

## IODINE DEFICIENCY IN A COMMONLY USED STOCK DIET

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It was shown in previous work by the authors ('35) that the addition of 1% cod liver oil to a supposedly complete diet produced thyroid glands in female rats which were significantly smaller than those of their litter-mate controls. Since it is known that the thyroid gland is sensitive to iodine, the possibility that the iodine present in cod liver oil was responsible for the changes suggested itself. Dr. Roe E. Remington, in a personal communication, suggested, as a result of his observations, that the changes might possibly be due to the addition of iodine (in cod liver oil) to an iodine deficient diet.

The present work was carried on to find out whether the change could be found in males; whether time had any influence upon the change; and whether the change was due to the iodine in the administered cod liver oil.

### MATERIALS AND METHODS

Male Wistar albino rats were used. The animals were weaned at 3 weeks of age and immediately placed on the diets. The basal diet consisted of: casein, 15%; whole milk powder, 10%; sodium chloride, 0.8%; calcium carbonate, 1.5%; butter (unsalted), 5.2%; and whole ground wheat, 67.5%. The mixture was prepared fresh at least every other day.

The animals were divided into three series. Series A consisted of twenty-five males which received the basal diet plus

2% pure cod liver oil (U.S.P.). Twenty-five litter mates received only the basal diet. The animals of this group remained on the diet until 4 months of age and were then autopsied.

In series B twenty-six males received the basal diet plus 2% cod liver oil. Twenty-six litter mates were sustained on the basal diet. The animals of this group were kept on the diet until the age of 3 months and then killed.

Series C consisted of five groups of male rats. Twenty-five were sustained on the basal diet; twenty-six had 1% oil added to their diet; twenty-six received 2% oil; twenty-six received 0.0184% potassium iodide, and twenty-six animals received 0.0184% potassium iodide plus 2% cod liver oil in addition to the basal diet described above. The rats of this series were autopsied at 2 months of age. The source of the iodine was Morton's iodized salt which the manufacturers state contains 0.023% of potassium iodide.

The progress of each animal was carefully watched, and each was weighed weekly. At the time of autopsy in the 4-month and 3-month series, the body and tail lengths were measured in addition to the weights of the head, suprarenal glands, hypophysis, thyroid, thymus and testes. In the 2-month series the lengths of the body and tail were measured as well as the weights of the head and the thyroid gland. The organs removed were fixed in Bouin's fluid. Sections of the thyroid glands of the 2-month series were stained with hematoxylin and eosin.

A comparison of the results from the various groups of animals was made, using modern biometrical methods. As is customary the difference between the two means divided by the probable error of the difference is spoken of as the significance ratio in this paper.

#### OBSERVATIONS

##### *Thyroid gland*

*Series A.* The mean thyroid weight (table 1) of the rats without oil in their diet was 0.02620 gm. The average weight

of the thyroid of those animals which received oil was 0.02025 gm. The 22.71% decrease in the oil group was definitely significant. The significance ratio for the difference was 7.88.

*Series B.* The thyroid gland of the animals in this group (table 2) showed a definite weight change. The mean value

TABLE 1  
Statistical summary of results of the 4-month group.  
(Measurements in grams and centimeters)

	MEAN		DIFFERENCE BETWEEN MEANS	SIGNIFI- CANCE RATIO
	Oil	No oil		
Hypophysis	0.00868±0.00015	0.00838±0.00012	0.00030±0.00019	1.59
Thyroid	0.02025±0.00044	0.02620±0.00061	0.00595±0.00075	7.88
Thymus	0.53233±0.01790	0.52694±0.01819	0.00539±0.02552	0.21
Suprarenals	0.02827±0.00051	0.02742±0.00045	0.00085±0.00068	1.25
Testes	2.9447 ±0.0494	3.1237 ±0.0462	0.1790 ±0.0676	2.65
Body weight	300.8 ±5.2	290.8 ±5.1	10.04 ±7.28	1.38
Head weight	23.08 ±0.30	22.50 ±0.25	0.58 ±0.39	1.47
Body length	22.32 ±0.11	22.18 ±0.11	0.14 ±0.15	0.91
Tail length	20.68 ±0.15	20.51 ±0.17	0.16 ±0.23	0.71

TABLE 2  
Statistical summary of results of the 3-month group.  
(Measurements in grams and centimeters)

