506, 1911; *ibid.*, viii, p. 265, 1911; *Journ. of Exp. Med.*, xiii, p. 455, 1911.

[4] *Loc. cit.; Journ. of Pharmacol. and Exp. Ther.*, ii, p. 15, 1910.

[5] von Fürth and Schwarz: *Pflüger's Archiv.* cxxiv, p. 113, 1908.

[6] von Cyon and Oswald: *Pflüger's Archiv*, lxxxiii, p. 199, 1901; Asher and Flack: *Zeitschr. f. Biol.*, lv, p. 83, 1910.

[7] Baumann: *Zeitschr. f. physiol. Chem.*, xxi, p. 487, 1896; *ibid.*, xxii, p. 1, 1896; *Münch. med. Wochenschr.*, xl, 1896.

[8] E. Pick and F. Pineles: *Zeitschr. f. exp. Path. u. Ther.*, vii, p. 518, 1909–10.

[9] *Arch. f. exp. Path. u. Pharm.*, lx, p. 115, 1908.

[10] Pick and Pineles: *loc. cit.*

of hydrolysis where also the greater part of the organically combined iodine is found. What relation the activity bears to the iodine content therein has however not been determined. As stated above we have evidence that some of the iodine is split off as iodide, but we have no direct evidence that all the organically combined iodine found in the products of hydrolysis is still in the same complex or in the same structural relationship as in the original thyreoglobulin. A number of iodized amino-acids have been studied qualitatively as to physiological activity. In no case has thyroid activity been detected. The most conclusive results as to the inactivity of 3,5-iodo-laevo-tyrosine are those reported by Strouse and Voegtlin.[11] Other observations on the inactivity of various iodized proteins, which on hydrolysis yield 3,5-iodo-tyrosine, also bear out these conclusions. The studies on other iodized amino-acids do not lead to definite conclusions. Thus von Fürth and Schwarz[12] prepared and studied what they considered iodized phenylalanine, histidine and tryptophane. They reported all these substances as physiologically inactive, but gave no data indicating that they had really separated iodo-derivatives of these substances. Pauly[13] however actually separated pure tetra-iodohistidine anhydride and tri-iodo-imidazol and reported that these substances increased the respiratory and pulse frequencies, although uniodized imidazol had no such action. These considerations lead us to conclude that for the present the validity of the third and fourth assumptions is unknown to us and that the true answers thereto are part of the problem in hand.

EXPERIMENTAL PART.

The mode of attack has already been outlined above. The details as to the methods employed and the preparation of the substances studied are given below.

A. Preparations.

Dried hog thyroids. Hog thyroids[14] were freed mechanically from fat as much as possible and dried on glass plates in a current of air at 30–35°C.

[11] *Journ. of Pharm. and Exp. Ther.*, i, p. 123, 1909.

[12] *Pflüger's Archiv*, cxxxiv, p. 113, 1908.

[13] *Ber. d. deutsch. chem. Gesellsch.*, xliii, p. 2243, 1910.

[14] The raw material for this research was supplied by the Armour Laboratory Department.

The mass was then ground to a coarse powder and fat removed by ether in the cold. The remaining dry mass was then finely powdered. Duplicate determinations on this gave 0.243 and 0.250 per cent iodine.

Thyreoglobulin. This was prepared as previously described.[15] Duplicate determinations on this gave 0.462 and 0.468 per cent iodine.

Iodothyrin (*a*) was prepared by the usual Baumann process from the above thyreoglobulin. The extraction with 95 per cent alcohol of the melanoidin precipitate was made in a continuous hot extractor. Duplicate determinations gave 5.81 and 5.85 per cent iodine.

Iodothyrin (*b*) was obtained in the same way from the melanoidin precipitate which separated in the complete hydrolysis of some of the same thyreoglobulin by 30–35 per cent sulphuric acid. This on analysis gave 7.51 per cent iodine.

Iodothyrin (*c*) was obtained from 40 grams of the same globulin by hydrolysis for three days at room temperature and for twenty-four hours at boiling temperature with 20–25 per cent phosphoric acid. Phosphoric acid was used as it was thought that possibly the oxidative action of sulphuric acid might have an injurious effect. This amount of globulin yielded 3.30 grams of melanoidin, containing 1.75 per cent iodine. The iodothyrin extracted from this represented 24 per cent of the weight and contained 4.44–4.46 per cent iodine. Thus only 61 per cent of the iodine in the melanoidin fraction was recovered in the alcohol extract.

