The Thiocyanate Space and Iodide Space in the Thyroid Gland

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Both thiocyanate and iodide have marked effects on the thyroid and both ions have been used to measure the distribution of available water in the body. It is clear that iodide space may not always be a measure of water distribution because the thyroid cell is able to concentrate iodide. The present paper is concerned with the iodide space and the thiocyanate space in the thyroid of the dog. We define thiocyanate space and iodide space as the ratio of the concentration of these substances in protein-free organs to that in protein-free blood plasma. After we had finished the determinations reported here we discovered the paper by Baumann & Metzger (1949) dealing with the same problem, but since these authors used an entirely different technique, and ours are the only experiments in which the volume of the thyroid was determined, we felt that our data were worth publishing.

EXPERIMENTAL

Method of estimation of thiocyanate. It appeared undesirable to hydrolyse tissue with ethanolic KOH according to Brodie & Friedman (1937). We began working with the thiocyanate method of Crandall & Anderson (1934) using trichloroacetic acid to precipitate the proteins. This method depends on measuring the colour of ferric thiocyanate which is red. In determining thiocyanate in blood plasma we were disturbed by the admixture of a green colour. Since ferrous thiocyanate is green, we supposed that the green colour was due to the reduction of ferric iron to ferrous iron by reducing substances of the tissues. We reduced the error due to ferrous iron by increasing the concentration of ferric iron ten times and working rapidly. Furthermore, in order to eliminate the possibility of the green colour interfering with the estimation of ferric thiocyanate, we estimated the latter by means of a Coleman photoelectric spectrophotometer set at 5000 Å. Graphs were made, plotting the log. of the reciprocal of transmission against mg. of Na thiocyanate in various dilutions of a standard solution. This graph was a straight line and was used in determining the thiocyanate concentration of the experimental samples. By this method we determined the thiocyanate space in the dog thyroid gland to average 0.45 (45%).

Animals. Dogs were selected with no externally visible thyroids, but one had a noticeable enlargement of the thyroid on thyroidectomy. They were divided into two groups of thirteen dogs each. Group 1: each dog was injected in the femoral vein with 30 ml. of 1% (w/v) NaI (except where specified otherwise, Table 1). After 2-3 hr. (Table 1), 5 ml. of blood were drawn from the femoral vein

under pentothal anaesthesia into a centrifuge tube containing 3 mg. of K oxalate as a film on its walls, and the thyroid gland removed. Group 2: each dog was injected in the femoral vein with 30 ml. of a solution of 1% NaI to which had been added 1 g. of NaCNS/100 ml. Blood and thyroid were removed after 3 hr. as with group 1.

Thiocyanate estimation in the protein-free filtrate of oxalated blood plasma. Plasma (1 ml.) was delivered into a 15 ml. centrifuge tube and diluted to 5 ml. with distilled water. 20% (w/v) Trichloroacetic acid (5 ml.) was then added and the two solutions thoroughly mixed to ensure complete precipitation. After standing for 10 min. the precipitate was centrifuged for 5 min. and the protein-free supernatant fluid transferred directly to the spectrophotometer cell with addition of 0.5 ml. of conc. ferric nitrate reagent (50 g. $\text{Fe}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ and 100 ml. conc. HNO_3 in 100 ml. water). The reading was made rapidly and compared with the graph.

Handling of the thyroid gland. The thyroid gland was dissected out immediately after the dog was killed and the volume was determined by placing the gland in a 10 ml. graduated cylinder containing exactly 1 ml. of distilled water, and noting the change in volume. The gland was then removed from the water, transferred to a watch glass and frozen solid in the ice-making compartment of the electric refrigerator. The frozen tissue was quickly sliced, finely diced with a sharp scalpel and crushed in a supercooled diamond mortar and placed in a Wisconsin homogenizer. After homogenizing for 7 min. the tissue was transferred to a centrifuge tube and the homogenizer washed with small amounts of distilled water which were added to the tissue until a total volume of 5 ml. had been reached. 20% Trichloroacetic acid (5 ml.) was then added and the procedure followed as with plasma.

