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THE METABOLISM OF IODIDE BY THE THYROID GLAND AND BY THE UTERUS DURING EARLY PREGNANCY IN THE RAT

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The activity of the thyroid gland varies during the oestrus cycle in the rat and the peak of activity seen at oestrus may be related to a discharge of thyroid-stimulating hormone (TSH) in association with the discharge of luteinizing hormone (LH) during late pro-oestrus that leads to ovulation (Brown-Grant, 1962, 1963). During pregnancy or pseudopregnancy spontaneous ovulation does not occur, but there is some evidence that periodic ripening of follicles may continue (Swezy & Evans, 1930) and ovulation can be induced by the administration of oestrogen (Everett, 1947; Sawyer, Everett & Markee, 1949). More recently much attention has been directed towards changes in the ovary and in the anterior pituitary in the very early stages of pregnancy and pseudopregnancy. Briefly, it has been shown that oestrogen, as well as progesterone, is necessary for the implantation of the blastocyst or the formation of deciduomata in the ovariectomized pregnant or pseudopregnant rat. In the intact rat, it is suggested that oestrogen is secreted by the ovary on Day 3 of pregnancy apparently in response to the secretion of LH by a pituitary in contact with a normal central nervous system between Days 2 and 3 of pregnancy. This release of LH occurs 24 hr before the expected LH release that would result in ovulation in an unmated rat showing a 4-day oestrus cycle (Mayer, 1959, 1963; Alloiteau, 1961; Nutting & Meyer, 1963; Psychoyos, 1963; Shelesnyak & Kraicer, 1963). The evidence for this sequence of events is not, however, accepted by all workers in the field (Yochim & De Feo, 1963; Zeilmaker, 1963). It seemed to be of interest to know whether this postulated surge of LH secretion was associated with any increase in TSH secretion as appears to be the case during the oestrus cycle. Various indices of thyroid activity were therefore measured in early pregnancy and pseudopregnancy. In addition, observations were made on some transient changes in the distribution of radio-iodide in the genital tract.

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METHODS

Adult virgin females from a closed colony of Wistar rats were used at 3–5 months of age and weighing between 180 and 230 g. The rats were fed on a pellet diet (Diet 41 B, obtained from E. Dixon & Sons, Ltd., Ware, Hertfordshire) and tap water *ad lib.* The diet is said to contain 117 μg/kg iodine. Temperature was controlled between 72 and 80° F and the lighting was on from 8.00 a.m. to 10.00 p.m. and was controlled by an automatic time switch. Vaginal smears were taken daily between 9 and 10 a.m. for at least 12 days before any experiment was carried out. Only animals showing a regular 4-day cycle (more than 95% of rats examined) were used. Females were placed with normal or vasectomized males in the evening of the day of pro-oestrus and removed the next morning. Mating was confirmed by the finding of sperms in the vaginal smear or the presence of copulation plugs; this day is Day 0 of pregnancy or pseudopregnancy and the day of the first smear with leucocytes is Day 1. Results obtained by other workers that are referred to in this paper have been altered to conform with this system of timing where necessary.

Thyroid gland activity was studied with radioactive iodine (^{131}I) in three ways. The uptake by the gland 2½ hr after the injection of ^{131}I, the thyroid-serum (T/S ratio) for ^{131}I in animals pre-treated with methylthiouracil, and the rate of release of ^{131}I from the gland *in vivo* were determined as previously described (Brown-Grant, 1962), except that a scintillation counter instead of a Geiger–Müller tube was used for measurements *in vivo*. The release curve counts were made in the afternoon; the decrease, after correction for isotope decay, was expressed as the percentage of thyroidal ^{131}I released per day for each animal and the results for all animals averaged. The uterus-plasma (U/P) and the oviduct-plasma (O/P) ratios for ^{131}I were determined 2 or 2½ hr after the injection of radio-iodide. Tissue samples were trimmed, blotted and weighed to 0·1 mg after expressing any obvious luminal fluid in the case of the uterus. All samples weighed less than 500 mg. The ^{131}I content was determined by counting in a well-type, NaI crystal scintillation counter of constant efficiency for tissue samples between 2 and 500 mg weight and the ratio calculated as: counts/100 sec/100 mg tissue divided by counts/100 sec/0·1 ml. plasma. The thyroid-serum ratios were in fact thyroid-plasma ratios also, but the term T/S ratio is retained for this organ as this is now the conventional expression used in the published literature. In the experiments on mice, tissue-blood instead of tissue-plasma ratios for ^{131}I were determined.

Statistical comparisons were made by means of the *t* test or by analysis of variance using Snedecor's *F* ratio (variance ratio). Not significant indicates a value for $P > 0{\cdot}05$. Values quoted in the text and in tables are group means $\pm$S.E. of the mean.