Metaprotein (A_4). The filtrate from the melanoidin fraction above was neutralized with NaOH and the metaprotein separated and dried over sulphuric acid in a vacuum desiccator. This weighed 1.62 grams and contained 1.51–1.53 per cent iodine.

Primary albumose (A_5). The filtrate from above was half saturated with zinc sulphate after slightly acidifying with sulphuric acid. The precipitate obtained was dialyzed until free from sulphate. In this fraction there were recovered 3.1 grams containing 0.22–0.225 per cent iodine.

Secondary albumose (A_6). Obtained from the filtrate from above by complete saturation with zinc sulphate. The precipitate after dialyzing as above yielded 4 grams dry substance containing 0.069 per cent iodine.

The table (I) below gives a summary of the distribution of iodine in the different fractions above.

TABLE I.

	WEIGHT RECOVERED	PER CENT OF IODINE THEREIN	WEIGHT OF IODINE	PER CENT OF TOTAL IODINE IN THE GLOBULIN
Melanoidin precipitate	3.30	1.74	0.0575	30.9
Metaprotein.....	1.62	1.52	0.0246	13.2
Primary albumose.....	3.01	0.22	0.0066	3.5
Secondary albumose...	4.0	0.0695	0.0027	1.5
Undetermined iodine..				50.9

[15] This *Journal*, ix, p. 121, 1911.

Phosphotungstic acid precipitate. Another 40 grams of thyreoglobulin were boiled with 25 per cent phosphoric acid for ninety-three hours. The filtrate from the melanoidin precipitate and metaprotein, after removal of the phosphoric acid by $Ba(OH)_2$ and the excess of barium by sulphuric acid, was concentrated under diminished pressure to about 250 cc. This was then freed from proteose and peptone by the Kutscher tannin method.[16] The filtrate finally obtained here after removal of the excess of lead was boiled with $BaCO_3$ to remove the ammonia. The dissolved barium was again removed by sulphuric acid. The filtrate after acidifying with H_2SO_4 to 5 per cent strength was precipitated with phosphotungstic acid in the usual way. The precipitate after thorough washing with 2.5 per cent phosphotungstic acid solution was freed from phosphotungstic acid, barium and sulphate in the usual way. Duplicate determinations on the dry amino-acid mixture gave 0.0107 per cent and 0.0093 per cent iodine.

Another phosphotungstic acid precipitate from a hydrolysis by H_2SO_4 was worked up in the same way. This dry residue contained 0.0068 per cent iodine. The two samples were mixed and designated as P.T.A. Ppt. 1. This mixture contained 0.0073 per cent iodine.

Phosphotungstic acid filtrate (1). This was freed from phosphotungstic acid in the usual way. The amino-acid solution was evaporated to dryness. Duplicate determinations on the dry amino-acid mixture gave 0.0024 per cent iodine.

Phosphotungstic acid precipitate (2). This was obtained in the same way as the above from the partial hydrolysis by 10 per cent sulphuric acid of 141.6 grams of thyreoglobulin containing 0.511 per cent iodine. The purified dry residue by analysis contained 0.0043 per cent iodine.

Phosphotungstic acid filtrate (2). The filtrate from the above was treated in the usual way. The dry purified amino-acid mixture left gave in duplicate determinations 0.0045 and 0.0043 per cent iodine.

Tetra-iodohistidine anhydride. Histidine was prepared from ox erythrocytes by the method of Frankel.[17] Various methods were employed in trying to iodize the dichloride or the base itself but in no case were there indications of true absorption of iodine, but rather decomposition of the histidine. While this work was under way Pauly[18] published his observations with the same conclusions as to the difficulty or inability to iodize histidine directly. At the same time, as stated above, he published his observations on tetra-iodohistidine anhydride. Following the methods given by Pauly[19] the preparation of the methyl ester of histidine dichloride was carried out and from this the histidine anhydride by the Pauly modification[20] of the Fischer and Zuzuki method. The histidine anhydride was recrystallized from hot water a number of times to obtain the more

[16] *Zentralbl. f. Physiol.*, xix, p. 504, 1905.
[17] *Monatsh. f. Chem.*, xxiv, p. 230, 1903.
[18] *Ber. d. deutsch. chem. Gesellsch.*, xliii, p. 2243, 1910.
[19] *Zeitschr. f. physiol. Chem.*, lxiv, p. 75, 1910.
[20] *Loc. cit.*

readily soluble laevorotatory form. This was then iodized according to the Pauly method. One determination on the snow-white product gave 63 per cent iodine (theoretical 65 per cent). The slightly lower value may be due to an admixture of a small amount of di-iodohistidine anhydride.

