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THE IODIDE CONCENTRATING MECHANISM OF THE MAMMARY GLAND

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The ability of the thyroid to accumulate iodide against a concentration gradient and to maintain a high gland/plasma concentration ratio is well known and forms an important aspect of the physiology of this gland. These properties are not, however, unique to the thyroid; iodide concentrating mechanisms in the salivary glands and in the gastric mucosa have been known for some time. More recently, Logothetopoulos & Scott (1956) have described a placental iodide concentrating mechanism, and Halmi, Stuelke & Schnell (1956) have suggested that, at least in the rat, there may also be some hepatic concentration of iodide. A further extra-thyroidal iodide concentrating mechanism is that of the mammary gland which has been studied in man by Honour, Myant & Rowlands (1952) and in the rabbit by Brown-Grant (1956*a*). In this species the concentration of ^{131}I in the milk was shown to be abolished by large doses of thiocyanate. It seems possible that studies of these extra-thyroidal mechanisms may help towards a better understanding of the workings of the thyroid 'iodide trap'. In the present paper further *in vivo* studies on the mammary glands of rabbits and of other laboratory animals will be presented. A preliminary account of some of the findings has already been published (Brown-Grant, 1956*b*).

METHODS

Animals. All experiments were performed on anaesthetized lactating female animals. The anaesthetic agent was pentobarbitone sodium B.P., 40–60 mg/kg body weight, administered intravenously or subcutaneously. Rabbits of mixed strains, 2·6–3·6 kg body weight, were used; the animals were fed on a pellet diet (Parkes diet 18, with a mineral supplement not containing iodide), hay and tap water *ad lib.* The experiments were performed 9–12 days post partum; the litters were removed overnight and allowed to suck on the morning of the experiment. The animals were anaesthetized about an hour later, the abdominal hair clipped and the animals placed in a supine position on a heated operating table. There are generally eight functioning breasts in the lactating rabbit and usually the lower six or sometimes the middle four were used.

Milking was carried out by suction, aided by gentle manual compression of the breasts as described by Popják, Hunter & French (1953) after the intravenous injection of 200 m-u. oxytocin ('Pitocin', Parke, Davis and Co.) in 0·2 ml. of 0·9% NaCl solution. The breasts were routinely emptied as far as possible before the subcutaneous injection of 10 μc of ^{131}I (as carrier-free NaI in a volume of 0·2–1·5 ml. of distilled water) at the beginning of the experiment (0 min). Milking was repeated at 45–75 min intervals for periods up to 6 hr. With the dose of oxytocin used there was no falling off in the visible response of the mammary glands or of the milk yield with repeated injections at these intervals. The usual milk yield under these conditions was about 0·25–1·0 ml. of milk/breast/hour, corresponding to a calculated daily yield per rabbit of 48–192 ml. This may be compared with the average daily figure of 127 g (range 44–282 g) obtained by Cross & Harris (1952) in chronic experiments with rabbits on a once-daily suckling regime. Although precise measurements of the milk yield were not made in detail, there was no indication of any marked or systematic change during the course of any one experiment. Changes in the radio-iodide concentration were not associated with changes in the milk yield. Blood was drawn from the marginal vein of the ear into tubes containing a small amount of dry heparin powder and centrifuged to obtain plasma samples for assay.

Experiments were also performed on albino guinea-pigs (Dunkin-Hartley strain), 630–820 g body weight, 7 or 8 days post partum and maintained on Parkes diet 18 with supplement, hay and tap water *ad lib.* plus a small daily ration of green food; on rats (Wistar albino strain) 290–320 g body weight, 10–12 days post partum, and on albino mice (Porton strain) 37–49 g body weight, 10 or 11 days post partum. The rats and mice were maintained on Thomson's (Rowett) cubes with mineral supplement and tap water *ad lib.* The experimental procedure consisted of suckling in the morning and anaesthesia about one hour later (ether plus pentobarbitone) followed by saline or thiocyanate subcutaneously at 0 min and ^{131}I subcutaneously at +30 min. At +120 min the animals were milked after the injection of oxytocin, bled and killed. Individual, or in the case of the mice, pooled samples of milk and plasma were analysed for ^{131}I and thiocyanate content.