RESULTS

Changes in thyroid gland activity during early pregnancy

Groups of twelve to twenty-two animals were used and, in each experiment, the uptake of ^{131}I by the thyroid was determined in animals at different stages of the cycle and of pregnancy. Analysis of variance showed that there was a just-significant difference ($P = 0{\cdot}05$) between experiments but that the differences between stages of the oestrus cycle and between pregnant and non-pregnant animals were highly significant ($P < 0{\cdot}001$). The results from all experiments were combined and the values obtained from 104 rats are shown in Table 1. During the cycle uptake was highest at oestrus as expected; values for Day 0 of pregnancy and for oestrus did not differ significantly nor did values for Day 1 and

metoestrus or Day 2 and dioestrus or Day 3 and pro-oestrus. On Day 4, however, the values were below those expected if the rats had not been pregnant ($P < 0{\cdot}001$). There was no evidence of increase in uptake between Days 2 and 3 or between Days 3 and 4 of pregnancy. The mean value for animals tested on Days 1 to 4 of pregnancy ($5{\cdot}61 \pm 0{\cdot}27$) was significantly lower ($P < 0{\cdot}02$) than the mean value for non-pregnant animals ($6{\cdot}99 \pm 0{\cdot}39$) as was the mean value for animals on Days 5 to 8 of pregnancy ($5{\cdot}34 \pm 0{\cdot}27$, $P < 0{\cdot}01$).

TABLE 1. The uptake of ^{131}I (percentage of injected dose) during the oestrus cycle and in pregnant and pseudopregnant rats. Stages of the cycle are indicated as OE, MO, DO and PrO in subsequent tables. Values are mean ± S.E. of the mean

Stage	Oestrus	Met-oestrus	Di-oestrus	Pro-oestrus	Day 0	Day 1	Day 2
% dose	9·17	6·44	5·28	5·49	7·28	7·30	6·09
S.E.M.	0·57	0·60	0·56	0·50	0·58	0·65	0·46
Number	16	8	9	10	7	4	7

Stage	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
% dose	4·71	5·39	5·09	4·94	6·25	5·75
S.E.M.	0·55	0·25	0·41	0·49	0·87	0·44
Number	9	9	10	7	4	4

Pseudopregnancy

Stage	Day 2	Day 3	Day 4	Day 5	Day 6
% dose	4·45	4·84	4·07	4·55	4·06
S.E.M.	0·44	0·32	0·19	0·37	0·40
Number	4	8	4	4	4

TABLE 2. Thyroid-serum (T/S) ratio for ^{131}I during the oestrus cycle and in pregnancy

Stage	Oe	MO	DO	PrO	Day 0	Day 1
T/S	123·1	68·1	74·4	64·9	97·9	95·4
S.E.M.	18·6	7·8	7·2	15·4	10·1	7·9
Number	7	5	5	5	4	4

Stage	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
T/S	58·1	76·6	75·1	55·1	77·2	69·0
S.E.M.	6·6	11·0	8·9	5·9	10·2	12·1
Number	6	6	6	4	4	4

Similar experiments were carried out in which the T/S ratio for radio-iodide was determined and the results obtained are shown in Table 2. The general pattern is the same as that of the uptake measurements. The highest values were obtained from animals in the oestrus phase of the cycle. The mean value for oestrus rats that had mated the night before (Day 0 animals) was rather lower than that for oestrus animals but the difference was not significant. The mean value on Day 4 of pregnancy (day

of expected oestrus) was much less than the oestrus value and there was no evidence of a significant increase between Days 2 and 3 or between Days 3 and 4. The general trend was towards a fall in T/S ratio during early pregnancy and the mean value for Days 2 to 7 (68·8 ± 4·0) was significantly lower ($P < 0.05$) than the mean value during the normal cycle (86·3 ± 7·8).

Values for the rate of release of ^{131}I from the gland *in vivo* during the cycle and in early pregnancy are given in Table 3. Release rates were highest at oestrus and decreased during pregnancy. The rate of release was not significantly higher on Day 3 than on Day 2 or on Day 4 than on Day 3.

TABLE 3. The rate of release (percentage gland content/day) of ^{131}I from the thyroid gland during the oestrus cycle and early pregnancy in the rat. Values are mean ± S.E.M. of results from six rats

Stage	Oestrus	Metoestrus	Dioestrus	Pro-oestrus	Day 0	Day 1
% release	10·3	5·9	5·9	10·7	10·2	6·3
S.E.M.	1·6	1·4	1·9	2·1	1·5	1·8
Stage	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
% release	5·2	6·7	6·8	4·1	4·5	10·3
S.E.M.	1·3	1·9	2·0	1·1	2·0	1·7
Stage	Day 8	Day 9	Day 10	Day 11	Day 12	
% release	4·9	4·1	5·2	3·0	4·4	
S.E.M.	0·8	0·8	1·7	1·5	1·2	
Stage	Cycle	Days 1–4	Days 5–8	Days 9–12		
% release	8·2	6·2	6·0	4·2		
S.E.M.	0·7	0·8	0·8	0·7		
P for difference from cycle values	—	N.S.	= 0·05	< 0·001		

Changes in thyroid gland activity during pseudopregnancy

The uptake of ^{131}I by the gland was measured in twenty-four rats on Days 2, 3, 4, 5 and 6 of pseudopregnancy induced by mating with vasectomized males (Table 1). As in pregnant animals, there was no evidence of any increase in uptake between Days 2 and 3 or between Days 3 and 4. The mean value (4·46 ± 0·16) was significantly lower ($P < 0.001$) than the mean value for non-pregnant rats. The rate of release of ^{131}I from the gland was determined in seven rats during the oestrus cycle. The animals were then mated with vasectomized males and measurements continued throughout pseudopregnancy and until the animals returned to normal cycles. The duration of pseudopregnancy in three animals was 10 days, in three animals 11 days, and in one animal 12 days. Some animals showed a typical sequence of pro-oestrus, oestrus, and metoestrus smears at the

