

and the writer indicate that a similar material is elaborated by this organism. In view of the established<sup>3</sup> greater pathogenicity of the S than of the R strains of *B. tuberculosis avium*, certain possible implications of these observations are obvious. They are being examined and will be discussed in a later more detailed description of this work.

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### Total and Thyroxine Iodine Content of Thyroid Gland After Injection of Saline Anterior Pituitary Extracts.

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The striking hyperplasia of the thyroid gland produced experimentally by injection of anterior pituitary extracts<sup>1</sup> affords an unusual opportunity to study the intermediate iodine metabolism of the hyperplastic thyroid gland under adequately controlled conditions. It is now well established that following parenteral administration of anterior pituitary extracts there is a decrease in the total iodine content of the thyroid gland, as shown in the dog,<sup>2, 3</sup> guinea pig,<sup>4</sup> duck,<sup>5</sup> and chick.<sup>6</sup> Closs, Loeb and MacKay<sup>4</sup> investigated the nature of this decrease in total iodine further by alcoholic fractionation of pooled thyroid glands of guinea pigs previously treated with anterior pituitary extract. They found a consistent and significant decrease in the alcohol-insoluble or "organic" fraction of the total thyroid iodine. Grab<sup>7</sup> employed the Reid Hunt acetonitrile test to compare the protective potency of thyroid glands of dogs treated with anterior pituitary preparations with that of untreated dog thyroids. The results were variable, but as the average potency

<sup>3</sup> Winn, W. A., and Petroff, S. A., *J. Exp. Med.*, 1933, **57**, 239.

<sup>1</sup> Literature reviewed by Loeb, L., *Klin. Woch.*, 1932, **11**, 2121.

<sup>2</sup> Loeser, A., *Klin. Woch.*, 1932, **10**, 2047; *Arch. exp. Path. u. Pharmacol.*, 1931, **163**, 530.

<sup>3</sup> Grab, W., *Arch. exp. Path. u. Pharmacol.*, 1932, **167**, 413.

<sup>4</sup> Closs, K., Loeb, L., and MacKay, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 170; *J. Biol. Chem.*, 1932, **96**, 585.

<sup>5</sup> Schockaert, J. A., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 89.

<sup>6</sup> Unpublished data.

<sup>7</sup> Grab, W., *Arch. exp. Path. u. Pharmacol.*, 1932, **167**, 313.

of thyroid glands of injected animals was lower, Grab also concluded that the decrease in iodine content occurs chiefly at the expense of the physiologically active or organic iodine fraction. Paal and Huber,<sup>8</sup> however, who used much the same procedure on rats found an increase in the potency of glands after treatment.

By utilizing the Leland and Foster method for the determination of thyroxine,<sup>9</sup> a more satisfactory partition of the iodine compounds known to be present in alkaline hydrolysates of thyroid gland can now be made. It was hoped that by using sufficiently large animals (sheep), it would be possible to estimate the total iodine, inorganic iodine and thyroxine content of a single lobe, thus permitting the removal of one lobe before treatment as a control for the second lobe, removed after treatment. The changes in the second lobe could then be compared with similar data available for normal human<sup>9, 10</sup> and hog<sup>6</sup> thyroid glands and for diffuse and nodular toxic and non-toxic goiters.<sup>10</sup>

*Procedure.* Five adult female sheep weighing about 150 pounds were obtained and one lobe of the thyroid gland removed, without preliminary iodine feeding and without using iodine at operation. The operated animals were kept in a separate enclosure on our experimental farm (May-June, 1932). They were permitted to graze freely, oats and water, but no salt or mineral feed, being added to the diet. Beginning 5 days after hemithyroidectomy, 4 sheep were injected intraperitoneally with an average of 50 cc. of a saline anterior pituitary extract (equivalent to 17 gm. of fresh beef anterior pituitary lobes) every day for 10 days. The day after the last injection, the second lobe of the thyroid gland was removed.

The thyroid glands thus obtained were weighed, a small piece removed for sectioning, the remainder minced, dried at 60°-65°C. to constant weight and pulverized. Duplicate samples of 10 mg. were weighed out on a micro-balance for determination of *total iodine* by a micro-method previously described by one of us.<sup>5</sup> The remainder of the lobe was weighed, the desiccated gland thoroughly extracted with iodine-free water,<sup>11, 10</sup> and the *iodine in the aqueous extract* determined. The water-insoluble residue was analyzed for *thyroxine iodine* by the method of Leland and Foster.<sup>9</sup>

<sup>8</sup> Paal, H., and Huber, W., *Arch. exp. Path. u. Pharmacol.*, 1931, **162**, 521.