Estimation of radioactivity. Carrier-free radio-iodine (^{131}I) obtained from the Radiochemical Centre, Amersham, was used in all experiments. Known volumes of the fluids to be assayed were diluted to 10 ml. with distilled water and counted in an M6 liquid counter with conventional scaling apparatus to a statistical accuracy of at least ±4%. Many assays were performed in duplicate. All activities were expressed as counts/100 sec after appropriate corrections for background counts and isotope decay.

Chemical estimations. Thiocyanate, expressed as μg KSCN/ml., was determined in duplicate by the method of Bowler (1944); absorption was measured with a 1 cm light path at 460 mμ. Recovery of thiocyanate added to rabbit plasma *in vitro* was 102% (mean of 6 expts.) and from rabbit milk was 98% (mean of 5 expts.). Discrepancy between duplicates was only rarely greater than 5%. Chloride estimations were performed in duplicate on milk and plasma by the method described by Consolazio, Johnson & Marek (1951).

Chemicals. The following salts were dissolved in distilled water and injected subcutaneously in volumes of 1–4 ml. in the case of rabbits and guinea-pigs and 0·2 ml. in the case of rats and mice, in the doses indicated in the text: KBr, $KBrO_3$, KCl, KI, KIO_3, KNO_3, KSCN and NaCl, all A.R. grade, and $KClO_4$ (L.R. grade). 4-Methyl-2-thiouracil (British Drug Houses, L.R. grade) was dissolved in weak alkali and injected intraperitoneally and subcutaneously.

RESULTS

The milk/plasma (M/P) ratio for ^{131}I *in normal rabbits*

Three rabbits were injected with radio-iodine and milk, and plasma samples collected as described for periods of 3–6 hr. A high concentration of ^{131}I relative to the plasma was found in the first milk sample at 45 min after injection and in all subsequent samples. One of these experiments is illustrated

in Fig. 1. The M/P ratios were based on the mean plasma concentration during the period of milk secretion determined from the graph by interpolation at the mid point of this period except for the first period immediately after injection; for this, a value of two-thirds the plasma level found at the first determination was used. The three experiments all gave similar results and the actual findings are shown in Table 1.

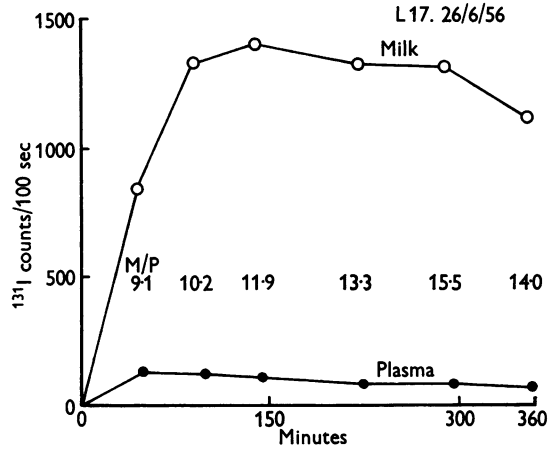

Fig. 1. Plasma (●) and milk (○) radioactivity after the injection of 10μc ^{131}I subcutaneously at 0 min in a normal rabbit. Calculated M/P ratios are shown.