Iodized tryptophane. Tryptophane was prepared from commercial casein by the Hopkins-Cole method.[21] Several attempts were made to iodize the pure crystals by the method of Neuberg,[22] but in no case was a substance obtained containing more than 6.3 per cent iodine. The preparation finally made for physiological testing was obtained by dissolving one milligram molecule of tryptophane in 4 cc. of $\frac{N}{2}$ NaOH, cooling by immersing in ice water and, while keeping cool and stirring well, adding drop by drop 6 cc. of aqueous N iodine solution. The mixture was allowed to stand at ice box temperature for twenty-four hours, then filtered off. The precipitate was well washed with cold water and dried over sulphuric acid in a vacuum desiccator. The product obtained is light brown in color, readily soluble in alkalies, reprecipitated on acidifying and liberates a very small amount of iodine to chloroform on shaking therewith. Duplicate determinations on this gave 41.5 and 41.9 per cent iodine (the theoretical for mono- and di-iodotryptophane are 38.4 per cent and 55.7 per cent respectively).

B. Methods.

Determination of iodine. The Hunter[23] method with slight modifications was employed. The material to be analyzed, taken in quantities of 0.05–2 grams was mixed with 15 grams of fusion mixture and covered with 10 grams of fusion mixture as suggested by Hunter. To conduct the fusion the Roger's ring burner was found to be much more satisfactory in ensuring a uniform rapid heating without overheating. With the size of the flame once determined one finds ten minutes to be ample time to give a satisfactory, easily removable fusion. In the treatment with alkaline hypochlorite it was considered best to warm to 40°C. for ten minutes. In acidifying it is very important to make sufficiently acid and then always to the same degree. Sulphuric acid of 25 per cent strength was used here and since the same amounts of fusion mixtures and hypochlorite were used in each case the acidity was well controlled by always adding the same amount of acid. In removing the excess of chlorine gentle boiling was continued for forty minutes after the negative test of the vapors by starch iodine paper. In this way the blank test on the reagents never was more than 0.1 cc. of a $\frac{N}{200}$ $Na_2S_2O_3.5H_2O$ solution.

Physiological testing by the Hunt method. The method employed was that of feeding the same quantity of iodine, in the different combinations, to white mice in such a manner as to make as certain as possible the entire consumption of the material fed. In order to do this each mouse was first

[21] *Journ. of Physiol.*, xxvii, p. 418, 1901.

[22] *Biochem. Zeitschr.*, vi, p. 276, 1907.

[23] This *Journal*, vii, p. 321, 1910.

fed for three or four days with cracker dust made into pellets of known weight. At the close of this preliminary feeding the unconsumed material was weighed and from this the average amount eaten per day determined. For ten days following this period each mouse then received this weight of cracker dust, with the incorporated iodine-containing substance, in the form of pellets. The control mice were fed in the same way with plain cracker dust pellets. At the end of the 10-day feeding period the acetonitrile was injected subcutaneously. Each dose administered in series I, II and III was contained in 1 cc. of fluid; in series IV–IX, in 0.5 cc.; in series X, in 0.66 cc. In most cases the animals consumed the food very well. All the mice used were raised in the laboratory building on a diet of milk and crackers with occasional bits of lettuce until used for the experiment. Care was taken to compare mice of as nearly the same age as possible. In the tables below the litter number of each mouse is given. The ages of the mice of the various litters were as follows: Litter 2, 119 days; litter 3, 102 days; litter 4, 100 days; litter 5, 80 days; litters 6 and 10, 99 and 113 days respectively; litter 9, 115 days; litters 11–12, 125 and 135 days respectively; litters 13–14, 144 and 151 days respectively; litter 28, 85 days; litters 29–30, 59 and 66 days respectively; litters 31, 32 and 34, 95, 85 and 97 days respectively; litters 33–35, 101 and 89 days respectively; litters 36–37, 89 days; litter 38, 79 days; litters 56–60, 91–103 days.