TABLE 4. The rate of release of ^{131}I from the thyroid gland (percentage gland content/day) in seven female rats before, during and after pseudopregnancy. Values are mean ± S.E. of the mean. The number of observations made at that stage of the cycle or of pseudopregnancy is also given

Stage	Oe	MO	DiO	PrO	Day 0	1	2	3	4	5	6
% release	15·3	6·3	10·6	13·8	11·8	4·7	9·2	9·3	7·5	7·7	3·5
± S.E.M.	1·4	1·5	1·2	0·9	1·4	2·3	2·0	2·0	1·8	1·7	1·2
No. of observations	8	8	9	10	7	7	7	7	7	7	7

Stage	7	8	9	10	11 + 12	PrO + Oe	MO	DiO	PrO	Oe
% release	6·9	7·3	5·2	7·5	3·8	11·7	1·4	4·0	7·9	12·6
± S.E.M.	1·4	1·8	2·1	2·0	2·0	1·8	0·9	1·8	1·6	1·9
No. of observations	7	7	7	7	5	14	7	5	5	5

end of pseudopregnancy while others showed two pro-oestrus smears followed by a metoestrus smear and others 2 days of cornified smears followed by a metoestrus smear. All values for days on which the rats showed pro-oestrus or oestrus smears at the end of pseudopregnancy have been combined in Table 4. The values during the cycle are as expected; analysis of variance showed no significant difference between rats but a highly significant difference ($P < 0{\cdot}001$) between stages of the cycle. The value for the period of sterile mating (Day 0) ($11{\cdot}8 \pm 1{\cdot}4$) is less than those previously obtained during oestrus ($15{\cdot}3 \pm 1{\cdot}4$) but the difference is not significant. Values for Days 1 to 12 of pseudopregnancy are lower than during the normal cycle ($6{\cdot}7 \pm 0{\cdot}6$ as compared with $11{\cdot}6 \pm 0{\cdot}8$, $P < 0{\cdot}001$). There was no evidence of any increase in release rate between Days 2 and 3 or between Days 3 and 4 of pseudopregnancy. The increase at the end of pseudopregnancy (values for pro-oestrus plus oestrus are significantly higher ($P < 0{\cdot}01$) than values during pseudopregnancy) is presumably related to the recurrence of ovulation at the time and is seen again at the next ovulation (Table 4).

Concentration of iodide by the uterus during pregnancy

The first indication that the level of thyroid gland activity was reduced during early pregnancy in the rat was obtained from experiments in which the $2\frac{1}{2}$ hr uptake of ^{131}I by the gland was measured. This index of thyroid activity, taken alone, may be misleading if there are changes in the extra-thyroidal distribution of iodide. Variations in the renal excretion of iodide, diversion to lactating the mammary gland or to the foetus in late pregnancy, for example, may result in decreases in thyroidal ^{131}I uptake independently of alterations in pituitary TSH secretion (Brown-Grant, 1961). It seemed possible, as at this stage of the investigation no measurements of T/S ratio or release rates had been made, that some such change might be responsible for the decreased thyroidal uptake in early pregnancy. One possibility was that iodide was being diverted to the uterus where considerable changes are occurring at this time. Portions of uterus were removed when rats were killed for thyroid uptake measurements and their ^{131}I content measured and compared with that of plasma and the percentage of the injected dose of ^{131}I contained in the uterus calculated. The ^{131}I of the uterus, as percentage of dose, was at no time during the first third of pregnancy sufficient to account for the decrease in thyroidal ^{131}I uptake that was observed. It was soon obvious, however, that the uterus-plasma (U/P) ratio for ^{131}I was considerably above unity on Days 3 and 4 of pregnancy whereas during the cycle it was consistently below one. The changes in U/P ratio were later examined in more detail. Information was obtained in two types of experiments: when thyroidal ^{131}I uptake was also

measured, the animals were killed 2½ hr after ^{131}I injection and when the T/S ratio was determined the animals were killed 2 hr after ^{131}I injection, having received methylthiouracil 45 min before the ^{131}I. In both types of experiment three samples of uterus were obtained: an upper third (oviduct end), a middle, and a lower third including the cervix. The U/P ratios for each sample were calculated separately and then averaged to obtain a mean value for each rat. Particularly in pregnant animals, the values for the upper third (oviduct end) were in general somewhat higher than those for middle and lower thirds. The values obtained in different experiments and values for animals with and without thiouracil pre-treatment were not significantly different and are combined in Table 5. The period during which the uterus showed a greatly raised iodide concentration relative to plasma is limited to Days 3 and 4 of pregnancy. The U/P ratio for Day 5 (0·80 ± 0·03) does, however, differ significantly ($P <$ 0·01) from the mean value (0·64 ± 0·03) obtained during the oestrous cycle.