TABLE 1. Milk/plasma ratios for ^{131}I, determined as described in the text in 3 normal and 1 methylthiouracil-treated rabbit: upper figures indicate time in min after injection of ^{131}I at which milk was collected; lower figures are M/P ratios

Rabbit no.	Serial milk/plasma ratios					
	1	2	3	4	5	6
L1	36	73	120	180	—	—
	6·1	8·4	8·5	8·6	—	—
L16	45	95	150	240	285	—
	15·3	32·5	33·5	33·8	33·4	—
L17	45	90	140	220	290	355
	9·1	10·2	11·9	13·3	15·5	14·0
L18*	45	90	145	210	270	310
	13·8	15·9	17·6	17·6	17·0	18·6

* Pretreated with methylthiouracil

A fourth rabbit was injected with 100 mg of methylthiouracil intraperitoneally 75 min, and again subcutaneously 15 min before the administration of ^{131}I. A further 100 mg was injected subcutaneously during the course of the experiment. These doses are known to produce a complete inhibition of the organic binding of ^{131}I by the rabbit thyroid (Brown-Grant & Gibson, 1955), but had no effect on the establishment and maintenance of the M/P ratio, as may be seen from the figures for rabbit L18 in Table 1.

The effect of various anions on the M/P ratio for ^{131}I

The experimental procedure was as follows. After the collection of the first and second sets of milk and blood samples at about 45 and 90 min after the administration of ^{131}I, the rabbits were injected subcutaneously with the salt under investigation. The standard dose used was 100 mg of KSCN; this amount is known to produce a rapid and complete discharge of ^{131}I from the thyroid of

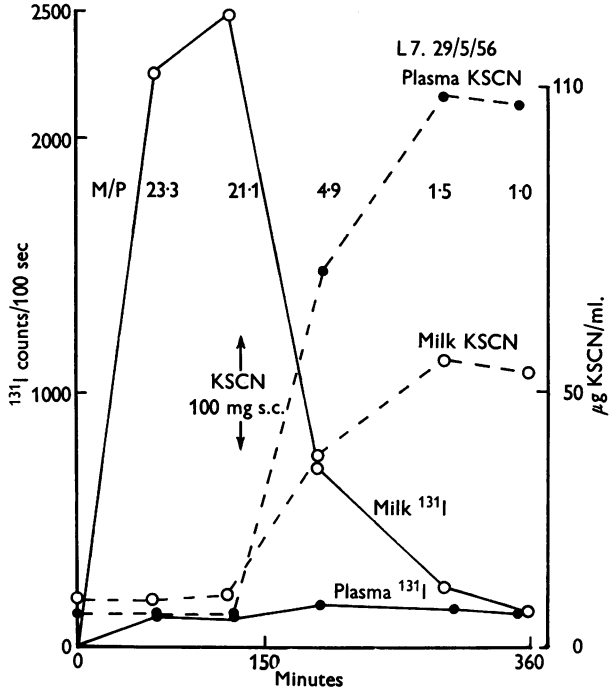

Fig. 2. Plasma and milk radioactivity before and after the injection of 100 mg KSCN: there is a rapid fall in the level of ^{131}I in the milk and a rise in the plasma level; the M/P ratio falls to unity. Milk and plasma thiocyanate levels are also shown; this anion is not concentrated in the milk. O——O, milk ^{131}I; ●——●, plasma ^{131}I; O---O, milk thiocyanate; ●---●, plasma thiocyanate.

the thiouracil-treated rabbit (Brown-Grant & Gibson, 1955). Except where specifically mentioned molar equivalents of the other potassium salts were used in these tests. The M/P ratio was determined for periods up to 4 hr after injection of these salts.

Thiocyanate. Two rabbits were injected with 100 mg of KSCN. There was a rapid and marked fall in the M/P ratio for ^{131}I, which in one case fell to unity. Fig. 2 illustrates one of these experiments and details are given in Table 2. A similar but less marked effect was seen after the injection of 10 mg of KSCN (Expt. 3, Table 2).

Perchlorate, iodide, iodate, bromate and nitrate. The results of experiments in which these anions were administered are given in Table 2, Expts. 4–9. Perchlorate had the most marked effects, a dose equivalent to 12·5 mg of KSCN resulting in a fall in the M/P ratio almost as great as that seen after 100 mg KSCN and considerably greater than that produced by 10 mg of KSCN. Iodide and iodate (2 experiments) reduced the M/P ratio considerably; nitrate and bromate produced small but definite effects. The experiment with KI is shown in Fig. 3.