C. Discussion of the physiological tests.

Thyreoglobulin. Series I shows that thyreoglobulin possesses the full activity per unit of iodine when compared with the dried thyroid from which it was prepared. This is also confirmed by series IV where a decomposition product obtained from the globulin still shows the complete activity per unit of iodine. The whole of the physiological activity of the gland is therefore quantitatively in the thyreoglobulin.

Metaprotein. As stated above, this still shows the full activity per unit of iodine although the percentage concentration of iodine has increased from 0.465 per cent in the thyreoglobulin to 1.52 per cent in the metaprotein.

Iodothyrin. None of the iodothyrin preparations tested was found to bring about a resistance to acetonitrile more than three-fourths of that produced by the thyroid-tissue fed mice. The indications are that these preparations are all about equally inactive. Iodothyrin is therefore less active per unit of iodine than the thyreoglobulin. See series III and V.

Primary albumose. This is still very active, as shown by series IV and VII; although the full activity per unit of iodine is not

shown to be present in every case tested. In this connection it may be mentioned that the results in series VI are of no value. This series VI, however is an illustration of irregular results, due in all probability to impure acetonitrile. The acetonitrile was taken from a freshly opened bottle and found to smell decidedly of hydrocyanic acid. Before using, it was shaken twice with saturated potassium carbonate solution, dehydrated with P_2O_5 and twice distilled from fresh P_2O_5. Finally it was redistilled and the fraction collected between 79 and 83°C. This distillate was used in series VI. For the later series this distillate was again purified in the same way three times and finally redistilled twice without the addition of P_2O_5. Here the distillate was collected between 80.5 and 81.5°C.

Secondary albumose. This is much less active per unit of iodine than either the iodothyrin preparations or the primary proteoses. Series VII shows this, where the maximum dose resisted is only 40 per cent of the maximum dose resisted by the thyroid-tissue fed mice.

Amino-acids from the phosphotungstic acid precipitate and the phosphotungstic acid filtrate respectively. The results in series IX indicate that the former possess very little physiological activity as measured by the Hunt method. On the whole, however, the results here are very unsatisfactory as the mice did not eat the amino-acid mixtures well, there being two or more days' feeding left. The results indicate that these amino-acid fractions contain very little thyroid activity. This is better shown in series X where only one-tenth the quantity of iodine-containing substances was fed. Although the mice fed with dried thyroid tissue resisted an amount over two and a half times that of the control mice, still the mice fed with the same amount of iodine, but in the form of amino-acids, resisted very little, if any, more of the acetonitrile than the control mice. In other words, these amino-acid fractions show a very slight physiological activity, if indeed they possess any activity whatever.

Tetra-iodohistidine anhydride and iodotryptophane. These substances when fed in amounts representing ten times the amount of iodine fed as thyroid tissue do not appreciably increase the resistance to acetonitrile. See series II and VIII.

Table II gives a summary of the results above. The relative physiological activity is expressed (on the basis of feeding the same amount of iodine in each case) as follows: representing in each case, by 100, the largest dose of acetonitrile from which the thyroid-tissue fed mice recovered, then the other figures represent the proportions the limiting doses of the otherwise fed mice bear thereto

TABLE II.

	RELATIVE ACTIVITY	IODINE IN THE SUBSTANCE	TOTAL IODINE IN THE TISSUE
		per cent	*per cent*
Thyroid tissue	100	0.247	100.0
Thyreoglobulin	100	0.465	100.0
Metaprotein	100	1.520	13.2
Iodothyrin	50–75	4.46–7.51	18.3
Primary albumose	80–100	0.220	3.5
Secondary albumose	40	0.0695	1.5
Amino-acids precipitated by phosphotungstic acid	0(+?)	0.0043	
Amino-acids not precipitated by phosphotungstic acid	0(+?)	0.0044	
Tetra-iodohistidine anhydride	0	65.00	
Iodotryptophane	0	41.70	