	MEAN		DIFFERENCE BETWEEN MEANS	SIGNIFI- CANCE RATIO
	Oil	No oil		
Hypophysis	0.00824±0.00012	0.00826±0.00015	0.00002±0.00019	0.10
Thyroid	0.02010±0.00053	0.02596±0.00052	0.00586±0.00074	7.93
Thymus	0.6225 ±0.01738	0.68070±0.01829	0.05820±0.02523	2.31
Suprarenals	0.02718±0.00036	0.02784±0.00050	0.00065±0.00062	1.06
Testes	2.99259±0.03221	3.15876±0.06913	0.16617±0.07626	2.18
Body weight	274.2 ±3.7698	273.0 ±4.7011	1.2 ±6.026	0.20
Head weight	21.89 ±0.1970	21.69 ±0.2835	0.20 ±0.3452	0.58
Body length	22.08 ±0.1140	21.81 ±0.1219	0.27 ±0.1669	1.62
Tail length	20.39 ±0.1016	20.29 ±0.1231	0.10 ±0.1596	0.63

for the no-oil animals was 0.02596 gm. while that for the rats with 2% oil was 0.02010 gm. The decrease in the oil group amounted to 22.57%. The difference was significant, the significance ratio being 7.93.

*Series C.* The mean autopsy weight (table 3) of the several groups of this series were: no-oil, 0.01646 gm.; KI-no oil,

0.01271 gm.; 1% oil, 0.01464 gm.; 2% oil, 0.01427 gm.; and KI-2% oil, 0.01262 gm. The no-oil glands were larger than the other glands. The KI-no oil glands were decreased 22.80% when compared to the no-oil group; the 1% oil group, 11.04%; the 2% oil group, 13.31%; and the KI-2% oil group, 23.31%. All of these decreases were significant as shown by the following significance ratios: KI-no oil, 7.46; 1% oil, 3.51; 2% oil, 4.14; and KI-2% oil, 8.24 (no-oil group used for comparison).

TABLE 3  
*Means of 2-month group. (Measurements in grams and centimeters)*

	NO OIL	KI-NO OIL	1% OIL	2% OIL	KI-2% OIL
Thyroid	0.01646 ±0.00038	0.01271 ±0.00033	0.01464 ±0.00035	0.01427 ±0.00037	0.01262 ±0.00027
Body weight	173.48±3.37	183.92±4.11	186.42±4.07	185.62±3.42	179.38±4.18
Head weight	15.90±0.23	16.26±0.27	16.26±0.26	16.24±0.25	15.90±0.28
Body length	19.29±0.11	19.50±0.12	19.48±0.13	19.43±0.11	19.27±0.14
Tail length	16.86±0.12	17.30±0.14	17.37±0.14	17.11±0.12	17.13±0.14

TABLE 4  
*Significance ratios of 2-month group. (No oil compared with other groups)*

	KI-NO OIL	1% OIL	2% OIL	KI-2% OIL
Thyroid	7.46	3.51	4.14	8.24
Body weight	1.96	2.45	2.53	1.10
Head weight	1.01	1.04	1.01	0.01
Body length	1.27	1.14	0.90	0.13
Tail length	2.40	2.74	1.49	1.48

The histological structure of the thyroid was studied in six animals of each group. No changes were noted.

#### *Other measurements*

The average measurements of body weight, head weight, body length, tail length, hypophysis, thymus, suprarenal glands and testes showed no differences between the tests and controls which were of certain significance in any of the three series. The average measurements are given in the tables and since the results are negative no further discussion is needed.

## INCIDENCE OF INFECTIONS

All animals were examined carefully for signs of infections. Very few pathological changes were noted. There was no observed difference in the amount of respiratory or middle ear infection in any of the series studied.

## DISCUSSION

Strikingly apparent was the effect of adding cod liver oil to the basal diet used in the present experiment. The thyroid glands of male rats, sustained on diets to which 1% cod liver oil and 2% cod liver oil had been added, at 4 months, 3 months, and 2 months of age were significantly smaller than those of animals without oil in the diet. The same phenomenon has been observed in the case of female rats at 4 months of age (Freudenberger and Clausen, '35). Interesting enough was the fact that no significant changes were found in any of the other structures studied in the 4-month and 3-month series. On this basis the gonads, hypophysis, thymus, and suprarenal glands were not studied in the 2-month series.

Naturally, the question arose as to which constituent, or constituents, of the cod liver oil was responsible for these definite and consistent weight changes in the thyroid gland with no significant alteration in any of the other structures studied. The adequacy of the diet together with the close parallelism of the various groups of animals both as to body growth and weights of the several organs (except thyroid), most certainly spoke against the possibility of any form of an avitaminosis.

