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THE SITES OF IODIDE CONCENTRATION IN THE OVIDUCT AND THE UTERUS OF THE RAT

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SUMMARY

Previous studies have shown that under the influence of progesterone the concentrations of radioiodide in the uterus and oviduct of the rat are maintained at levels higher than that in the plasma. In the present experiments the uterus and oviducts from rats killed 2 h after the injection of $Na^{125}I$ were autoradiographed by a technique which permits the localization of diffusible radioactive material. In intact non-pregnant rats and ovariectomized rats not injected with progesterone, uniformly low grain densities were observed over sections of oviduct and uterus with the exception of the epithelium and lumen of the oviduct where some increase in grain density was observed. In intact and ovariectomized rats treated with progesterone and in rats killed on Day 3 or 4 of pregnancy, grain densities over the epithelium and lumen of the oviduct were very high but the fimbria of the oviduct were consistently unlabelled. The stroma underlying the oviduct epithelium was also labelled. In the uteri of these animals the principal site of concentration of radioiodide was the luminal epithelium, but for technical reasons it was not possible to exclude the stroma immediately adjacent to the luminal epithelium as a less active site of concentration of iodide. No other site in the uterus concentrated radioiodide. The luminal epithelium occupies less than 3% of the volume of the uterus in ovariectomized rats: if this tissue is taken as the sole site of iodide concentration in the uterus, the levels reached in these cells must be at least a hundred times that of the plasma when the overall uterus:plasma concentration ratio for radioiodide is 4 or more.

INTRODUCTION

Two hours after the injection of radioactive iodide into intact rats, the uterus: plasma concentration ratio for radioiodide (U:P ratio, calculated as radioactivity/g uterus divided by radioactivity/ml plasma) is about 0·6–0·8 except on Days 3 and 4 of pregnancy when the U:P ratio is greater than unity and may be as high as 10·0. The non-pregnant rat has a slightly higher iodide concentration ratio in the oviduct (O:P ratio) than in the uterus: this ratio also increases during pregnancy on Days 3 and 4 and remains high for several days thereafter (Brown-Grant, 1965). In subsequent experiments it was established that the development of this iodide-concentrating ability is a response of the oviduct and uterus to progesterone. This response can be seen in ovariectomized rats given a single injection of progesterone alone and the administration of oestrogen to such animals will inhibit the development of the iodide concentrating mechanism (Brown-Grant, 1966*a*). Under standardized conditions there is a linear relationship between the logarithm of the dose of progesterone and the iodide concentration ratio for the uterus (Brown-Grant, 1967). This response provides the opportunity to investigate the site and mechanism of an action of progesterone on the oviduct and uterus of the rat. The uterus of the ovariectomized rat is metabolically relatively inactive, providing a low and stable baseline against which to measure effects caused by progesterone while the time interval between progesterone administration and the first detectable concentration of iodide is only 8 h.

In the present paper autoradiographic experiments to determine the precise sites within the oviduct and uterus of the rat at which iodide concentration occurs are presented. A preliminary account of some of these experiments has already been published (Brown-Grant & Rogers, 1967).

Materials and Methods

Adult female Wistar rats weighing 170–220 g were used. Six rats were killed on Day 3 or Day 4 of pregnancy (Day 0 is the day on which sperm were found in the vaginal smear). A further 17 were ovariectomized under tribromoethyl alcohol (Avertin, Bayer) anaesthesia: to minimize damage to the oviducts, no ligatures were applied: these animals were used 10–21 days later. Non-pregnant rats were given a single s.c. injection of 2·5 mg progesterone (Organon Laboratories Ltd.) in arachis oil, or oil alone at the dioestrous stage of the cycle, and radioactive iodide 22 h later. Ovariectomized rats also received oil alone or progesterone followed by ^{125}I.