TABLE 2. Milk/plasma ratios for ^{131}I before and after the injection of the various salts indicated in fifth column: upper figures indicate time and lower figures the M/P ratios as in Table 1. The dose was 100 mg of KSCN and the molar equivalent of other salts except in Expt. 3 (L8) where 10 mg of KSCN was injected, and in Expt. 4 (L13) where $KClO_4$ equivalent to 12·5 mg KSCN was injected

		Serial milk/plasma ratios							
Expt. no.	Rabbit no.	1	2	Time; and compound injected	3	4	5	6	7
1	L6	60	215	220	280	320	—	—	—
		24·9	24·4	KSCN	7·7	3·5	—	—	—
2	L7	60	120	130	190	290	360	—	—
		23·3	21·1	KSCN	4·9	1·5	1·0	—	—
3	L8	50	95	100	175	240	320	—	—
		27·9	23·7	KSCN	15·3	9·5	10·3	—	—
4	L13	45	90	95	150	225	290	335	—
		11·8	12·6	$KClO_4$	6·9	3·6	3·1	3·5	—
5	L11	55	105	110	150	225	295	—	—
		25·4	22·4	KI	5·0	1·5	1·5	—	—
6	L9	45	105	110	165	225	—	—	—
		15·8	16·7	KIO_3	11·8	8·2	—	—	—
7	L21	45	90	95	135	205	265	315	360
		37·4	36·1	KIO_3	29·8	12·1	5·7	3·6	2·9
8	L10	45	100	105	150	210	285	345	—
		26·4	22·0	KNO_3	20·1	19·3	16·3	13·3	—
9	L20	45	90	100	135	195	255	315	—
		17·8	15·6	$KBrO_3$	15·5	14·6	13·1	12·9	—
10	L14	45	90	95	135	180	255	305	60
		8·4	13·2	KBr	18·2	21·2	24·1	24·0	21·1
11	L12	45	95	100	160	205	280	—	—
		16·8	17·8	KCl	16·8	18·2	16·8	—	—
12	L19	45	105	115	155	220	280	330	—
		22·0	22·2	NaCl	20·5	25·2	28·7	26·1	—

Bromide and chloride. Three experiments were performed in which KBr, KCl and NaCl were injected. There were no significant effects on the M/P ratio (Expts. 10–12 of Table 2), although as illustrated in Fig. 4 there was a slight rise in the chloride content of the milk following KCl or NaCl administration.

The changes in the level of plasma radioactivity over the period of these studies are of interest. In the control experiments, as after bromide or chloride, there was a gradual fall from the peak value seen at 45 min (Figs. 1, 4). In contrast, after the administration of anions effective in reducing the M/P

ratio there was a definite rise in the plasma ^{131}I level (Figs. 2, 3). This is probably the result of the simultaneous inhibition of the salivary and gastric secretion of radio-iodine and the reabsorption of ^{131}I from the small intestine. Similar changes have been described in the rat by Brown (1956).

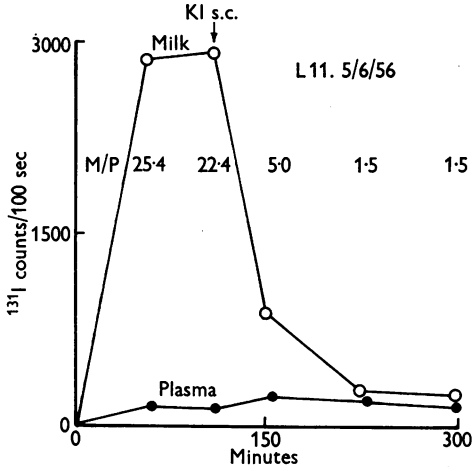

Fig. 3. The effect of KI (M-equiv of 100 mg KSCN) on the M/P ratio for ^{131}I in the rabbit: plasma ^{131}I levels rise as after KSCN administration (Fig. 2): ●, plasma; ○, milk.

