IODINE METABOLISM OF SALIVARY GLANDS

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It has been known for many years that the salivary glands secrete iodide in the saliva at a concentration higher than in the plasma (Elmer, 1938). Using radioiodide, Schiff *et al.* (1947) showed that at physiological concentrations of iodide, the saliva/plasma iodide concentration ratio (S/P ratio) for the mixed saliva of humans is usually above 20 and may be as high as 100.

Chromatographic analysis of the saliva or salivary glands has shown that in mice (Fletcher *et al.*, 1956), in hamsters (Logothetopoulos and Myant, 1956*a*), and in humans (Cohen and Myant, 1959), all the iodine freshly concentrated from the plasma is in the form of iodide. Though Fawcett and Kirkwood (1954) have shown that homogenates of rat submaxillary gland synthesize monoiodotyrosine (MIT), this synthesis cannot be a necessary step in the transport of iodide from the plasma into the saliva, since thiouracil inhibits MIT synthesis but has no effects on the iodide-concentrating mechanism. In any case, it is not certain that the synthesis observed by Fawcett and Kirkwood occurs in the living animal, since it occurs *in vitro* only in the presence of a high concentration of copper ions. Taurog *et al.* (1957) have found a radioactive substance, probably an oxidation product of iodide, in homogenates of rat submaxillary gland incubated with radioiodide. However, this substance cannot play a part in iodide transport since it is not formed in mouse submaxillary glands, which concentrate iodide, and its formation in rat salivary glands is inhibited by thiouracil. It has been suggested that the salivary glands play a special role in the degradation of diiodotyrosine and thyroxine (Fawcett and Kirkwood, 1954), but this is most unlikely since both substances are metabolized equally well in the presence or absence of the salivary glands (Tong *et al.*, 1955; Myant, 1956). It seems, therefore, that in most species the transport of iodide into the saliva is the primary, if not the only, pathway for metabolism of iodine in the salivary glands.

Apart from the importance of the iodide-concentrating mechanism to the problem of the active transport of anions in general, this mechanism has aroused interest because of its similarity to the first step in the synthesis of thyroxine in the thyroid gland. Iodide is known to be concentrated in the thyroid before taking part in the iodination of tyrosine to form the primary components of the thyroxine molecule. In humans, the thyroid/plasma iodide concentration ratio in the presence of thiouracil, which blocks hormone synthesis but not iodide accumulation, is about the same as the S/P ratio for the mixed saliva. In both the thyroid and the salivary glands iodide accumulation is inhibited by perchlorate, thiocyanate, and nitrate. Moreover, Edwards *et al.* (1954) have shown that the order of effectiveness of these anions is the same in salivary glands and in the thyroid. Also, it seems likely that in both glands the inhibition is due to competition between iodide and the inhibiting anion for a common transporting process. The evidence for this is that thiocyanate (Crandall and Anderson, 1934) and perchlorate (Edwards

et al., 1954) have been shown to be concentrated by salivary glands and that thiocyanate has been shown to be concentrated by the thyroid (Logothetopoulos and Myant, 1956*b*).

From all this evidence it is difficult to avoid the conclusion that the mechanism responsible for concentrating iodide in the salivary glands is the same as that in the thyroid. The only observation casting doubt on this conclusion is that thyrotrophic hormone (TSH) apparently does not act on the salivary glands, whereas in the thyroid it is known to stimulate the iodide-concentrating mechanism and to induce hyperplasia. In some unpublished work (Myant),

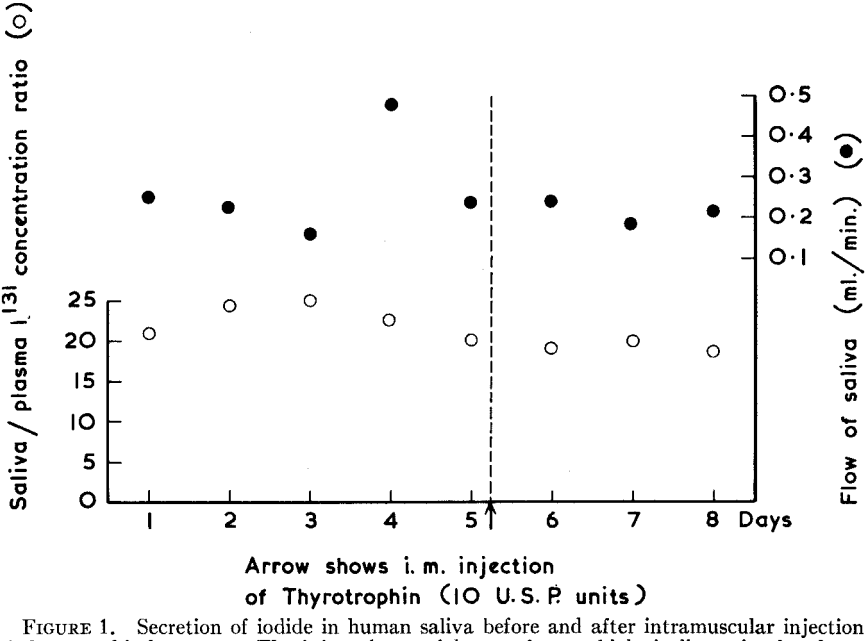

FIGURE 1. Secretion of iodide in human saliva before and after intramuscular injection of thyrotrophic hormone. The injected material came from a biologically active batch, as shown by its effect on uptake of I^{131} by the thyroid in humans.