All animals received a single i.m. injection of $Na^{125}I$ (Radiochemical Centre Ltd., Amersham) at doses ranging from 50 to 250 μCi, and were killed by exsanguination under ether anaesthesia 2 h later. The ^{125}I-content of weighed samples of uterus and oviduct and of a known volume of plasma were determined in a well-type NaI crystal scintillation counter. The U:P and O:P ratios were calculated from these values. Some rats received a single s.c. injection of 2 mg $KClO_4$/100 g body weight 1 h after the ^{125}I and 1 h before killing.

Autoradiographs were prepared by a method described by Rogers (1967) and based on the cryostat technique of Appleton (1964). Small fragments of uterus and oviduct were rapidly frozen in isopentane cooled in liquid nitrogen. They were then sectioned at 4 μm in a cryostat at -24 °C. The cryostat was a Pearse-type (S.L.E.E. Ltd) modified as described by Rogers & John (1969). The sections were collected on slides which had been coated with a layer of Ilford G5 emulsion to a thickness of 3–4 μm by a dipping technique. The cutting and collection of the sections took place under safelighting (S 902 filter, Ilford) and the emulsion-coated slides had previously been allowed to reach thermal equilibrium within the cryostat. After collection the sections on the frozen emulsion layers were exposed for 1–3 weeks in light-tight boxes at -79 °C in the early experiments and at -40 °C in later ones. After exposure the slides were allowed to thaw and dry at room temperature. Histological fixation in 4% formaldehyde in phosphate buffer (pH 7·4) was followed by development in ID-19 diluted with an equal volume of distilled water at 20 °C for 5 min, a 1% acetic acid stop bath, and photographic fixation in 30% (w/v) sodium thiosulphate solution for 8 min. The sections were stained in Harris's haematoxylin. In this way, by holding the tissue frozen from immersion in isopentane up to the end of autoradiographic exposure, diffusion of the radioactive iodide is kept to a minimum. Control slides to exclude the possibility of positive and negative chemography (Rogers & John, 1969) were exposed with each experiment. No chemographic artifacts were encountered.

To measure the relative volume of different tissues of the uterus two cryostat sections of uterus from four separate animals were examined at a magnification of ×48, with a grid of 100 squares in one eyepiece. The tissue under each of the intersections of the grid was recorded. From these results the relative volumes occupied by luminal and glandular epithelia, lumen, stroma of the lamina propria, and muscle coat were calculated by the methods described by Weibel & Gomez (1962).

RESULTS

Iodide distribution in the absence of progesterone

Tissues from four oil-injected control rats (two intact, two ovariectomized) were examined. The U:P ratios for ^{125}I together with the O:P ratios, where available, are listed in Table 1. The values for these control animals all fell within the range expected from previous experiments.

Autoradiographs from the uteri of these rats showed a uniform, low random distribution of silver grains over the tissue (Pl. 1, figs 1 and 2). No tissue or area seemed to be labelled above the general level. The grain densities over the tissue were, however, higher than in emulsion away from the section. This distribution of silver grains was consistent with a fairly uniform distribution of radioiodide throughout the intra- and extracellular compartments.

In the oviduct a similar low labelling was seen in general over each section but higher grain densities were observed over the luminal epithelium and over the lumen itself. There was an impression from some sections that this region of higher grain density extended to the stromal cells immediately beneath the epithelium. The grain densities increased progressively from the fimbria, which were labelled only at background levels, up to the uterine end of the oviduct.

Iodide distribution in the uterus under the influence of progesterone

The U:P ratios of the animals studied are listed in Table 1. They all fell within the range found in previous experiments and were all greater than unity.