TABLE 5. Uterus-plasma (U/P) ratios for radio-iodide during the oestrus cycle and in pregnancy

Stage	Oestrus	Met-oestrus	Di-oestrus	Pro-oestrus	Day 0	Day 1	Day 2
U/P ratio	0·58	0·64	0·65	0·74	0·61	0·57	0·64
S.E.M.	0·02	0·02	0·02	0·09	0·02	0·03	0·02
Number	12	8	7	9	5	5	9

Stage	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
U/P ratio	4·79	2·39	0·80	0·68	0·66	0·65
S.E.M.	0·46	0·26	0·03	0·02	0·02	0·02
Number	13	9	9	7	8	4

Various additional experiments were performed to determine the origin of the raised ^{131}I concentration in the uterus during Days 3 and 4. First, the fact that similar values were obtained in rats with and without methylthiouracil treatment appeared to exclude the possibility that the accumulation of ^{131}I was the result of organic binding of iodine. Further evidence that the radioactivity was present as iodide in free equilibrium with the blood was obtained in experiments in which rats on Days 3 and 4 of pregnancy were injected with $KClO_4$ (2 mg/100 g body weight intraperitoneally) 1½ hr after the injection of ^{131}I and killed 1 hr later. The mean U/P ratio in four rats treated in this way was 0·59 ± 0·01 as compared with a mean value of 2·08 ± 0·17 in five rats injected with saline solution. The difference was highly significant ($P < 0·001$). Perchlorate at the same dose level injected 20 min before ^{131}I also prevented the establishment of a high U/P ratio for ^{131}I; five control rats gave a value of 2·04 ± 0·22 and four rats pre-treated with perchlorate a value of 0·60 ± 0·05 ($P < 0·001$).

These results suggested that the raised ^{131}I concentration could have resulted from an active transport of iodide from the blood by the uterus. It was necessary, however, to exclude the possibility that the iodide was reaching the uterus by some other route to give rise to the high U/P values observed. One possibility considered was that urine of high ^{131}I content was in some way reaching the uterus; the finding that the upper third of the uterus generally showed a higher U/P ratio than the cervical third was evidence against this hypothesis, but in turn raised the possibility that the activity was reaching the uterus as an iodide-rich secretion by the oviduct. Such a source for iodide would be consistent with the restriction of the period of high ^{131}I content to Days 3 and 4, as fertilized eggs are thought to enter the uterus on Day 3 (thus allowing free access of oviductal secretion) and implantation to occur late on Day 4 (Noyes, Dickmann, Doyle & Gates, 1963), after which the rapid growth of the blastocyst would prevent the free spread of oviductal fluid along the uterine lumen. The hypothesis of an origin from the oviduct was strengthened by the finding that the oviduct/plasma (O/P) ratio for ^{131}I was between 3 and 5 at this stage of pregnancy. These two possibilities were excluded, however, by a series of experiments carried out on four rats on Days 3 and 4 of pregnancy in which under ether anaesthesia ligatures were tied around the uterus at different levels, care being taken to avoid damage to the blood vessels as far as possible. The abdominal incision was closed and the rats allowed to recover from the anaesthetic. Twenty minutes later ^{131}I was injected and the animals killed $2\frac{1}{2}$ hr later. The U/P ratios for individual portions of uterus were determined and the results are shown in Fig. 1. Values for the U/P ratio greater than unity were obtained from portions of uterus isolated from the oviduct or from the vagina or from both.

The high U/P ratios observed appeared to be the result of active iodide transport by the uterus itself. The beginning of the period of pregnancy at which these results were obtained corresponded to the time of entry of the eggs into the uterus and the end to the time when implantation had begun. Results obtained in pseudopregnant rats (see next section) excluded the possibility that the effect observed was obligatorily related to the presence of *fertilized* eggs in the uterus, but it was possible that the presence of eggs or of some substance released by them or secreted by the oviduct was necessary for the change to occur. Three rats were taken on the morning of Day 0 of pregnancy and under ether anaesthesia ligatures were tied round the uteri as shown in Fig. 2. On Day 3 they were injected with ^{131}I and killed $2\frac{1}{2}$ hr later. The U/P ratios for ^{131}I obtained are shown in Fig. 2. Continuity of oviduct and uterine lumen after 12.00 on Day 0 of pregnancy is not necessary for the appearance of a raised U/P ratio for ^{131}I on Day 3.