injections of TSH into humans were observed to have no effect on the secretion of iodide in the mixed saliva. Observations on the S/P ratio were made on three patients during the second week after they had been given about 100 mc. of I^{131} for treatment of thyroid cancer. FIGURE 1 shows the results obtained in 1 patient. The S/P ratio for the mixed saliva and the rate of flow of saliva induced by chewing were measured daily at the same time of day. On the fifth day, 10 U.S.P. units of TSH* were given by intramuscular injection, and the measurements were repeated daily for 3 more days. Although the S/P ratio and the rate of flow of saliva varied from day to day, the results show clearly that neither was influenced by the injection of TSH. Similar results were obtained from the other 2 patients.

* Armour Laboratories, Chicago, Ill.

Leblond and Grad (1948) observed atrophy of the secreting tubules of the rat's submaxillary gland after thyroidectomy. This is contrary to what would be expected if TSH stimulates the salivary glands, since thyroidectomy causes an increase in the secretion of TSH. In rabbits, an increase in the secretion of endogenous TSH has no detectable effect on the weights of the salivary glands, as shown in a series of experiments on young adult rabbits (Myant, unpublished) in which the submaxillary glands were removed and weighed three weeks after removal of the thyroid. Although TSH secretion was increased, as shown by the histological appearance of the pituitaries, the weights of the submaxillary glands did not differ significantly from those of litter mates with intact thyroids.

In spite of this negative evidence, the failure of TSH to stimulate the salivary glands is not inconsistent with the hypothesis that the accumulation of iodide is brought about by the same mechanism in the thyroid and the salivary glands. In the salivary glands, the mechanism may be inaccessible to TSH. Again, TSH might influence iodide transport in the thyroid by altering the amount or the availability of some substance which limits the rate at which iodide can be accumulated. There is no reason to assume that iodide accumulation is limited in the salivary glands by the same factors that limit it in the thyroid.

The power to concentrate iodide is present in a wide variety of tissues, as well as in the thyroid and the salivary glands. It is present in the stomach (Elmer, 1938), in the small intestine (Pastan, 1957), in the lactating mammary gland (Honour *et al.*, 1952), in the placenta (Logothetopoulos and Scott, 1956), and in the mucous glands of the soft palate of cats and dogs (Cohen and Myant, 1959). In this connection, it is also worth noting that the concentrating power of the salivary glands varies considerably with species and with glands in the same species. In rats, for instance, none of the salivary glands concentrates iodide; in mice and hamsters, in which the submaxillary glands possess a very active concentrating mechanism, the sublingual glands do not concentrate iodide. In each of these tissues in which the effect of thiocyanate has been investigated, it has been found that thiocyanate inhibits accumulation of iodide at low concentrations which have no measurable effect on other metabolic processes. This suggests that all the tissues mentioned above, despite differences in their origin and histological structure, share with the thyroid and salivary glands a common mechanism for concentrating iodide. If this possibility is accepted, the distribution of the mechanism must be taken into consideration in any attempt to understand how the salivary glands concentrate iodide.

One approach to this problem is to try to identify the cells of the salivary gland in which the concentrating mechanism is present. A limited amount of information has been gained by means of autoradiography. Serial autoradiographs (FIGURE 2) of the submaxillary glands of mice and hamsters injected with radioiodide show selective blackening over the secretory ducts but never over the acini (Logothetopoulos and Myant, 1956*a*). The concentrating mechanism must therefore be located in the rodded cells lining the secretory ducts and not in the acini. This raises the possibility that iodide is concentrated in the saliva by the duct cells which would reabsorb water and

electrolytes other than iodide from a primary fluid secreted by the acini. The hypothesis that the ducts modify a fluid derived from the acini is supported by the experiments of Burgen and Seeman (1958) on potassium secretion in dog saliva. However, Fletcher *et al.* (1956) have shown that slices of mouse submaxillary gland, when incubated in a buffer, concentrate iodide from the surrounding medium. It is difficult to see how this could take place if iodide