The pattern of distribution of iodide within the uterus was identical in all the animals in this group, whether ovariectomized or intact, pregnant or non-pregnant. A uniform low level of labelling, similar to that seen in both intact and ovariectomized control animals, was found over all the tissues of the uterus with the exception of the luminal epithelium which was very heavily labelled (Pl. 1, fig. 3). It was difficult in most sections to differentiate between grain densities over the luminal epithelium itself and those over the very narrow lumen. However, in the few sections where the lumen was wider than usual the grain densities were lower over the lume than over the epithelium, suggesting that a considerable proportion of the silve grains over the lumen itself may have been due to crossfire from the adjacen epithelium (Pl. 2, fig. 4). Grain densities did not fall off abruptly to the level of th general tissue background at the base of the luminal epithelial cells, but graduall over 20–100 μm (Pl. 2, fig. 5). The absence of labelling over glandular epithelium wa a constant finding.

Table 1. *Uterus:plasma (U:P) concentration ratios of [^{125}I]iodide in the rats used i these experiments, together with oviduct:plasma (O:P) ratios where available*

Treatment	U:P ratio	O:P ratio
Ovariectomized control	0·70	1·87
Ovariectomized control	0·79	1·81
Intact control	0·80	—
Intact control	0·85	1·45
Ovariectomized, progesterone-treated	3·89	6·50
Ovariectomized, progesterone-treated	2·20	4·15
Ovariectomized, progesterone-treated	2·59	3·24
Intact, progesterone-treated	3·52	3·18
Intact, progesterone-treated	4·76	3·17
Intact, progesterone-treated	3·44	—
Day 3 of pregnancy	4·18	4·66
Day 4 of pregnancy	2·45	4·75
Day 4 of pregnancy	2·85	—
Day 4 of pregnancy	2·14	—
Day 4 of pregnancy	5·44	—
Day 4 of pregnancy	8·35	—
Intact control plus perchlorate	0·71	0·82
Ovariectomized control plus perchlorate	0·79	0·73
Ovariectomized, progesterone plus perchlorate	0·86	0·77
Day 3 of pregnancy, plus perchlorate	0·69	0·86

Iodide distribution in the oviduct under the influence of progesterone

Considering the ovariectomized animals first, the grain densities over sections oviduct followed the general pattern described for both intact and ovariectomiz control animals but were very much higher over the epithelium and the lumen. T fimbria, the muscle coat, and the connective tissues had a random distribution silver grains over them at a density similar to that seen in these tissues in the contr animals. Labelling in the lumen and epithelium increased progressively down t oviduct: less heavy but nonetheless significant labelling was seen over subepitheli stromal cells, particularly in the uterine half of the tube (Pl. 2, fig. 6).

In view of the possibility that ovariectomy had interfered with the blood supp of the oviduct, and hence the pattern of distribution of iodide within it, the aut radiographs of the pregnant and non-pregnant intact rats were examined to see any difference could be found in the grain distributions. No such difference was see The fimbria were not labelled above the general tissue background in the pregna rats while the lumen and epithelium of the oviduct were again increasingly heavi labelled as the uterine end of the oviduct was approached (Pl. 2, fig. 7).

The relative volumes of the tissues comprising the uterus

The uterus was considered to be made up of five compartments: the lumen, the luminal epithelium, the glands, the stroma of the lamina propria, and the muscle coats. Table 2 lists the percentage area of transverse sections of uterus from four rats that was occupied by these five compartments. It can be seen that the lumen itself formed less than 2 % of the total cross-sectional area, and luminal epithelium less than 3 %. Since the rat uterus is very long in relation to its diameter it is reasonable to take these values for the relative volumes occupied by each compartment in the whole uterus.

Table 2. *The relative areas of a cross section of the uterus, expressed as % total area, occupied by various tissue elements in cryostat sections from four ovariectomized rats*

	Rat no.				
Tissue	1	2	3	4	Mean
Luminal epithelium	2·3	3·5	2·7	3·0	2·9
Lumen	0·9	3·0	2·7	0·7	1·8
Glandular epithelium and glandular lumen	4·2	7·9	5·0	3·3	5·2
Stroma of lamina propria	56·4	54·2	43·2	51·3	52·2
Muscle coat	36·2	31·3	46·3	41·7	38·0