These results show that both the thyroid activity and iodine may be concentrated from thyroid tissue in the thyreoglobulin as well as in the metaprotein and iodothyrin from the latter. Per unit of iodine, however, we have full activity retained in the thyreoglobulin and metaprotein only. In the primary albumose fraction we have a lowering in the percentage concentration of iodine and also a slight lowering in the physiological activity per unit of iodine. In the secondary albumose this is still more marked. In the amino-acid fractions the activity is extremely low if present. In view of the researches of Hunt and Seidell with various iodine compounds and in view of the results obtained here, we cannot attribute the protective action in any of these cases to iodine itself, but to a specific iodine-containing complex in the thyreoglobulin. It is significant to note that the highest physiological activity per unit of iodine is found in the original protein and in the more complex products of hydrolysis. Since the lowest products of hydrolysis are still less active per unit of iodine than the secondary albu-

mose it indicates either that the iodine group is altered in the hydrolysis, or that the iodine-containing group when in simpler combination or when separated, does not possess the full specific thyroid activity. That the iodine-containing group when once separated would not possess the full activity is not at all unlikely, but we would be inclined to expect it to show some activity; at least when given in amounts such as were employed by Strouse and Voegtlin with iodotyrosine and by the author in the experiments with tetra-iodohistidine anhydride and iodotryptophane. The indications as to the presence of tyrosine and tryptophane in iodothyrin are very favorable, both from the chemical studies on iodothyrin and also from similar studies on iodine-free melanoidins.[24] It is not likely that the iodine is split off and then later added to the melanoidin fraction; it is more likely that it is already present in the globulin in the melanoidin-forming groups and remains in the original position in these groups, but that the groups themselves are changed in regard to each other and thus the activity affected to some extent; a poly-iodo derivative may be changed to a mono-iodo derivative and then may show decided differences in physiological activities. If this were not the case we would expect artificially iodized melanoidins to show a decided thyroid activity. Furthermore, it is not likely that sufficient hydriodic acid is split off in the early stages of the hydrolysis to yield as much iodine as is contained in the melanoidin fraction. Finally, it is not at all improbable that we here have to do with a specific iodophore group just as in hemoglobin we have the chromophore group containing the iron. The negative results with artificially iodized proteins speak strongly in favor of this view.

CONCLUSIONS.

1. The full activity of thyroid tissue is contained in the thyreoglobulin fraction when this activity is measured by the Hunt method.

2. The full activity per iodine unit is still present in the metaprotein fraction from this globulin, although the iodine content in the metaprotein fraction has been increased over threefold that of the globulin itself.

[24] Samuely: *Hofmeister's Beiträge*, ii, p. 355, 1902.

3. The other products of the hydrolysis studied, primary albumose, iodothyrin and secondary albumose, show a gradual decrease in activity per unit of iodine in the order given.

4. The amino-acid fractions precipitated and not precipitated by phosphotungstic acid from the partially hydrolyzed thyreoglobulin still contain very small amounts of iodine and per unit of iodine are either extremely low in activity or entirely inactive.

5. Tetra-iodohistidine anhydride and iodotryptophane do not possess thyroid activity as determined by the Hunt method.

I wish to express my thanks to Prof. A. P. Mathews for suggestions made in the course of the work.

SERIES I. *February 25–March 8.*

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETONITRILE	DEATH OCCURRED AFTER	DOSE OF ACETONITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	*hrs.*	*mg. per gm.*
(*a*) ♂	4		0.4	1¼	
(*b*) ♀	4				0.35
(*c*) ♀	4		Gravid,	not injected	
(*d*) ♀	4		Escaped	from cage	
(*e*) ♀	4	1 mg. dried hog thyroid (=0.00247 mg. I)	4.49	3½	
(*f*) ♂	3		died while	feeding	
(*g*) ♂	3		4.0	30	
(*h*) ♀	3	0.531 mg. thyreoglobulin (=0.00247 mg. I)	4.0	1½	
(*i*) ♀	3				3.79
(*j*) ♀	3				3.50

