DIFFERENTIAL OVARIAN UPTAKE OF $[^{131}I] \text{ALBUMIN}$ INJECTED INTO ONE UTERINE HORN IN THE GUINEA-PIG

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Changes in the permeability of the guinea-pig uterus to $[^{131}I]$albumin, injected into the uterine lumen during the oestrous cycle have been investigated to see whether such changes could be linked to the supposed time of release and action of the uterine lytic factor. Since albumin would presumably be cleared mainly by the uterine lymphatics, the existence of utero-ovarian lymphatic connections might account for the local nature of the lytic effect.

Thirteen virgin guinea-pigs (500 to 950 g) from the Institute’s own colony were used in these experiments. They were anaesthetized with tribromoethanol (‘Avertin’, Bayer Products Ltd) supplemented with ether; ‘Avertin’ abolishes the squirming movements seen when only ether is used. The left carotid artery was cannulated; the cannula (Portex Ltd, i.d. 0·63 mm) was taken through the skin to the back of the neck and the ventral neck incision closed. The cannula was flushed with heparinized saline (100 units/ml 0·9% saline) but no further heparin was given. The uterus was exposed by a mid-ventral abdominal incision, and both Fallopian tubes were ligated near the junction with the uterus. $[^{131}I]$Albumin (H.S.A., The Radiochemical Centre, Amersham), 10 to 20 µCi in 50 µl 0·9% saline was injected into the uterine lumen of the right or left side, near the body of the uterus and the uterine horn was ligated around the point of the needle to prevent loss of isotope. The opposite uterine horn was also ligated, at about the same level. The abdominal incision was closed and the animal was allowed to regain consciousness. Blood samples (0·5 to 1 ml) were taken from the carotid cannula, quickly counted on a ‘Panax’ type A.C. 300/6 thallium-activated sodium iodide crystal detector and returned to the animal. The amount of radioactivity in carotid blood was monitored for 3 to 4 hr. The animals were then killed, the ovaries and uterine horns were removed, blotted on tissue and weighed, and their radioactivity was counted. Samples of other tissues, lung, kidney, mesometrial fat, peritoneal fluid from control and injected sides and the inguinal lymph nodes, were also weighed and counted. The mesometrial fat of the experimental side showed very high activity (often more than $1 \times 10^4$ counts/min/g) but there was little difference in the activity of the peritoneal fluid from injected or control sides (both were usually less than $1 \times 10^3$ counts/min/ml).

In a few instances, the area of mesometrium on the injected side was spread onto X-ray film overnight to prepare simple autoradiograms in an attempt to locate any possible direct utero-ovarian connections. No such connections were
found, but the autoradiograms showed that the radioactivity was not diffuse, but was limited to definite tracts within the mesometrium, presumably corresponding to the lymphatic drainage.

Graphs of arterial radioactivity (counts/min/ml plotted against time after injection) showed that the circulating level of $^{131}$I rose slowly, reaching a plateau 2 to 3 hr after the injection. The levels reached showed considerable individual variation, ranging from $15 \times 10^3$ to $50 \times 10^3$ counts/min/ml. There was no correlation between the stage of the oestrous cycle and the rate of rise or the final blood concentration. Blood samples from two animals at the end of the experiment were centrifuged and 1 ml plasma was dialysed against Krebs-Ringer solution, pH 7-4, overnight. Over 95% of the radioactivity present was contained within the dialysis membrane. This control experiment showed that almost all of the radioactivity in plasma was bound to protein. In two additional experiments, when 5 µCi [$^{131}$I]albumin were injected into the femoral vein, the radioactivity in carotid blood rose rapidly and declined very slowly during the 3- to 4-hr experimental period, and was still 80% of the peak concentration. Arterial radioactivity provides a very indirect measure of uterine permeability, and factors affecting general lymph flow probably masked any cyclical variation.