DISCUSSION

Validity of the autoradiographic technique

The technical difficulties involved in autoradiographing a labelled ion such as [^{125}I]iodide are considerable and have been discussed at length elsewhere (see, for instance, Rogers, 1967, and Stumpf & Roth, 1969). The method used in these experiments, modified from that of Appleton (1964), has been criticized on the grounds that transient thawing of the section is inevitable at the moment of picking up the section on the frozen emulsion layer – thawing caused by the slight pressure needed to make the section adhere to the emulsion. An alternative technique, with freeze-drying of the cryostat sections before application to the emulsion, has been developed by Stumpf & Roth (1964). In experiments in this laboratory (Rogers & Brown-Grant, 1971) the localization of [^{125}I]iodide in the submaxillary gland of the mouse has been studied by both techniques. The precision of localization achieved was identical in material processed by the two methods, suggesting that no significant translocation of the iodide ion took place as a result of using the Appleton technique.

The resolution achieved in autoradiographs of diffusible material has been claimed to equal that of incorporated material examined by conventional methods of autoradiography (Appleton, 1966). These calculations assume that the tissue section remains at its nominal thickness throughout exposure whereas in fact it is certain to freeze-dry almost completely within the first 24 h. When the consequent decrease in actual section thickness is taken into account, the resolution is found to be slightly poorer than for conventional techniques of autoradiography. If one defines the resolution of an autoradiograph in terms of the decrease in grain density that occurs with increasing distance from the edge of a uniformly labelled disk densities for ^{125}I should fall to half their value in less than 2–3 μm frc the disk with the techniques employed in this experiment. In th mentioned above on [^{125}I]iodide in the submaxillary gland of the mo of this order was obtained. The fall-off in grain density at the basal end epithelial cells of the uterus was different from the pattern predicted c tion that the radioactivity was confined to the epithelial cells. It is poss degree of iodide concentration is taking place in the stroma immediate epithelium (Pl. 2, fig. 5). It is very difficult to exclude diffusion at th block of uterus during freezing as a cause of this gradual fall-off in beneath the epithelium. It is suggestive evidence, however, that in all tl conducted into iodide distribution in the uterus to date, no autoradiog produced which shows an absolutely clear localization to epithelium alc concurrent experiments into iodide distribution in the mouse salivary & Brown-Grant, 1971) much sharper resolution has consistently been c an identical technique, even in the centre of blocks of tissue of comp

Iodide concentration in the uterus

In contrast to the oviduct, no iodide concentration above general t seen in the uteri of either intact or ovariectomized control rats (Pl. 1 this agrees well with the observed U:P ratios (Brown-Grant, 1966*a*, 19 which have been consistently less than unity for ovariectomized rats of progesterone treatment and do not decrease after treatment with chlorate per kg body weight known to inhibit this iodide concentrat (Brown-Grant, 1965). After treatment with progesterone, the iodid mechanism appears to be restricted to the luminal epithelium of t possibly the immediately subjacent stromal cells. Where the lumen is escape crossfire from the adjacent epithelium, labelling over the lum considerably lower than in the epithelium itself (Pl. 2, fig. 4). This with the oviduct where unmistakable labelling is seen within tl restriction of labelling to the epithelium lining the lumen of the uter stromal cells is quite clearcut: even near the lumen, glandular epit unable to concentrate the iodide ion. The concentrations of iodide dif ally between luminal epithelium and other tissues that no attempt ha measure the grain densities. If the general tissue level of radioiodide is give a reasonable grain density, that over the epithelium is too hi counting and is approaching emulsion saturation.

If one assumes that all the iodide in excess of the general tissue level in the luminal epithelium, which forms about 3% of the total uterine ovariectomized rats, the concentration of iodide in these epithelial hundred times the plasma level when the overall U:P ratio is 4·0. The values indeed. The biological significance of this surprising local co iodide is quite unknown. Brown-Grant (1966*a*, *b*) was unable to sl relationship between the presence or absence of a high iodide conce uterus and implantation of the blastocyst.

