Lack of Synergistic Relationship Between Thyroid and Salivary Gland Function. (21965)

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Kirkwood *et al.*(1-3) have postulated a direct relationship between activity of thyroid gland and ability of salivary glands to clear iodine. This group also believes that salivary glands play an important role in deiodination of such intermediates as diiodotyrosine (DIT) and in the control of the circulating blood level of thyroid hormone. They have demonstrated a monoiodotyrosine iodinase system in rat salivary glands, and concluded that the reverse of this reaction might actually be taking place in the intact glands. To test this hypothesis, Fawcett and Kirkwood(3) followed the deiodination of DIT in salivariectomized and intact rats, and found that DIT was not deiodinated to any appreciable extent in the salivariectomized animals.

Our preliminary observations(4) support the conclusion reached by Freinkel and Ingbar(5,6) that the iodide clearance function of salivary glands is probably not influenced directly by the thyroid, even though the amount of iodide cleared by these glands appears to correlate with the activity of the thyroid. The following data are offered as additional evidence for this conclusion, and for the belief that the deiodination of such thyroid analogues as diiodotyrosine does not take place in the salivary glands.

Methods. Carrier-free doses of I^{131} labeled diiodotyrosine, triiodothyronine, thyroxine and iodide were administered intravenously 15 μc/kg body weight to mongrel dogs maintained under sodium pentobarbital anesthesia. Saliva samples were collected under Mecholyl stimulation, and chromatographed for 18 hr on paper, together with 20% trichloracetic acid filtrates of plasma in both 80% phenol and in 68:2:30 butanol-acetic acid-water mixture. In the rat experiments, 50 μc doses of I^{131} diiodotyrosine (DIT) were injected into jugular veins of rats anesthesized with sodium pentobarbital. Blood samples were withdrawn by cardiac puncture and by tail-vein sampling one hour after the DIT injection. 20% trichloracetic acid filtrates were prepared from these specimens, and chromatographed for 18 hr with the same solvents used in dog experiments. After chromatographs were dry, each strip was cut into 10 mm horizontal sections, and these were counted in a Nancy Wood Scintillation Well Counter to an accuracy of $\pm$ 5%. Activities for each spot were then totalled and used for subsequent calculations of the % deiodination taking place and of iodide clearance.

Results. Irrespective of which of the 4 substances was administered, only I^{131} iodide was

TABLE I. Constancy of Salivary Clearance of I^{131} Iodide Irrespective of whether Iodide or DIT I^{131} Was Administered, and whether Exogenous Thyroid Hormone Was Fed in the Diet.

	Clearance*					
	Iodide injected		DIT injected		Iodide injected	
Time	Dog A	Dog B	Dog C	Dog D	Dog E before thyroid feeding	Dog E after thyroid feeding
15 min.	—	—	33	40	17	19
2 hr	26	20	34	31	19	26
4 "	27	19	20	40	—	—
8 "	22	19	20	25	14	16
12 "	24	20	24	33	—	—
24 "	27	17	—	—	16	21

* Clearance is expressed as cc of plasma cleared of I^{131} iodide per min. and is given by the expression: $\text{Clearance} = \frac{(\text{cpm/cc saliva})(\text{cc saliva/min.})}{(\text{cpm/cc plasma})}$.

TABLE II. Rate of DIT Deiodination Determined from Comparison of I^-/DIT Counts on Plasma Paper Chromatograms.

Time	% I^{131} activity appearing as iodide in plasma
30 min.	30
1 hr	59
2 "	70
4 "	75
6 "	75
8 "	80
12 "	78
24 "	80

found on the saliva chromatograms, indicating that thyroid analogues are not cleared by the salivary glands in detectable amounts. By comparing the I^{131} iodide activity found on plasma and saliva chromatograms, clearance into the saliva remained relatively constant over a 24 hr period as shown in Table I. Furthermore, feeding of thyroid substance at 0.5% of diet for 2 months, failed to influence the clearance of I^{131} iodide in the dog (Table I). Therefore the amount of iodide cleared by salivary glands appears to be a function of the plasma level of iodide, and increased blood levels of thyroid hormone do not alter this clearance.

Kirkwood *et al.* have implied that salivary glands are the principal site of deiodination. We repeated this experiment in salivariectomized and intact dogs. It was found that salivariectomized animals deiodinate DIT at the same rate, and to the same extent as control animals subjected to mock surgery. This deiodination takes place very rapidly, but 10-20% of the circulating activity 24 hr later is still DIT (Table II).