Iodine determination. The protein-free supernatant from 1 ml. of plasma was evaporated and fused in a nickel crucible with two pellets of NaOH. 10 ml. of an azide-carbonate solution (500 mg. NaN_3 and 500 mg. Na_2CO_3 in 100 ml. twice distilled water) was added and the solution brought to boiling and transferred to the microdistilling flask. The iodine was then determined by the method of McClelland & Bratton (1938). The iodine in the protein-free centrifugate of the thyroid gland was determined in the same manner.

RESULTS AND DISCUSSION

The iodide space in the thyroid gland of dogs injected with sodium thiocyanate (Table 2) was as large as that in dogs not so injected (Table 1).

It is evident that the iodide space, averaging 3.7-4.26 (grand average 3.98) times the total volume

Table 1. *Iodide space in thyroid gland of dogs injected with sodium iodide solution*

Dog wt. (kg.)	Vol. of NaI (1% w/v) injected (ml.)	Interval between injection and taking of samples (hr.) (min.)		Thyroid vol. (ml.)	Iodide space
8.0	30	2	10	0.8	0.66
16.2	30	2	05	2.6	1.70
15.6	30	2	10	2.2	2.44
9.4	30	2	05	0.9	2.10
7.8	30	2	10	1.5	2.20
11.5	30	2	30	0.6	2.30
22.0	30	2	30	2.4	2.40
7.1	30	1	45	1.2	2.44
15.0	20	3	30	0.9	5.26
8.0	30	2	30	0.4	5.40
6.2	30	2	45	1.0	5.60
8.5	10	3	15	1.0	6.00
24.0	20	2	40	1.3	10.80
Av.					3.70

of the thyroid gland cannot be a measure of the available water in the gland. The thyroid may concentrate iodide just as the brown seaweed does. On the other hand, thiocyanate seems not to interfere with the absorption of iodide by the thyroid gland when equal quantities of their sodium salts are injected intravenously and measured after 2-3 hr. Perhaps if much larger quantities of thiocyanate or much smaller quantities of iodide had been injected, or the samples taken earlier than 2 hr. after the injection, some effect on the absorption of iodide

(with one exception) smaller than the blood samples we usually take for iodine analysis. The iodide space in the thyroid gland was more than nine times as large as the thiocyanate space and thus the conclusions of various workers that the thyroid selectively absorbs iodide is confirmed.

The variation in individual values of the 'iodide space' in Tables 1 and 2, added to the fact that it is usually greater than unity indicates that it is not a measure of the available water in the thyroid, but a measure of the concentrating activity of the thyroid cells. Until we know more of the concentrating mechanism it would seem premature to discuss this point. Since the literature on this subject is covered by Leblond (1948), and Stanley & Astwood (1948), we need only refer to a more recent paper. The generalization of Baumann & Metzger (1949) that all elements of Group VII of the Periodic Table except iodine cause goitre is not in harmony with all the literature. In fact, Shrewsbury, Hatfield, Doyle & Andrews (1944) conclude that fluoride prevents goitre, but they did not make iodine analyses.

Table 2. *Iodide space in thyroid of dogs after injection of sodium iodide and sodium thiocyanate*

(Samples taken 3 hr. after injection of NaI (300 mg.) and NaCNS (300 mg.).)

Dog wt. (kg.)	Thyroid vol. (ml.)	Iodide space
7.0	0.7	0.64
15.0	9.0	0.73
6.0	0.4	0.78
7.7	0.7	0.94
10.8	0.6	0.94
6.5	0.5	1.79
6.4	0.8	3.40
8.2	1.1	4.94
7.0	0.7	5.45
8.7	0.4	7.05
14.0	1.5	8.57
8.4	0.4	9.28
5.5	0.6	10.81
Av.		4.26

might have been obtained. We used large quantities of iodide to make the analytical work more accurate. Most of the iodine in the thyroid is bound to proteins and the volumes of these dog thyroid glands were

SUMMARY

1. The thiocyanate space in the thyroid gland of dogs averaged 0.45 (45%). The iodide space in the thyroid gland of dogs not receiving thiocyanate averaged 3.7 (370%) and of those receiving thiocyanate 4.26 (426%) 2-3 hr. after the injections. In other words, these analyses do not support the idea that thiocyanate interferes with the absorption of iodide by the thyroid gland.