Iodide concentration in the oviduct

The O:P ratios in control ovariectomized animals were consistently greater than unity, indicating the presence of an iodide-concentrating mechanism in the absence of additional exogenous progesterone, since the O:P ratios in such animals are less than unity if they are treated with perchlorate which blocks iodide concentration (Table 1). It is interesting to note that detectable levels of circulating progesterone, presumably of adrenal origin, can be found in ovariectomized rats (Feder, Resko & Goy, 1968). It is reasonable to assume that these levels, although low, are sufficient to maintain a concentration mechanism for iodide in the oviduct. An increase in circulating progesterone produces a greatly enhanced concentrating mechanism for iodide (Table 1).

The pattern of distribution of labelled iodide in the oviduct was similar in control and progesterone-treated ovariectomized rats. There is clearly no iodide concentrating mechanism in the fimbria, even after the administration of 2·5 mg progesterone. The luminal epithelium appears to achieve progressively higher concentrations of iodide as the uterine end of the oviduct is approached and equally high levels are found in the lumen. The subepithelial stroma also concentrates iodide, though to a lesser degree, in the uterine half of the tube. Brown-Grant (1967) has excluded the possibility that this iodide secreted into the lumen of the oviduct is responsible for the raised iodide levels seen in the uterus, by an experiment involving multiple ligation of the uterus. The effect on the ovum of this high concentration of iodide within the oviduct is not known. Unfortunately no ova were seen in the sections prepared from pregnant rats.

Iodide concentration in the uterus as a model system for studying progesterone action

The uterus of the ovariectomized rat provides a valuable experimental system for studies of the early effects of progesterone. The hormone produces a specific and easily monitored effect, limited to one group of cells in the uterus, and detectable as early as 8 h after the injection of progesterone. Brown-Grant, John & Rogers (1972) studied the rates of incorporation of precursors into RNA and protein in the uterus in the first few hours after progesterone administration in an attempt to define mechanisms linking the arrival of the hormone and its measurable effect – stimulation of iodide concentration. John & Rogers (1972) investigated the distribution of progesterone and that of the synthetic progestogen, megestrol acetate, in the uterus over the time period during which the iodide concentrating mechanism develops.

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DESCRIPTION OF PLATES

PLATE 1

Fig. 1. An autoradiograph of the uterus of an ovariectomized rat killed 2 h after the injection of [^{125}I]iodide: the animal did not receive progesterone. At low magnification, individual silver grains are not resolved. There is no blackening visible over luminal epithelium (LE), glands (G) or stroma (S). (×185.)

Fig. 2. Part of the luminal epithelium from fig. 1 at higher magnification, showing the low grain density over luminal epithelium. This photograph is directly comparable with fig. 5. (×750.)

Fig. 3. An autoradiograph of the uterus of an ovariectomized rat killed 24 h after progesterone injection and 2 h after [^{125}I]iodide injection. Note the very high grain densities over the luminal epithelium (LE). There is no evidence of concentration of radioiodide in glands (G), stroma (S), or muscle coat (M). (×75.)