Slices of some tissues and organs of the dog were then incubated in plasma with carrier-free DIT at 37°C. At the end of 3 hour incubation period, trichloracetic acid filtrates of the media were chromatographed on paper, and the relative amounts of iodide and DIT I^{131} determined. Of all tissues analyzed, only the liver and kidneys possessed significant deiodinase activity (Table III). On a weight basis, liver appeared to be more active than kidney, and considering the relatively greater size of this organ, it might be assumed that the liver is the principal deiodination site. To determine this experimentally, however, the deiodination of DIT was followed in plasmas of nephrectomized dogs. It was found that deiodination takes place just as rapidly and completely in the nephrectomized animal as in the control. Additional evidence for the relatively minor role played by the kidney is given by the fact that appreciable amounts of DIT can be found in the urine of intact animals for several hours after the administration of DIT.

TABLE III. Relative Distribution of DIT Deiodinase in Tissues of Dog.

Tissue	% I^{131} activity as iodide	Wt of tissue slice (mg)
Liver	17.6	346
Kidney	6.5	404
Spleen	2.3	320
Submaxillary gland	2.5	294
Parotid "	2.4	356
Sublingual "	2.4	289
Skeletal muscle	2.7*	270

* Approximately 2% of Abbott Laboratory DIT preparation is in the form of iodide as determined by our chromatographic methods.

Because the data obtained in our dog studies conflict with those of Kirkwood *et al.* for the rat, we attempted to repeat their rat experiments, using the same experimental technics which they described(3). In the first experiments however, we employed cardiac rather than tail-vein blood samples. We also prepared trichloracetic acid filtrates of plasma and chromatographed these together with the whole plasma samples on the same paper, using the butanol-acetic acid-water mixture employed by Kirkwood. Deiodination took place as rapidly and extensively in salivariectomized rats as in animals subjected to mock surgery. In subsequent experiments, tail-vein samples were compared with cardiac samples taken from salivariectomized and control animals, and it was observed that essentially all the activity present in the tail-vein samples could be attributed to DIT. Our explanation for this discrepancy is that under sodium pentobarbital anesthesia, the animals become acutely cyanotic and the blood pressure falls markedly. Therefore, tail-vein samples are not representative of the systemic circulation, and any DIT passing into the tail might become trapped and never recycled to the liver for deiodination. Slices and homogenates of

the principal tissues of the rat (excluding the thyroid) were also assayed for deiodinase activity. As in the case of the dog, only the liver and kidneys were found to possess activity.

Summary. It is believed that the salivary glands play no active role in metabolism of such thyroid analogues as DIT in the dog. The amount of iodide cleared into the saliva appears to be strictly a function of the circulating plasma level of iodide ion and is not influenced by exogenous thyroid hormone. This clearance will depend of course upon the rates with which exogenous diiodotyrosine, thyroxine and triiodothyronine become deiodinated. Since neither dog nor rat salivary glands appear to contain a DIT deiodinase system such as that found in liver and kidney tissue, it is concluded that these glands are not involved in the metabolism of DIT nor in the control of the circulating blood level of this analogue.

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Received June 28, 1955. P.S.E.B.M., 1955, v90.

Incorporation in Adrenal Cortex of C^{14} Labeled Fractions of *Klebsiella pneumoniae*.*† (21966)

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Crude polysaccharides from *Klebsiella pneumoniae* type B produce severe joint and mild cardiac valvular lesions in the guinea pig(1). To investigate the pathogenesis of the lesions, C^{14} labeled polysaccharides have been prepared and the distribution of the label followed in the animal tissues. The present report deals with (a) the adrenal cortical localization of the polysaccharide fractions; (b) comparison of incorporation of the polysaccharide with the whole organism and certain crude protein fractions in the adrenal; and (c) attempts to establish the mechanism.

Methods and materials. *Klebsiella pneumoniae* type B organisms were grown for 16 hours on peptone-glucose agar plates containing either 1-C^{14} sodium acetate or uniformly labeled d-glucose in concentrations ranging from 2 to 25 μc per ml of media. Bacteria were gently scraped from the agar. Crude polysaccharides were obtained by acid (A) and alkaline (B) extraction technics of Wong (3). The debris (C) remaining after acid extraction was separated into 2 fractions by cold trichloracetic acid to a concentration of 5% (w/v); the supernatant (D) was dialyzed against running water and evaporated to dryness over a steam bath. A portion of the trichloracetic acid precipitate (E) was washed with cold trichloracetic acid, then extracted 2 times with trichloracetic acid at 90°C for 10 minutes as for isolating nucleic acid (F)(4). Fractions (E) and (G) were insoluble in water and 0.85% sodium chloride and have not been studied in animals. The maximum absorption of the nucleic acid fraction (F) was at 260 mμ. Except for (D), the fractions were dissolved in 1% concentration in 0.85% sodium chloride, autoclaved and injected in less than 1 ml total volume into the ear veins of male and female guinea pigs

* A portion of the data herein presented was given at the meeting of the Fed. Am. Soc. for Exp. Biol.(2), San Francisco, April 1955.

† This investigation was supported, in part, by a research grant (H-1842) from the National Heart Institute, National Institutes of Health, U. S. Public Health Service.