The radioactivity in the inguinal lymph nodes was never more than twice the background count, which was usually about 250 counts/min. This indicated that, as in preliminary experiments using Evan's blue and Berlin blue-gelatin, the major route taken by the absorbed material was along the lymphatics associated with the utero-ovarian vein. These join the aortic lymphatics near the junction of the renal veins and the inferior vena cava. In unligated uteri, much of the material injected into the uterine lumen is lost by way of the Fallopian tube and the cervix; very little is absorbed through the endometrium. In the present experiments, ligatures were used to ensure that the only means of exit for injected material was through the uterine epithelium. Material might then be expected to follow a similar path to that of a substance secreted by the endometrium.

The quantity of radioactivity lost from the injected uterine horn varied with the stage of the cycle. In four animals from Day 13 onwards (Day 1 = day on which leucocytes first appeared in the vaginal smear), the absorption of injected tracer was $94.0 \pm 2.1%$ (mean ± S.E.), while during the luteal phase, from Days 3 to 12 (nine animals) the absorption was $70.1 \pm 2.4%$. This difference is statistically significant ($P < 0.01$). Albumin injected into tissues is usually cleared by the lymphatic rather than the venous drainage; but surgical experiments in this and other species would seem to implicate the venous drainage of the mesometrium as the route for the lytic factor (Bland & Donovan, 1969; Fischer, 1969; McCraken, Baird & Goding, 1971), though the lymphatics have not been completely excluded. The small late changes demonstrated in these experiments might, therefore, reflect the increasing permeability of the uterine venous drainage to [$^{131}$I]albumin at the time of oestrus. The level of radioactivity in the ovaries ranged from 350 counts/min/ovary (excluding the background count of 150 counts/min) to 3000 counts/min/ovary. In relation to the plasma concentrations, the levels of radioactivity/unit weight of ovarian tissue
were surprisingly high (between 25 and 50\%). Whether the radioactivity was still \(^{131}I\)albumin was not determined. During the mid-luteal phase of the cycle, the concentration of radioactivity was similar in both ovaries (see Text-fig. 1). The paired \(t\) test showed that in the nine animals, from Days 3 to 12 of the cycle, there was no significant difference in ovarian radioactivity. From Day 13 onwards (four animals), the ovary adjacent to the injected uterine horn contained more radioactivity/g of tissue than the contralateral ovary (paired \(t\) test, \(P < 0.05\)).

At the start of these experiments, it seemed that the uterine luteolytic factor might be a protein or protein conjugate (Williams, Johnston, Lauterbach & Fagan, 1967; Schomberg, 1969) of fairly high molecular weight. However, later work has shown the active principle in sheep endometrial extracts to have

![Text-fig. 1. Differential ovarian uptake of \(^{131}I\). Ratio has been calculated as counts/min/g of ipsilateral ovary/counts/min/g of contralateral ovary, where the ipsilateral ovary is the ovary adjacent to the injected uterine horn.](image)

a molecular weight of less than 1500 (Caldwell, Rowson, Moor & Hay, 1969). Recently, much experimental evidence has accumulated that the luteolysin in several species (including the guinea-pig, see Donovan, 1971) is prostaglandin F-2\(\alpha\), which is a fatty acid of relatively small molecular weight.

These experiments demonstrate, at a particular stage in the oestrous cycle, a differential uptake by the ovaries of a substance transported from the uterine lumen through the endometrium. It is evident that one effect of the luteolytic agent is to cause changes in the vascular and/or lymphatic drainage of the uterus such that each ovary is made more readily accessible to secretions from the adjacent uterine horn. This would account for the local action of the luteolytic agent. The adverse effect of the uterus upon the continued growth and function of the corpus luteum is evident earlier in the cycle than the changes in permeability demonstrated in our experiments. However, the tracer used,
[\textsuperscript{131}I]albumin, is very different in nature and molecular weight from prostaglandin F-2\alpha, currently postulated as the lytic factor. These facts may indicate that the changes are progressive and non-specific so that, by the last few days of the cycle, they are of such magnitude as to permit the transmission of substances of relatively high molecular weight.

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REFERENCES