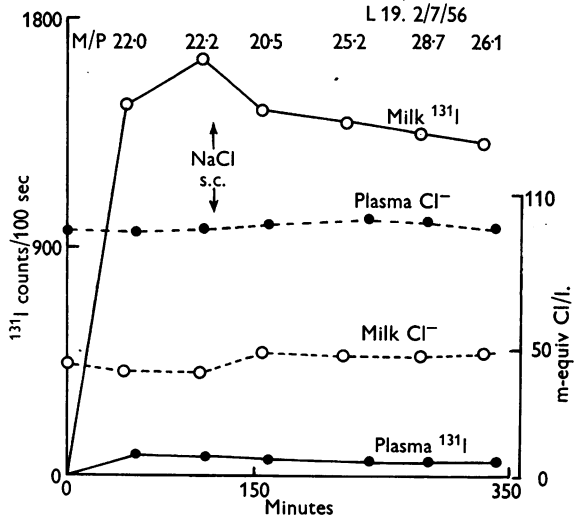

Fig. 4. The injection of NaCl (M-equiv of 100 mg KSCN) has no effect on the M/P ratio for ^{131}I: chloride levels in milk and plasma are also shown. ○——○, milk ^{131}I; ○- - -○, milk chloride; ●——●, plasma ^{131}I; ●- - -●, plasma chloride.

The M/P ratio for thiocyanate in rabbits

Of the various anions known to affect iodide concentrating mechanisms thiocyanate has been studied in the greatest detail as it was the first to be shown to have these effects and can be easily estimated in microgram quantities. Experiments to determine the M/P ratio for thiocyanate in the rabbit have been performed.

Five rabbits were injected with 100 mg of KSCN; in three experiments this replaced the usual ^{131}I injection at 0 min and in the other two the thiocyanate was injected at about 90 min during the course of an experiment on the ^{131}I M/P ratio. A sixth animal received 10 mg of KSCN during a radioiodine experiment. One of the second group of experiments is illustrated in Fig. 2. In no case was the milk level of thiocyanate consistently higher than the plasma after KSCN had been injected. The M/P ratio varied from 0·5 to 1·3 with two aberrant values of 2·3 and 2·4 in one rabbit. These two were probably due to inadequate emptying of the breasts at a time when the plasma level of thiocyanate was falling rapidly. Excluding these, the mean of thirteen determinations at plasma levels of from 8 to 132 μg KSCN/ml. was 0·95 ($\pm$0·11, S.E. of mean).

The M/P ratios for ^{131}I and thiocyanate in other species

The experimental details were described under Methods. Single samples of milk and plasma were obtained 90 min after the subcutaneous injection of ^{131}I (10 μc for guinea-pigs and 5 μc for rats and mice).

Guinea-pigs. The M/P ratios, determined as above, were 7·2 and 5·7 for two normal animals and 2·2 and 1·6 for two animals pretreated with 50 mg of KSCN. The M/P ratios for thiocyanate in these two animals were 0·7 and 1·7.

Rats. Two normal rats gave M/P ratios for ^{131}I of 23·9 and 21·0; two animals pretreated with 10 mg KSCN gave 2·1 and 2·5, the M/P ratio for thiocyanate being 0·3 and 0·4.

Mice. Pooled samples from a group of three normal mice gave an M/P ratio for ^{131}I of 3·7; a group of three, each pretreated with 20 mg of KSCN, gave 0·2 and an M/P ratio for thiocyanate of 0·2. The low values for the M/P ratios obtained in mice may be related to the fact that the experiments were not begun for some 3 hr after the animals had suckled their young and a significant amount of unlabelled milk may have been present in the glands at the time the experiments were begun.