SERIES II. *April 21–May 1.*

(*a*) ♂	3		0.55	4¼	
(*b*) ♂	5				0.30
(*c*) *j* (Ser. I)	3				0.40
(*d*) *i* (Ser. I)	3				0.45
(*e*) ♂	2	1 mg. dried hog thyroid (=0.00247 mg. I)	5.0	3½	
(*f*) ♂	2		4.0	4	
(*g*) ♂	2		4.5	23	
(*h*) ♀	4	0.00392 mg. tetraiodohistidine anhydride (=0.00247 mg. I)			0.55*
(*i*) ♀	4		0.60	<1¼	
(*j*) ♀	4		0.55	8½	
(*k*) ♂	3	0.0392 mg. tetraiodohistidine anhydride (=0.0247 mg. I)	0.55	5	
(*l*) ♀	4		3.55	48	
(*m*) *b* (Ser. I)	4				0.60

SERIES III. *May 19–29.*

(*a*) ♂	6–10		0.30	5†	
(*b*) ♀	6–10				0.45
(*c*) ♂	9		died while	feeding	
(*d*) ♂	9		0.50	8	

* Slight loss in injection.
† Not well when injected.

SERIES III—Continued.

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETONITRILE	DEATH OCCURRED AFTER	DOSE OF ACETONITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	*hrs.*	*mg. per gm.*
(*e*) ♂	9	1 mg. dried hog thyroid (=0.00247 mg. I)	3.5	7	
(*f*) ♀	6–10				2.9
(*g*) ♂	6–10		3.2	30	
(*h*) ♂	9	0.0424 mg. iodothyrin (*a*) (=0.00247 mg. I)	2.0	8	
(*i*) ♀	6–10		3.0	2	
(*j*) ♂	6–10				1.5
(*k*) ♂	9	0.0329 mg. iodothyrin (*b*) (=0.00247 mg. I)	<2.5	2	
(*l*) ♀	6–10		died while feeding		
(*m*) ♂	6–10		died while feeding		
(*n*) ♀	6–10	0.0554 mg. iodothyrin (*c*) (=0.00247 mg. I)	died while feeding		
(*o*) ♀	6–10		2.5	8	
(*p*) ♀	6–10				1.5
		SERIES IV. *June 19–29.*			
(*a*) ♂	11–12	1 mg. dried hog thyroids (=0.00247 mg. I)	3.0	3	
(*b*) ♀	11–12				2.5
(*c*) ♂	11–12		died while feeding		
(*d*) ♀	11–12				2.0
(*e*) ♂	11–12	0.163 mg. metaprotein (A_4) (=0.00247 mg. I)			2.0
(*f*) ♀	11–12				2.5
(*g*) ♀	11–12		3.0	3	
(*h*) ♀	11–12	1.123 mg. primary albumose (A_5) (=0.00247 mg. I)			2.5
(*i*) ♀	11–12		died while feeding		
		SERIES V. *August 2–12.*			
(*a*) ♂	13–14	1 mg. dried hog thyroid (=0.00247 mg. I)			2.5
(*b*) ♂	13–14		died while feeding		
(*c*) ♀	13–14				<3.0*
(*d*) ♀	13–14	0.0424 mg. iodothyrin (*a*) (=0.00247 mg. I)	2.8	3	
(*e*) ♂	13–14		died while feeding		
(*f*) ♀	13–14				2.0

* Slight loss in injection.

SERIES V—Continued.

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETO-NITRILE	DEATH OCCURRED AFTER	DOSE OF ACETO-NITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	*hrs.*	*mg. per gm.*
(*g*) ♀	13–14	0.0556 mg. iodothyrin (*c*) (=0.00247 mg. I)	died while feeding		
(*h*) ♀	13–14		2.5	3½	

SERIES VI. *November 24–December 4.*

(*a*) ♀	29–30	1 mg. dried hog thyroids (=0.00247 mg. I)	2.5	<24	
(*b*) ♂	29–30		3.0	1½	
(*c*) ♂	29–30		2.0	<18	
(*d*) ♂	29–30	1.123 mg. primary albumose (A_5) (=0.00247 mg. I)	died while feeding		
(*e*) ♀	28		2.0	36–40	
(*f*) ♂	29–30				2.5
(*g*) ♂	28	3.55 mg. secondary albumose (A_6) (=0.00247 mg. I)	1.5	< 4	
(*h*) ♀	28		2.0	24–36	
(*i*) ♂	28		1.25	20–36	