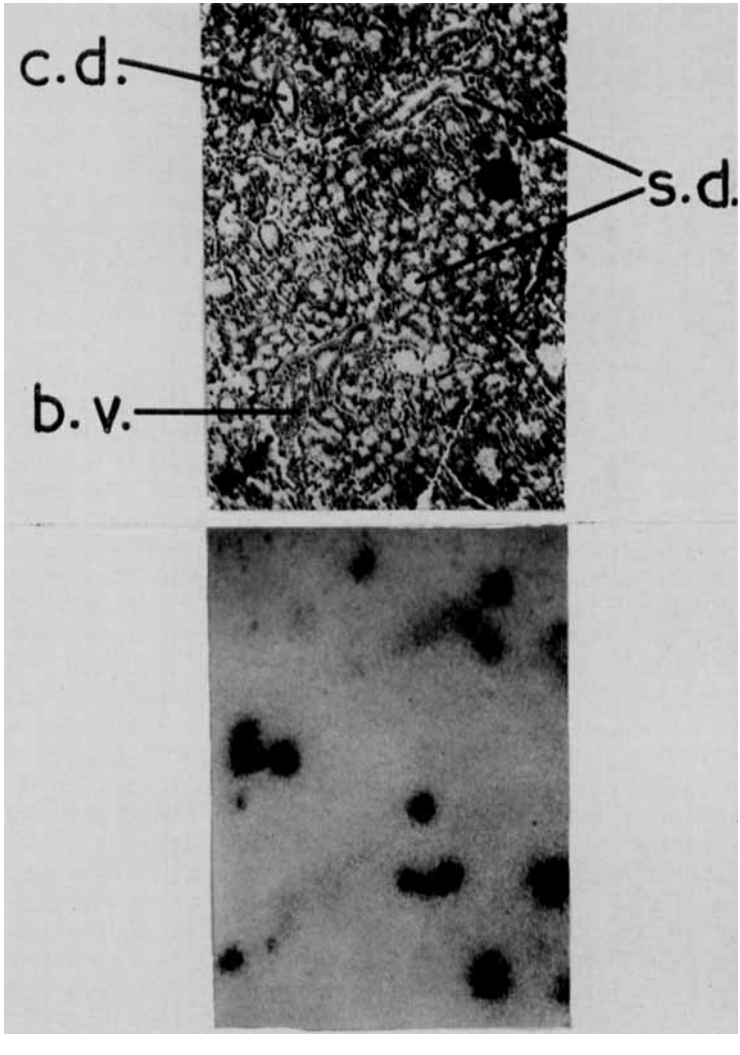

FIGURE 2. Contact autoradiograph of a section of hamster submaxillary gland removed from the animal 1 hour after injection of radioiodide. The pattern of blackening corresponds to the distribution of the secretory ducts (s.d.). The intensity of blackening over the acini and over some of the collecting ducts (c.d.) is no greater than that over the blood vessels (b.v.). Owing to the low resolving power of the method, it is not possible to say whether the blackening is more intense over the lumen or over the cells lining the secretory ducts.

accumulation is brought about by secretion in one part of the gland followed by reabsorption in another. Moreover, nothing as complicated as this could occur in other tissues, such as the mucous glands of the soft palate, in which iodide is also concentrated. However, as I have pointed out, there is reason to think that the mechanism for concentrating iodide in these tissues is similar to that in the salivary glands.

The simplest hypothesis is that the cells lining the secretory ducts transport iodide from the plasma to the lumen by a mechanism that is also operative in the cells of all those tissues in which iodide accumulation occurs. Fletcher *et al.* (1956), in their study of the metabolism of salivary glands *in vitro*, found that as the concentration of iodide in the medium was increased, there was a fall in the tissue/medium (T/M) iodide concentration ratio. Since the relationship between the T/M ratio and the iodide concentration in the medium conformed to Langmuir's adsorption equation, it was suggested that reversible adsorption of iodide to a substance in the salivary glands might be an essential step in the concentrating mechanism. Attempts to demonstrate, by means of dialysis experiments, the existence of a substance with a high affinity for iodide in homogenates of rat thyroid (Doniach and Logothetopoulos, 1955) and hamster submaxillary gland (Myant, unpublished) have not been successful. This does not disprove the existence of an adsorbing substance, since its stability might depend on the maintenance of an intracellular environment. However, an adsorbing substance in the cells of the salivary gland would not, by itself, account for the transport of iodide from the plasma, across the cell, and into the saliva. It would be hard to distinguish, simply from the relationship between iodide concentration and the concentration ratio achieved, a mechanism depending on adsorption from other mechanisms that include a step with a limited capacity for iodide. If, for example, the cell membrane was impermeable to iodide but permeable to some reversible carrier–iodide complex, the concentration ratio established across the membrane might be limited by the amount of carrier available, or by the rate at which it could return through the membrane for more iodide ions. A simple model for moving iodide across a boundary impermeable to iodide consists of a U tube containing a solution of iodide in each arm, separated by carbon tetrachloride in the bend of the tube. If a mild oxidizing agent is placed in the left arm and a reducing agent in the right arm, iodide will be oxidized to free iodine in the left arm and will then diffuse through the carbon tetrachloride to the right arm, where it will be reduced to iodide. Provided the oxidizing and reducing agents are insoluble in carbon tetrachloride, the concentration of iodide on one side of the U tube will become higher than that on the other side. FIGURE 3 illustrates an experiment in which ferric chloride was the oxidizing agent and sodium arsenite the reducing agent. In this model, the concentration ratio finally established depends on the amounts of oxidizing and reducing agents added and the amount of iodide initially present in the U tube. When the amount of oxidizer in the left arm is nearly sufficient to oxidize all the iodide initially present, the relationship between the concentration of iodide at the beginning, and the concentration ratio finally established, deviates only slightly from the Langmuir equation. Demonstration that this relationship