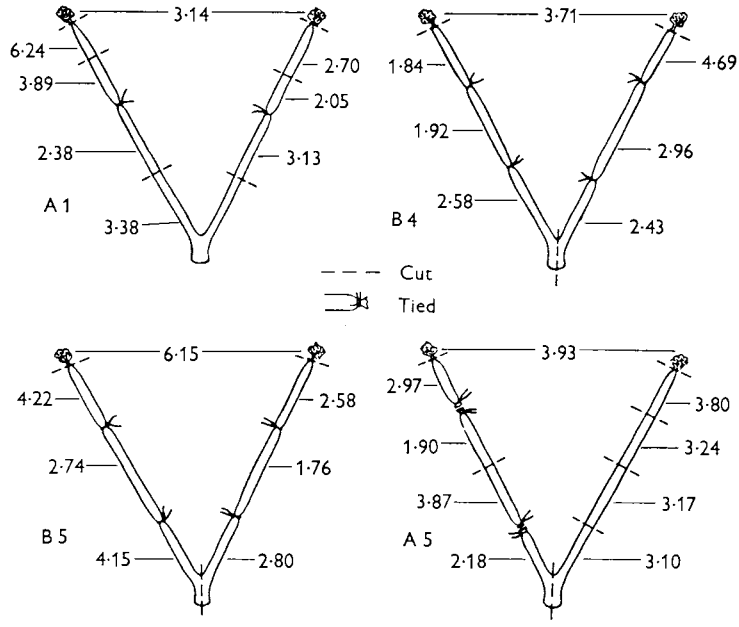

Fig. 1. Uterus-plasma and oviduct-plasma ratios for ^{131}I in rats injected on Days 3 or 4 of pregnancy. Ligatures were placed in position 20–30 min before the injection of radio-iodide.

Uterus-plasma ratios for ^{131}I in pseudopregnant rats

The U/P ratio was determined as described previously in twenty-four rats on Days 2, 3, 4, 5 and 6 of pseudopregnancy, and the results are shown in Table 6. The duration and magnitude of the changes are quite similar to those obtained in pregnant animals (Table 5). The U/P ratio on Day 5 (0·83 ± 0·05) is significantly ($P < 0·05$) different from the mean values in non-pregnant animals (0·64 ± 0·03).

The effect of perchlorate on U/P ratios for ^{131}I in non-pregnant rats

Although the U/P ratio for ^{131}I is low in rats that are not pregnant, it is possible that some portion of the organ is capable of concentrating iodide and is active, but that the proportion of this element relative to the mass of muscle and other tissue is so low in these uteri that this is not evident from an examination of the ^{131}I-concentration ratio for the whole organ. However, if such activity were present then pre-treatment with perchlorate might produce some evidence for this in that the U/P ratio might change. The U/P ratio was determined in sixteen rats (four at each stage of the oestrous cycle) that were injected with $KClO_4$ (2 mg/100 g body weight) intraperitoneally 20 min before the injection of ^{131}I. The mean U/P value

obtained (0·57 ± 0·02) was not significantly different from that obtained in non-pregnant animals without perchlorate treatment (0·64 ± 0·03).

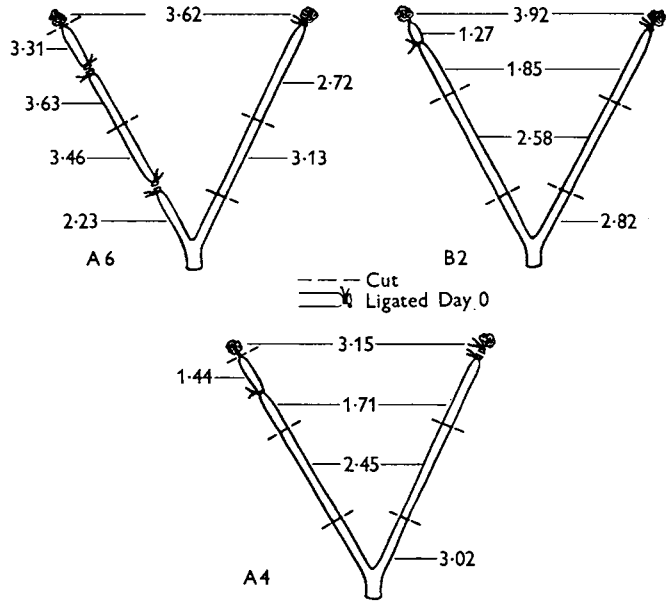

Fig. 2. Uterus-plasma and oviduct-plasma ratios for ^{131}I in rats injected on Day 3 of pregnancy. The ligatures were placed in position at about 11.00 a.m. on Day 0 of pregnancy.

TABLE 6. The uterus/plasma ratios for ^{131}I at different stages of pseudopregnancy in the rat

Day of pseudo-pregnancy	2	3		4	5	6
U/P ratio	0·60	4·08	3·03	3·40	0·93	0·67
	0·67	4·44	4·36	2·82	0·83	0·76
	0·61	5·71	2·74	3·26	0·89	0·64
	0·69	3·04	10·03	4·02	0·66	0·64
Mean	0·64	4·68		3·38	0·83	0·68

Oviduct-plasma ratios for ^{131}I in rats

Experiments in which ligatures were tied around the uterus showed that the high U/P ratios observed on Days 3 and 4 of pregnancy were not due to the secretion of iodide into the uterus by the oviduct. The finding that the O/P ratio could be greater than unity suggested that the oviduct might be capable of active transport of iodide. The O/P ratio was determined at different stages of the oestrous cycle and during pregnancy and pseudo-pregnancy to see if there were any changes under different conditions of stimulation by ovarian hormones. Analysis of variance of the results are given in Table 8. Uptake by the thyroid was highest on Day 0; there

obtained in a series of experiments showed that there were no significant differences between experiments, between pregnant and pseudopregnant animals, or between animals with and without methylthiouracil. The results obtained are shown in Table 7. During the oestrus cycle, the highest values were seen in the dioestrus stage and analysis of variance showed that differences between stages were highly significant ($P < 0{\cdot}01$). During pregnancy and pseudopregnancy the values for Days 1 and 2 were similar to those seen during metoestrus and dioestrus and values for Days 3 and 4 were even higher. The values on Days 5, 6 and 7 remained elevated in contrast to the U/P ratio values, but were somewhat lower than those for Days 3 and 4. Pre-treatment with $KClO_4$ reduces the O/P ratio for animals killed in metoestrus, dioestrus or pro-oestrus or during pregnancy to or below the level observed in oestrus animals (Table 7).