<sup>9</sup> Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

<sup>10</sup> Gutman, A. B., Benedict, E. M., Baxter, B., and Palmer, W. W., *J. Biol. Chem.*, 1932, **97**, 303; *Trans. Am. Assn. Study Goiter*, 1932, 31.

<sup>11</sup> Harington, C. R., and Randall, S. S., *Quart. J. Pharm. and Pharmacol.*, 1929, **2**, 501.

Anterior pituitary extract was prepared by soaking the carefully trimmed anterior pituitary lobes for 5 minutes in 95% alcohol, rinsing with sterile saline solution, mixing the thoroughly ground anterior lobes with twice their weight of sterile physiological salt solution and centrifuging. The pink, turbid supernatant fluid was kept on ice until ready for use. The potency of the extracts used was proven by tests on chickens about 8 weeks old and averaging 400 gm. in weight (Table I).

TABLE I.  
Effect of Saline Anterior Pituitary Extracts on Thyroid Gland of Chicken.

	Chicken No.	Dry weight one lobe mg.	Total iodine one lobe $\gamma$	Total iodine % dry gland
<i>First extract</i>				
Controls	1	14.8	115	.776
	2	12.4	85	.685
2 cc. injected daily 5 days	3	31.0	7	.023
	4	27.3	4	.015
<i>Second extract</i>				
Controls	5	5.6	24	.430
	6	7.5	22	.300
3 cc. injected daily 6 days	7	23.9	1	.004
	8	21.2	1.5	.007

*Results.* On histological examination, characteristic hyperplastic changes, including hypertrophy of the Golgi apparatus, were observed in every thyroid gland after anterior pituitary injections. They were more marked in the glands showing more pronounced chemical changes. We are indebted to Dr. A. Severinghaus for the special stains.

The chemical results (Table II) indicate a consistent decrease in total iodine per cent of dry gland after injection of anterior pituitary extract, under conditions which led to an increase in iodine in our untreated control sheep. The mean decrease of 50% is much less than that found in ducks and chicks but comparable to that reported in dogs (75%)<sup>3</sup> and guinea pigs (65%)<sup>4</sup>. There was a proportionate and consistent decrease in thyroxine iodine per cent of dry gland following anterior pituitary injection. The reduction in the absolute content of total iodine and thyroxine iodine per lobe after injection is less marked than the relative decrease, due to the compensatory enlargement of the gland. The changes in water-extractable iodine (an approximation of the inorganic iodine content) are not regarded as significant. The figures for glands 5a and 8a are undoubtedly too high. Subsequent experience has shown that the

TABLE II.  
Effect of Saline Anterior Pituitary Extract on Thyroid Gland of Sheep.

Sheep No.	Dry weight, gm.	Dry matter, %	Total iodine % dry gl.	Thyroxine Iodine % dry gl.	Iodine in aqueous extract, % dry gl.	Thyroxine iodine % of org. I	Iodine in aqueous extract, % of tot. I	Total iodine per lobe, mg.	Thyroxine iodine per lobe, mg.
4 a	.498	24.9	.31	.065	.013	21.9	4.2	1.54	.324
b	.576	15.4	.07	.017	.006	26.6	8.6	0.40	.098
5 a	.675	24.9	.27	.082	.056†	38.3		1.82	.554
b	.677	20.0	.19	.044	.009	24.3	4.7	1.29	.298
6 a	.454	25.0	.28	.078	.025	30.6	8.9	1.27	.354
b	.672	17.4	.19	.040	.013	22.6	6.8	1.28	.269
7 a	.353	25.8	.44	.111	.015	26.1	3.4	1.55	.392
b	.542	17.0	.16	.030	.013	20.4	8.1	0.87	.163
Control sheep (not injected)									
8 a	.366	24.1	.12	.023	.052†	33.8		0.44	.084
b	.556	24.8	.23	.055	.020	26.2	8.7	1.28	.306

a = control lobe removed before anterior pituitary injection.  
b = lobe removed after anterior pituitary injection.

aqueous extract of desiccated thyroid contains small amounts of protein iodine and occasionally, as in these 2 instances, large amounts of organic iodine may be extracted with water.