holds in a biological system is clearly not proof that the mechanism depends on adsorption of iodide.

If iodide transport required the formation of a carrier–iodide complex and an intact cell membrane capable of maintaining a concentration gradient, the fact that homogenates do not concentrate iodide *in vitro* would be readily explained. However, there is no experimental support for such a mechanism. As I mentioned earlier, chromatographic analysis of salivary glands has shown

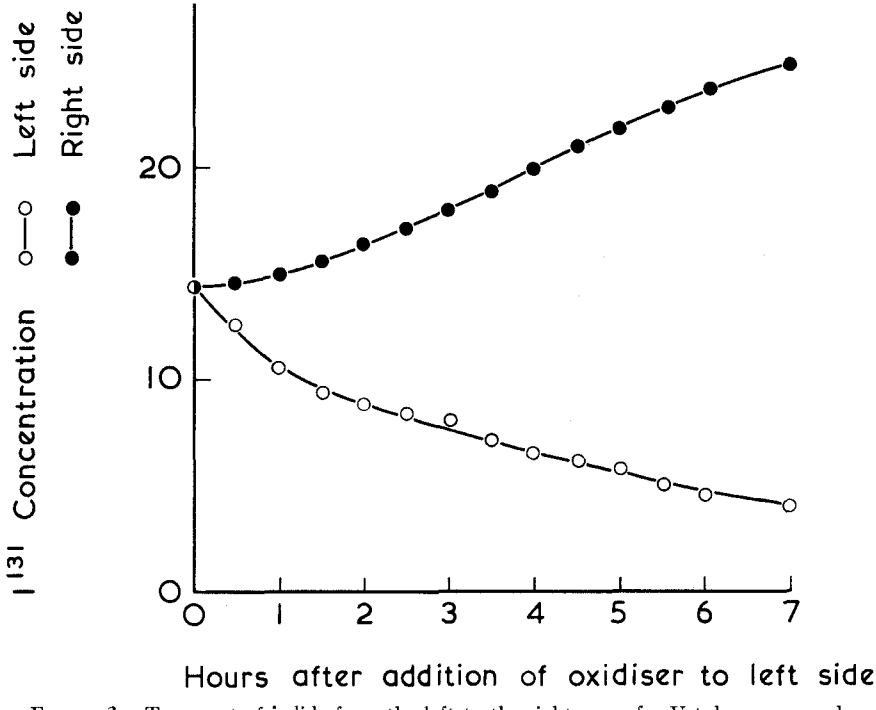

FIGURE 3. Transport of iodide from the left to the right arm of a U tube across carbon tetrachloride in the bend of the tube. At the beginning of the experiment, each arm contained 1 ml. of 0.1 *M* radioactive KI in 0.1 N H_2SO_4. At zero time, 2 ml. of 0.1 *M* ferric-chloride was added to the left arm and 2 ml. of 0.1 *M* sodium arsenite was added to the right arm. The radioactivity was then measured in serial samples taken from each arm.

only iodide and two other iodinated compounds that are not concerned in iodide transport. If a carrier is concerned in iodide transport, the complex with iodide either must be very unstable, or must be present at a very low concentration.

In conclusion, the salivary glands of most species, along with several other tissues, including the thyroid gland, possess a powerful mechanism for concentrating iodide. The accumulation of iodide is inhibited by several anions which appear to act by competition with iodide. Thyrotrophic hormone, known to stimulate the iodide-concentrating mechanism in the thyroid, does not affect iodide accumulation in the salivary glands. In the salivary glands,

the mechanism is confined to the secretory ducts. Iodide is probably transported from the plasma, through the cells lining the secretory ducts, and into the saliva. Active transport of iodide may require either the presence of a substance inside the cell capable of adsorbing iodide, or the formation of a carrier–iodide complex to which the cell wall is permeable, or both may be necessary.

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