The chemical state of the ^{131}I in rabbit milk

Two procedures were used to investigate the nature of the radioactive compounds in milk and in homogenates of breast tissue in distilled water. The protein-bound radioactivity was determined by precipitation with 5 vol. of

10% (w/v) trichloroacetic acid (TCA) followed by centrifuging and two further washings with TCA. The precipitate was dissolved in 2 N-NaOH and counted, as was the pooled supernatant fluid in many cases. When radio-iodide was added to milk or homogenate *in vitro* and allowed to stand at room temperature for 1–2 hr, it was found that an average of 1·6% (mean of 5 expts. on milk) and 0·2% (2 expts. on homogenates) was present in the precipitate. Twelve samples of milk collected from six rabbits gave an average of 4·4% TCA-precipitable activity; homogenates from four animals that had received ^{131}I contained an average of 8·5% of TCA-precipitable radio-iodine. In comparison, the figures for duplicate samples from a rabbit treated with methylthiouracil and ^{131}I were 1·2% for milk and 1·1% for homogenate.

Known volumes of milk and homogenate from animals which had been injected with ^{131}I and others to which radio-iodine had been added *in vitro* were dialysed in 'Visking' membrane (Viscase Ltd.) against running tap water for 15–18 hr. It was found that of the radio-iodine added *in vitro* to milk and homogenate 99·7% (3 expts.) and 99·8% (2 expts.) respectively was dialysable under these conditions. The corresponding *in vivo* figures were 85·3% (3 expts.) and 86·2% (3 expts.) for normal rabbits, but 99·4 and 100% for samples from a methylthiouracil-treated animal.

It appears that a small and variable but significant proportion of the radioactivity in the milk and mammary gland tissue of rabbits injected with ^{131}I was present not as iodide but in some form in which it was precipitated, at least in part, with the protein by TCA and was not freely dialysable. This fraction was not found in samples from a rabbit treated with methylthiouracil.

DISCUSSION

The method described allows repeated estimates to be made of the milk/plasma concentration ratio for radio-iodine and other substances in the anaesthetized rabbit. The values obtained for ^{131}I ranged from 6·1 to 37·4 in untreated animals, but did not vary greatly over periods of from 3 to 6 hr in any one animal under the conditions of these experiments. The establishment of a high M/P ratio for ^{131}I was not affected by large doses of methylthiouracil. A prompt fall in the ^{131}I content of the milk and a decrease in the M/P ratio was seen after the administration of certain anions. From the data available, the order of effectiveness is $ClO_4^- > SCN^- \geqslant I^- > IO_3^- > NO_3^- > BrO_3^-$. Bromide and chloride had no effect in the doses used in these experiments. These findings are in good agreement with the results on the relative potencies of these anions obtained by workers who studied other iodide concentrating mechanisms such as those of the thyroid (Wyngaarden, Wright & Ways, 1952; Wyngaarden, Stanbury & Rapp, 1953) and of the salivary gland (Rowlands, Edwards & Honour, 1953; Edwards, Fletcher & Rowlands, 1954). As Wyngaarden *et al.*

(1952) point out, however, no common mechanism of action for these anions can, or should be, assumed on the basis of results such as these.

The existence of an iodide concentrating mechanism in the mammary glands of lactating guinea-pigs, rats and mice has been demonstrated. In these species, too, large doses of thiocyanate greatly reduced the M/P ratio. The findings in the rat are of particular interest as from other work it appears that certain extra-thyroid iodide trapping mechanisms are not present in this animal. Logothetopoulos & Scott (1956) could not demonstrate a concentration of iodide across the placenta in rats such as they found in guinea-pigs and rabbits, though from the effect of thiocyanate they postulate that some form of active iodide transport mechanism may be present. Halmi *et al.* (1956) found no concentration of ^{131}I in the salivary glands, and Fletcher, Honour & Rowlands (1956) state that there is no concentration in saliva or salivary glands of the rat. Brown (1956) implies in his paper that there is significant secretion of iodide in the saliva and that this is reduced by thiocyanate, but presents no direct or conclusive evidence for the presence of a raised ^{131}I content of saliva. However, the findings in the present work suggest that the mammary glands in the rat are comparable in this respect with those of the other species so far studied. This conclusion is supported by the findings of Freinkel & Ingbar (1956) that ^{131}I is concentrated, as iodide, by surviving slices of rat mammary gland and that this gradient is abolished by thiocyanate and perchlorate. It should be noted, however, that Taurog, Potter, Tong & Chaikoff (1956) do not consider that their data from similar *in vitro* preparations indicate any significant concentration.