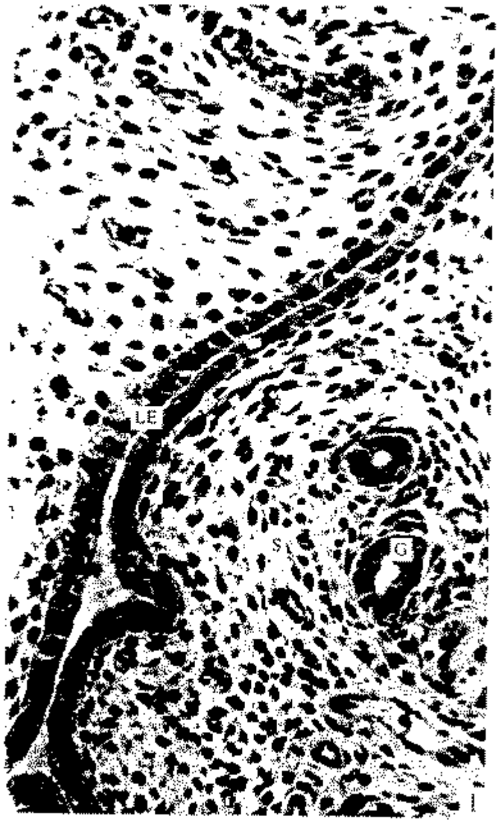
PLATE 2

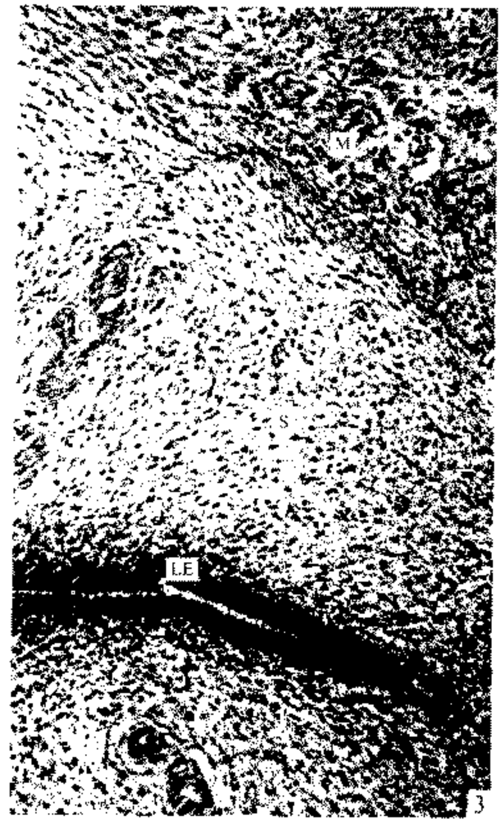
Fig. 4. An autoradiograph of the uterus of an ovariectomized rat killed 24 h after progesterone and 2 h after [^{125}I]iodide injection. This section, taken near the region where the oviduct enters the uterus, shows the relatively low grain density that was seen over the uterine lumen whenever the opposing layers of luminal epithelium were not in contact. (×30.)

Fig. 5. An autoradiograph of the uterus of an ovariectomized rat killed 24 h after progesterone and 2 h after [^{125}I]iodide injection. Note that the high grain density over the luminal epithelium falls off to low levels over the stroma but does so gradually over a relatively large distance. (×750.)

Fig. 6. An autoradiograph of a section through the oviduct of an ovariectomized rat killed 24 h after progesterone injection and 2 h after the injection of [^{125}I]iodide. Note the high grain densities over the lumen (L) and epithelium (LE) extending some way out over the stroma (S), but not involving the muscle coat (M). (×85.)

Fig. 7. An autoradiograph of a section through the oviduct of an intact rat treated with progesterone 24 h before, and with [^{125}I]iodide 2 h before, killing. High grain densities lie over the oviduct but the epithelium of the fimbria (F) is no more heavily labelled than the surrounding connective tissue. (×75.)


LE
S
G
1


M
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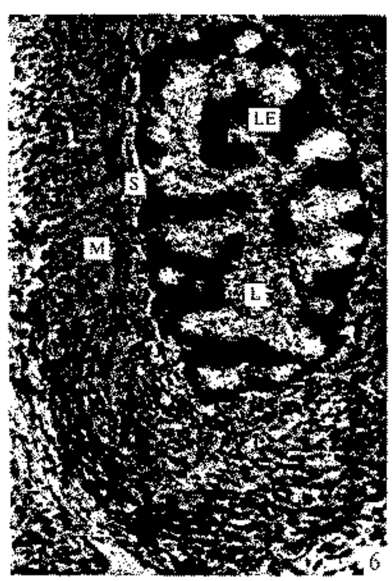

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