TABLE 7. Oviduct-plasma (O/P) ratios for ^{131}I during the oestrus cycle and during pregnancy. Perchlorate-treated rats received 2 mg $KClO_4$/100 g body weight intraperitoneally 20 min before the injection of ^{131}I. P values refer to t test of perchlorate-treated rats versus appropriate control group

Stage	Oe	MO	DiO	PrO	Day 0	Day 1
O/P ratio	0·79	1·37	2·86	0·95	0·71	1·51
S.E.M.	0·06	0·06	0·29	0·10	0·03	0·10
Number	4	5	6	5	2	5
Stage	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
O/P ratio	3·42	4·42	3·58	2·37	3·28	3·03
S.E.M.	0·22	0·32	0·75	0·27	0·39	0·37
Number	10	15	4	6	7	6
			Perchlorate-treated			
Stage	Oe	MO	DiO	PrO	Day 3	
O/P ratio	0·53	0·51	0·60	0·48	0·64	
S.E.M.	0·08	0·04	0·05	0·03	0·04	
Number	4	4	4	4	4	
P	$> 0{\cdot}05$	$< 0{\cdot}001$	$< 0{\cdot}001$	$< 0{\cdot}01$	$< 0{\cdot}001$	

Changes during early pregnancy in the mouse

Adult female albino mice of the Parkes strain were housed in groups of eight to twelve for 3–4 weeks before experiments were begun. Under these conditions the mice do not show regular oestrus cycles, but when males are introduced into the cages there is a peak of matings on the third night after their introduction. The day when a copulation plug was found was Day 0 and animals were injected with ^{131}I on Day 0 and Days 1 to 5 of pregnancy. The uptake of ^{131}I by the thyroid gland, the uterus-blood ratio and oviduct-blood ratio for ^{131}I were determined when the animals were killed $2\frac{1}{2}$ hr after injection. Upper, middle and lower thirds of the uterus were counted separately and the mean U/B ratio calculated as for rats. The value for the upper third was usually the highest. The results obtained

TABLE 8. The uptake of ^{131}I by the thyroid gland of the mouse, the uterus-blood ratio and the oviduct-blood ratio for ^{131}I during early pregnancy. Figures in brackets indicate number of animals studied. Values are mean ± S.E. of the mean

	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Thyroidal ^{131}I uptake (% dose)	9·37 ± 1·20 (7)	7·29 ± 1·13 (5)	6·29 ± 0·92 (12)	7·39 ± 0·52 (7)	7·99 ± 0·55 (7)	7·50 ± 0·69 (8)
Uterus-blood ratio	0·85 ± 0·05 (13)	0·92 ± 0·11 (5)	1·40 ± 0·11 (12)	1·28 ± 0·09 (7)	1·06 ± 0·10 (8)	0·88 ± 0·07 (8)
Oviduct-blood ratio	0·53 ± 0·06 (6)	1·48 ± 0·64 (4)	1·66 ± 0·30 (10)	1·70 ± 0·34 (6)	0·86 ± 0·28 (3)	1·23 ± 0·21 (5)

were no striking differences between Days 1 to 5 and though the mean uptake for pregnant mice (7·17 ± 0·30) was significantly less than the value on the day of mating ($P < 0·02$) the only single day that was significantly different was Day 2 ($P < 0·05$). The changes in uterus-blood ratios were not as marked as for the U/P ratios in rats, but the values observed on Days 2 and 3 were significantly different from Day 0 ($P < 0·01$ and $< 0·02$). Day 4 was just significantly different from Day 0 ($P < 0·05$). Oviduct-blood ratios were low on Day 0 and high later in pregnancy. Differences from Day 0 values were significant except in the cases of animals killed on Days 1 and 4 of pregnancy. The U/B and O/B ratios were not as high as those observed in rats, but the effect of perchlorate (2 mg $KClO_4$/100 g body weight 20 min before the injection of ^{131}I) provided additional evidence for the active transport of iodide on Day 2. The U/B and O/B ratios were determined for six control and six perchlorate-injected mice on Day 0 and six control and six injected animals on Day 2. Control and perchlorate-injected mice gave U/B values on Day 0 of 0·77 ± 0·05 and 0·61 ± 0·04 ($P > 0·05$) and of 1·21 ± 0·25 and 0·61 ± 0·17 ($P < 0·001$) on Day 2. The O/B values on Day 0 were 0·53 ± 0·06 and 0·44 ± 0·05 and on Day 2 1·31 ± 0·34 and 0·66 ± 0·22. The differences in O/B values were not statistically significant.