The distribution of large doses of thiocyanate has been studied in some detail in the rabbit and to a limited extent in other animals. The results indicate that inhibition of the iodide concentrating mechanism takes place without any comparable concentration of thiocyanate in the milk. In this respect the iodide concentrating mechanism of the mammary gland seems to resemble more closely the thyroid 'iodide trap' than does that of the salivary gland. While it is generally held that no significant concentration of thiocyanate occurs in the thyroid (Wood & Williams, 1949; Wood & Kingsland, 1950; Vanderlaan & Storrie, 1955; see, however, Baumann & Metzger, 1949) there is very clear evidence that thiocyanate is concentrated by the salivary gland and appears to *replace* iodide in the saliva, at least in man (Edwards *et al.* 1954). The fact that thiocyanate is not concentrated in the milk does not, of course, rule out a concentration in the mammary tissue itself. Estimates of the thiocyanate content of mammary homogenates were not performed because of erratic values for the recovery of thiocyanate added *in vitro*. The amount of thiocyanate normally present in rabbit plasma and milk is at the lower limit for reliable estimation by the method used in the present study. However, several determinations were made of the M/P ratio for thiocyanate in uninjected

animals and the results indicated that the milk levels were consistently higher than that of plasma. A mean ratio was found of $1{\cdot}7 \pm 0{\cdot}2$ (S.E. of mean of 7 expts.). This seems unlikely to be the result of technical errors; the reason for the high value is obscure, but a similar disparity between the distribution of endogenous and added thiocyanate has been observed in the case of the salivary glands of mice by Fletcher *et al.* (1956).

The main portion of the radio-iodine present in rabbit milk or gland homogenate behaved as iodide; a small but significant amount, not seen after methylthiouracil treatment, was present in some other form. The finding that this fraction is higher in homogenates than in the milk suggests that the biochemical processes involved were taking place in the gland tissue cells rather than in the milk after secretion. Similar findings have been reported from *in vitro* studies with rat mammary glands (Freinkel & Ingbar, 1956; Taurog *et al.* 1956). Taurog and his co-workers found the main product to be mono-iodotyrosine with traces of other compounds. The formation of organic iodine compounds by the mammary gland deserves further study.

The physiological significance of the iodide concentrating mechanism of the mammary gland is unknown. In so far as the reaction to various chemical inhibitors is concerned it resembles quite closely the thyroid 'iodide trap' and further, this tissue seems to have at least some capacity to iodinate amino acids. Any analogy that might be drawn between this, or other extrathyroidal iodide concentrating mechanisms, and the thyroid itself would be greatly strengthened if it could be shown that any of them was influenced by naturally occurring hormones known to act on the thyroid, in addition to responding to chemical agents with known effects. It is perhaps significant that so far no positive response to a hormonal stimulus has been reported.

SUMMARY

1. The milk/plasma ratio for ^{131}I has been studied for periods up to 6 hr after injection in anaesthetized rabbits and values between 6·7 and 37·4 were found. Methylthiouracil had no effect on the establishment of a high M/P ratio.

2. The M/P ratio was depressed by various anions, the order of potency being: $ClO_4^- > SCN^- \geqslant I^- > IO_3^- > NO_3^- > BrO_3^-$. Bromide and chloride had no effect.

3. A concentration gradient for ^{131}I between milk and plasma was also found in guinea-pigs, rats and mice and was shown to be reduced by thiocyanate.

4. No evidence was obtained for the concentration of thiocyanate in the milk during inhibition of iodide concentration by this anion.

5. A small proportion (up to 5%) of the radio-iodine found in rabbit's milk appears to be in organic combination. This fraction is not seen after methylthiouracil administration.

6. The iodide metabolism of the mammary gland is discussed and compared with that of the thyroid and other extra-thyroidal iodide concentrating tissues.

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