SERIES VII. *January 13–23.*

(*a*) ♀	31–34	1 mg. dried hog thyroid (=0.00247 mg. I)	died while feeding		
(*b*) ♂	31–34				2.0
(*c*) ♀	31–34				2.5
(*d*) ♂	31–34				3.0
(*e*) ♂	31–34	1.123 mg. primary albumose (A_5) (=0.00247 mg. I)			2.5
(*f*) ♀	31–34				2.0
(*g*) ♀	31–34		2.8	> 6	
(*h*) ♀	31–34		3.0	> 8	
(*i*) ♂	31–34	3.55 mg. secondary albumose (A_6) (=0.00247 mg. I)	2.0	2½	
(*j*) ♂	31–34		1.2	> 4	
(*k*) ♀	31–34		1.0	24	
(*l*) ♀	31–34				1.2

SERIES VIII. *February 17–27.*

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETONITRILE	DEATH OCCURRED AFTER	DOSE OF ACETONITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	*hrs.*	*mg. per gm.*
(*a*) ♀	33–35				0.45
(*b*) ♀	33–35				0.40
(*c*) ♀	33–35				0.35
(*d*) ♀	33–35		0.55	<18	
(*e*) ♂	33–35	1 mg. dried hog thyroids (=0.00247 mg. I)	4.0	2	
(*f*) ♂	36–37		died while feeding		
(*g*) ♀	36–37		3.0	6	
(*h*) ♀	36–37				2.0
(*i*) ♂	33–35	0.0059 mg. iodotryptophane (=0.00247 mg. I)	0.55	>36	
(*j*) ♂	33–35		1.6	2½	
(*k*) ♀	36–37		1.0	18	
(*l*) ♀	36–37		0.45	>24	
(*m*) ♂	33–35	0.059 mg. iodotryptophane (=0.0247 mg. I)	1.0	< 3	
(*n*) ♂	33–35				0.5
(*o*) ♀	33–35		0.70	<18	

SERIES IX. *March 12–22.*

(*a*) *a* Ser. VIII	33–35	1 mg. dried hog thyroid (=0.00247 mg. I)	4.0	< 6	
(*b*) *b* Ser. VIII	33–35				3.5
(*c*) *c* Ser. VIII	33–35		did not eat; not injected		
(*d*) *n* Ser. VIII	33–35				3.0
(*e*) ♀	38	33.6 mg. P. T. A. Ppt. 1 (=0.00247 mg. I)	died while feeding		
(*f*) ♀	38		died while feeding		
(*g*) ♂	38				0.8*
(*h*) ♂	38	100 mg. P.T.A. Filt. 1 (=0.0024 mg. I)	1.0	< 3*	
(*i*) ♂	38		0.8	<18*	
(*j*) ♂	38		0.6	<18*	

* Two or more days feeding left. This experiment is not reliable as animals were used which had recovered in previous experiments and the differences in age were too great for such young animals.

SERIES X.

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETO-NITRILE	DEATH OCCURRED AFTER	DOSE OF ACETO-NITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	*hrs.*	*mg. per gm.*
(*a*) ♂.....	56-60		0.5	<10	
(*b*) ♂.....	56-60		died while feeding		
(*c*) ♀.....	56-60				0.4
(*d*) ♂.....	56-60				0.35
(*e*) ♂.....	56-60	0.1 mg. dried hog thyroid (=0.000247 mg. I)	1.2	3½	
(*f*) ♂.....	56-60				1.1
(*g*) ♀.....	56-60				1.0
(*h*) ♀.....	56-60		not injected, gravid		
(*i*) ♂.....	56-60	5.74 mg. P. T. A. Ppt. 2 (=0.000247 mg. I)	0.5	48*	
(*j*) ♂.....	56-60		1.0	< 6†	
(*k*) ♂.....	56-60		0.8	<16	
(*l*) ♀.....	56-60				0.4
(*m*) ♂.....	56-60	5.61 mg. P. T. A. Filt. 2 (=0.000247 mg. I)	1.0	3½	
(*n*) ♂.....	56-60		0.8	<12	
(*o*) ♂.....	56-60		0.7	24	
(*p*) ♂.....	56-60				0.5

* Two days' feeding left.
† About one day's feeding left.