DISCUSSION

This study was undertaken in order to see whether a detailed examination of the level of thyroid gland activity during early pregnancy in the rat would provide any evidence of an increase in pituitary TSH secretion between Days 2 and 3 coincident with the period of increased LH secretion that some workers hold to be responsible for a necessary 'surge' of oestrogen secretion that precedes implantation in this species. No evidence was obtained, from a study of the thyroid uptake of ^{131}I, variations in the thyroid-serum concentration ratio for radio-iodide or the rate of release of ^{131}I from the thyroid gland, that any such increase in TSH secretion occurs between Days 2 and 3 of pregnancy or pseudopregnancy. If such a critical period of increased LH secretion occurs, then the mechanisms involved do not, in contrast to what seems to happen during the oestrous cycle (Brown-Grant, 1962, 1963), also cause a release of TSH from the pituitary. The general trend of all three indices of thyroid activity over the first 8 days of pregnancy was towards a decrease as compared with the mean level in rats showing regular ovarian cycles. Thyroid function during early pregnancy in the rat has not been studied in detail before, but previous workers, who have in the main studied the changes in the second and final thirds of pregnancy, have also observed a slight decrease in thyroid activity (Feldman, 1958; Iino & Greer, 1961). This decrease has

now been shown to begin very early in pregnancy. Changes in pseudopregnancy do not appear to have been studied before; the general trend, as judged by measurements of ^{131}I-release rates, is a depression throughout the 10–12 days of pseudopregnancy with a marked increase at the time of the first ovulation that occurs thereafter. Current views on the changes that occur in thyroid gland activity during pregnancy are perhaps unduly influenced by the well documented evidence that in the human the activity of the gland increases (Freedberg, Hamolsky & Freedberg, 1957). This change may be peculiar to the human. The pregnant macaque monkey shows a similar rise in the level of hormonal iodine in the plasma and in the level of thyroxine-binding globulin, but no apparent change in thyroid gland activity (Dowling, Hutchinson, Hindle & Kleeman, 1961). In the sheep, thyroid gland activity, the thyroxine-binding capacity of the serum and thyroxine metabolism are unchanged (Henneman, Reinecke & Griffin, 1955; Annison & Lewis, 1959; Robertson & Falconer, 1961) and no marked changes in thyroid activity are seen in goats (Flamboe & Reinecke, 1959) or in rabbits (Brown-Grant, 1956). The observation that relaxin, which is believed to be secreted during pregnancy in many species (Zarrow, 1961), may stimulate certain aspects of thyroid gland activity in some rodents (Plunkett, Squires & Richardson, 1960) led to the suggestion (Plunkett, Squires & Heagy, 1963) that this hormone might be concerned in the activation of the thyroid during pregnancy. In view of the fact that in many species thyroid function is unaltered or even depressed during pregnancy this hypothesis does not appear to be supported by the known facts.

The significance of the changes in the ^{131}I-concentration ratio of the uterus during early pregnancy and pseudopregnancy is unknown. It could be that they represent some important change that is related to the process by which the endometrium becomes capable of receiving the blastocyst or able to form deciduomata in response to a suitable stimulus. On the other hand, it may be that the changes in ^{131}I-concentration ratio are an epiphenomenon essentially unrelated to these processes. The discussion by Lutwak-Mann (1963) of the history of the carbonic anhydrase theory of blastocyst implantation indicates the need for caution in any consideration of these possibilities. Preliminary experiments (see note added in proof) have failed to demonstrate any deleterious effect on pregnancy of potassium perchlorate in large doses by mouth in either rats or mice. The temporal duration of the changes in the U/P ratio for ^{131}I is of some interest; in rats they cover precisely the time during which De Feo (1963) has shown that the uterus is sensitive to scratch stimulation of deciduoma formation and the time at which implantation normally occurs (Noyes *et al.* 1963). Further, Noyes *et al.*

(1963) present results obtained by transfer of ova in rats which show that transferred ova only implant when placed in the recipient uterus on Days 2, 3 or 4 of pseudopregnancy, results on Days 3 and 4 being much better than on Day 2. The changes in the uterus-blood ratio for ^{131}I in the mouse are less striking than in the rat. Their significance with regard to the process of implantation is equally problematical. It is of interest, however, that they appear to occur a day earlier in the mouse; fertilized eggs enter the uterus on Day 2 in the mouse as opposed to Day 3 in the rat (Noyes *et al.* 1963). The sensitive period for eliciting deciduoma formation does not appear to have been established in as much detail as for the rat. Noyes *et al.* (1963) state that implantation normally occurs on Day 3 and their results with transplanted ova show that the highest percentage of implantations is obtained when eggs are transferred on Days 2 and 3, the days when the U/B ratios for ^{131}I are highest in this species.

The concentration of iodide by the uterus is of considerable theoretical interest with regard to the active transport of iodide. Many instances of non-thyroidal iodide concentrating mechanisms have been described (Brown-Grant, 1961), but if, as seems possible, this phenomenon in the uterus can be shown to be the result of the action of a particular combination of ovarian hormones, it will be the first example of hormonal control of any of these of non-thyroidal iodide transport mechanisms apart from the peculiar effects (described by Brown-Grant & Taylor, 1963 and others) of hormonal deprivation in apparently stimulating iodide concentration by the submandibular glands of mice.