The data indicate a depletion of the thyroglobulin content of the thyroid glands after 10 daily injections of anterior pituitary extract, (Fig. 1) presumably due to increased liberation of thyroid hormone into the circulating fluid. This conclusion is in agreement with earlier studies on the total iodine content of the thyroid gland and its fractionation with alcohol.<sup>1-5</sup> It is also in harmony with interpretations of histological changes in the thyroid gland,<sup>1, 5</sup> and with the loss of weight, increase in metabolism,<sup>12, 13</sup> increased alcohol-insoluble iodine in the blood<sup>4, 14</sup> and other effects of anterior pituitary injections.

It has been suggested that, in view of the striking analogies between the effects of anterior pituitary injections and changes observed in Graves' disease, the pituitary gland and associated structures in the mid-brain are primarily affected in Graves' disease. The only observation made by us relevant to this problem is that we

<sup>12</sup> Siebert, W. J., and Smith, R. S., *Am. J. Phys.*, 1930, **95**, 396.

<sup>13</sup> Verzár, F., and Wahl, V., *Biochem. Z.*, 1931, **240**, 37.

<sup>14</sup> Schneider, E., *Deutsche Z. f. Chir.*, 1932, **236**, 391.

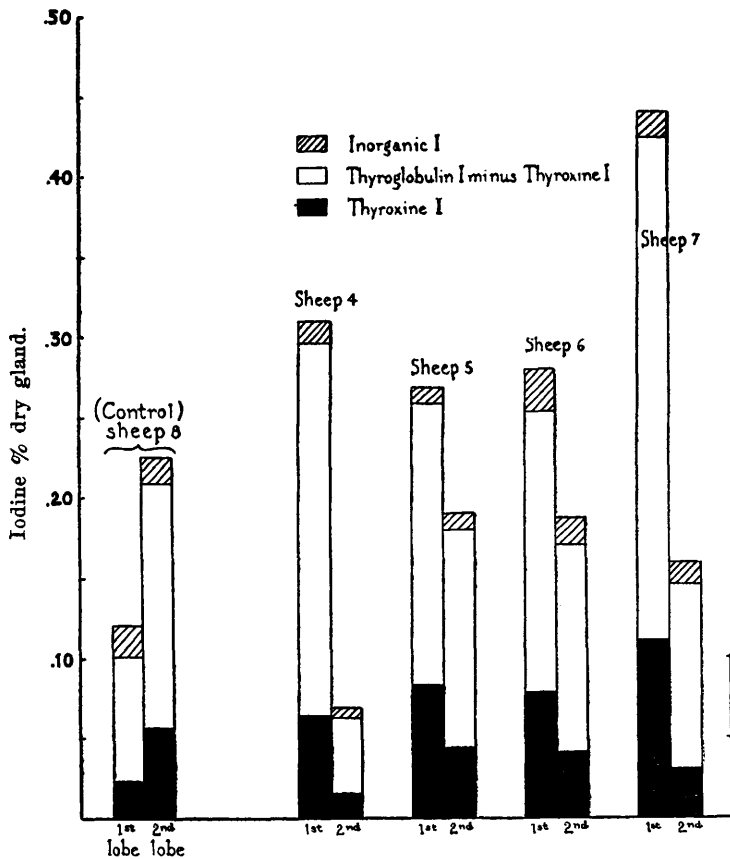


FIG. 1.

The effect of saline anterior pituitary extract on iodine fractions of the thyroid gland of sheep. The water-extractable iodine of 8a and 5a has been represented as equal to the percentages of water-extractable iodine in 8b and 5b.

found no significant decrease in thyroxine iodine per cent of organic iodine such as has been observed in many cases of severe Graves' disease.<sup>15, 10</sup> Since we succeeded in producing a decrease in total iodine comparable to the low levels found in Graves' disease in only one instance, this negative result does not preclude the possibility that such a change in thyroglobulin may occur.

*Summary.* After 10 daily intraperitoneal injections of a saline anterior pituitary extract, there is a decrease in the total iodine content of the thyroid gland of the sheep, with a proportionate decrease in its thyroxine content.

<sup>15</sup> Wilson, L. B., and Kendall, E. C., *Am. J. Med. Sci.*, 1916, 151, 79.