2. In the analysis of thiocyanate in living tissue, the formation of ferrous thiocyanate is to be avoided.

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Biochemistry of the Wood-rotting Fungi

6. VOLATILE METABOLIC PRODUCTS OF SPECIES OF *ENDOCONIDIOPHORA*

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The substances responsible for the characteristic odours of the fungi *Lentinus lepideus* Fr. and *Trametes suaveolens* (Linn.) Fr. have been isolated and characterized (Birkinshaw & Findlay, 1940; Birkinshaw, Bracken & Findlay, 1944). The odours are due mainly to aromatic esters, amongst which the methyl esters of *p*-methoxycinnamic, cinnamic, and anisic acids were identified in the products of *Lentinus lepideus*, and methyl anisate in the products of *Trametes suaveolens*. The latter fungus also produced anisaldehyde which was a contributor to the odour.

This work has now been extended to an investigation of the odoriferous products of species of *Endoconidiophora*. Some of the products isolated were briefly reported (Morgan, 1948) whilst the investigation was in progress; a complete account of this work forms the subject of the present communication. *Endoconidiophora* is a genus established by Münch to include the species of *Ceratostomella* which produce secondary spores in an endogenous manner (i.e. from within the terminal cell and not by abscission of the ends of the hyphae). It includes a number of species important as a cause of 'blue stain' or 'sap stain' in logs and sawn timber. *Endoconidiophora coerulescens* is an important cause of staining in sap wood in Europe, and according to Davidson (1944) *E. virescens* is one of the principal fungi causing stain in hardwoods in the southern United States. *E. fimbriata* is said to be of little importance as a stainer of timber.

The bluish tinge produced by these fungi is not due to a pigment, but is an optical effect caused by the scattering of light rays striking the very fine dark hyphae. The bluish colour of diluted milk is produced in a similar way. Whilst sap stain has little

effect on the mechanical properties of the commoner European soft woods, it may be more serious in soft tropical woods.

When some *Endoconidiophora* species are grown under laboratory conditions a pleasant fruity odour is evolved. Davidson (1944) refers to the banana-oil (amyl acetate) odour as a characteristic of *E. coerulescens*. He describes the odour of *E. virescens* as musty and penetrating. *E. coerulescens* was selected, from the species made available to us, as yielding the strongest odour, and was cultured in quantity on a liquid malt medium.

After a few days the odour permeated the incubator room. After harvesting, the fungal mycelium and culture fluid were subjected to steam distillation. The steam-volatile products were then distilled. Two main fractions were obtained. The lower boiling fraction was found to consist of isobutyl acetate ($\text{HMe}_2\text{CH}_2\text{O.CO.CH}_3$); the higher fraction contained methylheptenone (2-methylhept-2-en-6-one) and a mixture of methylheptenols (2-methylhept-2-en-6-ol) in which the dextrorotatory isomer and the racemic form were detected and characterized as allophanates. Since (+)-methylheptenol has been shown by Levene & Haller (1929) to be configurationally related to (+)-lactic acid, which is now denoted as L-lactic acid, the two methylheptenols isolated are correctly described as L-2-methylhept-2-en-6-ol and the corresponding DL-compound.

Two other species of *Endoconidiophora* were readily available and were examined by way of comparison. *E. virescens*, which gave a less pronounced odour than *E. coerulescens*, produced methylheptenone and L- and DL-methylheptenol, but no isobutyl acetate could be detected. None of these products was isolated from *E. fimbriata*, which