The sensitivity of the thyroid to the iodine intake is an established fact. This seemed to be by far the most likely constituent of the cod liver oil responsible for the changes. The effects of iodine on the thyroid gland have been studied by many investigators. Hayden, Wenner and Rucker ('23-'24) found thyroid enlargement in rats on a diet containing a small amount of iodine (9 to 10 parts per billion). In the same period McClendon and Williams made similar observations. By the addition of iodine, these authors found that

smaller thyroid glands were obtained. Marine ('24) maintained that simple goiter was caused by a deficiency of iodine. Tanabe ('25) confirmed Marine with results obtained from the rat. Krause and Monroe ('30) found that the addition of iodine to diets low in this constituent resulted in smaller thyroid glands in rats. Thompson ('32) reported enlargement of thyroids in rats on a rachitogenic diet. The addition of iodine, not vitamin D, produced smaller glands. Levine, Remington and von Kolnitz ('33) worked out a range of iodine dosage which was proved to be very sensitive. The range was between 0.14  $\gamma$  and 0.59  $\gamma$  ( $\gamma = 0.001$  mg.) daily. Small variations in the amount of iodine within this range produced marked changes in the thyroid glands. The authors came to the conclusion that the minimum daily intake of iodine to prevent goiter in the rat was from 1 to 2  $\gamma$ .

Cod liver oil as a source of iodine is without dispute. Holmes and Remington ('35), reporting on the iodine content of twenty representative samples of American cod liver oil, found the iodine content to average 8640 parts per billion parts of cod liver oil.

The evidence, from the data obtained with the 4-month and 3-month series, was very suggestive that the basal diet fed the animals was inadequate in iodine. That is, the rats received iodine which perhaps fell within the critical range of iodine intake mentioned above (less than  $\frac{1}{2}$   $\gamma$  daily).

In the 2-month group iodine in the form of potassium iodide was added to the basal diet to find out whether the glands could be reduced in size, as was the case with the administration of cod liver oil. The iodized salt was used in place of the normal salt in the basal diet. The amount of potassium iodide in the salt was stated as 0.023%. Since the amount of salt in the basal diet was 0.8%, the amount of potassium iodide was calculated to be 0.0184%. This value would give each animal approximately 14  $\gamma$  of iodine daily (assuming each animal ate 10 gm. of food daily). Such an iodine intake is far in excess of the physiological requirement for the rat.

The results showed that the administration of potassium iodide to the basal diet produced a much greater change in the weight of the thyroid gland than was found with the administration of either 1 or 2% of cod liver oil. It would seem, therefore, that the iodine in the cod liver oil was not available to the organism in sufficient quantities to render the basal diet completely antigoirogenic (did not remove the iodine intake from the critical range cited above). Thus, iodine in the form of potassium iodide was more effective in preventing goiter than the iodine in the cod liver oil.

An unexplainable bit of information was the fact that the 2% oil had practically no more effect than did the 1% oil. Certainly, one would think that if 1% cod liver oil was not sufficient to bring the intake of iodine out of the critical range, 2% oil would produce a change greater than that produced by the 1% oil. Such was not the case. Two per cent cod liver oil did not have the magnitude of influence in the 2-month series that it did in the 3- and 4-month series. This fact may or may not have significance.

Remington, Remington and Welch ('37) reported normal thyroid weights which were lower than those reported in the literature with the exception of those of McCarrison ('30). They called attention to the importance of an adequate amount of iodine in the diet and suggested that a stock diet such as that used by us was probably deficient in iodine. Our results would tend to confirm their contentions.

#### SUMMARY AND CONCLUSIONS

A total of 231 male Wistar albino rats was divided into three series. The animals of all series were placed on the various diets at 3 weeks of age. Series A consisted of twenty-five rats fed a well-balanced basal diet plus 2% cod liver oil, and twenty-five rats fed on the basal diet for controls. The animals were killed and autopsied at 4 months of age. Series B was composed of twenty-six test rats and twenty-six controls treated exactly as those of series A, except that the animals were killed and autopsied at 3 months of age. In series

C there were five groups of rats. Twenty-five were sustained on the basal diet; twenty-six had 1% oil added to the diet; twenty-six received 2% oil; twenty-six had 0.0184% potassium iodide added to the basal diet; and twenty-six animals received the same amount of potassium iodide plus 2% cod liver oil. The rats of this group were autopsied at 2 months of age.

No significant difference in any of the series was found in body weight, body length, tail length, incidence of infection, or in the weights of the head, suprarenal glands, hypophysis, thymus, or testes.

The thyroid gland was significantly smaller in all groups of animals which received either cod liver oil or potassium iodide, or both. The change was most marked in those animals which had potassium iodide present in their diets.

The conclusion was reached that the stock diet fed the animals of this colony is iodine deficient. This condition was compensated for in part by the addition of cod liver oil. Potassium iodide added to the stock diet was more efficacious in obviating the condition. There was apparently no difference between the sexes. There seemed to be a greater change in thyroid weight in the series of animals sustained on the diets for the longer periods of time, namely, 3 and 4 months.

Lastly, iodized salt should be substituted for ordinary salt in basal diets, at least in goitrogenic belts, in order to supply the thyroid gland (and the organism) with the physiological requirement of iodine. The result will be a lower normal value for thyroid gland weights.

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