The changes in oviduct-plasma concentration ratios for ^{131}I and the effects of perchlorate administration suggest that active transport of iodide may occur in this organ in rats in response to changes in the secretion of ovarian steroids. Active transport of iodide by the oviduct of birds has been described previously (see Brown-Grant, 1961 for references), but in a comprehensive review Blandau (1961) makes no reference to any previous studies with iodide in mammals. Observations, mainly morphological and histochemical, on possible variations in the oviduct during the ovarian cycle and in pregnancy are described in detail in this review. Apparently changes in the rat oviduct during the cycle have not been detected previously; the evidence obtained in the present study suggests that iodide concentrating ability may vary during the oestrus cycle.

There have been few published studies of the distribution and metabolism of iodide or other halides in the uterus in the last 10 years although workers with quite diverse interests have reported observations on this topic. Von Kaulla, Aikawa, Bruns, Wikle & Drose (1957) reported a high concentration of radio-iodide in human cervical mucus, though not in myo- or endometrium, after the intravenous injection of ^{131}I and more

recently Loo, Burger & Adamson (1963) have reported the bromination of phthalein dyes by the uterus of the pregnant spiny dogfish, *Squalus acanthias*.

SUMMARY

1. Thyroidal uptake of radio-iodide, thyroid-serum ratios for ^{131}I and the rate of release of ^{131}I from the thyroid have been measured during early pregnancy and pseudopregnancy in the rat.

2. No evidence was obtained of any increase in the secretion of pituitary TSH between Days 2 and 3 of pregnancy at the time of the postulated LH release concerned with implantation or of a persistence during pregnancy of the periodic increase in TSH release associated with cyclic ovulation. Thyroid activity is moderately depressed during early pregnancy and pseudopregnancy.

3. Concentration of iodide by the uterus has been demonstrated during Days 3 and 4 of pregnancy and pseudopregnancy in rats and Days 2 and 3 of pregnancy of mice.

4. The oviduct of rats is able to concentrate iodide. This is detectable during the metoestrus and dioestrus stages of the cycle and during early pregnancy or pseudopregnancy.

5. The possible significance of iodide concentration by the uterus and oviduct is discussed.

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Note added in proof. The effects of oral administration of potassium perchlorate throughout the period of early pregnancy, during which active transport of iodide by the uterus can be detected, has been studied in mice and rats. Mice were given tap-water, 0·2% (wt./vol.) $KClO_4$ in tap-water, 0·2% $KClO_4$ plus 0·2% KI in tap-water or a saturated solution of 4-methyl 2-thiouracil in tap-water to drink from the morning of Day 1 to the morning of Day 6 of pregnancy. Delivery of a live litter, duration of pregnancy and numbers of young were recorded. Seven of 9 control mice littered after a mean gestation period of 19·0 days and had a mean litter size of 6·7. Values for perchlorate treated mice were 7 out of 9 littering, 19·0 days and 8·3 young; for perchlorate plus iodide-treated mice, 5 out of 8, 18·8 days and 7·2 young and for thiouracil-treated mice 8 out of 8, 19·1 days and 4·9 young. No effect of the treatments on pregnancy was observed, except for a reduction in litter size in the thiouracil-treated females.

In the experiments on rats, control animals received 0·25% (wt./vol.)

KCl solution and treated animals 0·25% (wt./vol.) $KClO_4$ in tap-water from the morning of Day 2 until the morning of Day 8 of pregnancy. Both groups received tap-water before and after this period. The fluid intake was measured and the mean intake (mg of salt per rat per day) over the experimental period calculated. Pregnancy was successful in 15 of 18 control rats, mean duration 22·1 days, mean litter size 9·5 and mean KCl intake 83 mg/rat/day. Pregnancy was successful in 13 of 17 rats treated with perchlorate, mean gestation period 22·0 days, mean litter size 8·2 and mean $KClO_4$ intake from Day 2 to Day 8 69 mg/rat/day. No evidence was obtained that perchlorate treatment prevented or delayed implantation in these rats. The possibility that this level of $KClO_4$ intake by mouth was not adequate to block iodide concentration by the uterus was tested. Rats were given 0·25% KCl or 0·25% $KClO_4$ to drink from the morning of Day 2 of pregnancy. On the morning of Day 3 they were injected with thiouracil and 45 min later with ^{131}I. Two hours later, plasma, thyroid, uterus and oviduct samples were obtained as usual and also a sample of fore-stomach. Tissue-plasma ^{131}I concentration ratios were determined. The values obtained for 5 control and 6 perchlorate treated rats (control values given first) were: thyroid $54·0 \pm 6·0$, $0·56 \pm 0·06$; oviduct $2·67 \pm 0·33$, $0·44 \pm 0·04$; uterus $4·78 \pm 1·00$, $0·54 \pm 0·04$; stomach $5·85 \pm 1·31$, $0·49 \pm 0·04$. All differences were highly significant ($p < 0·001$). Mean $KClO_4$ intake was 90 mg/rat/day compared with a mean intake of 81 mg/rat/day over the same period in the chronic experiments and 69 mg/rat/day over the Day 2 to Day 8 period.

Administration of perchlorate by mouth has, under the conditions of these experiments, been shown not to interfere with implantation in mice or rats and this suggests that concentration of iodide in the uterus is not essential for implantation in these species.

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