

Fig.2. Variations de poids d'une larve maintenue à 93% humidité relative pendant 90 h. Les flèches montrent la régularité des phases d'absorption d'eau.

Ces gains de poids qui se produisent de façon cyclique alors que les conditions expérimentales sont invariables, ne peuvent résulter que d'une absorption active de l'eau.

Ainsi, à des degrés d'hygrométrie proches de son point d'équilibre hydrique (93% humidité relative), la larve de *Tinea*, peut absorber de la vapeur d'eau, absorption qui correspond à la reprise hydrique de type spasmodique mentionnée par Beament⁹. Si la lenteur du rythme du

phénomène tend à faire rejeter l'hypothèse d'une liaison avec un rythme respiratoire, elle nous suggère par contre, l'existence d'un processus métabolique ou hormonal cyclique.

Ces résultats posent le problème de la localisation des sites d'absorption d'eau chez cette larve: tégument, paroi rectale ou glandes labiales comme c'est le cas chez divers arthropodes^{10,11}.

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Semen-elicited accumulation of antibodies and leucocytes in the rabbit female tract

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Summary. A marked increase in vascular permeability to proteins, including IgG, was observed in the reproductive tract of both virgin and parous rabbit does following insemination, leading to accumulation of immunoglobulins in the tissues and fluids of the reproductive tract.

Antibodies to spermatozoa, mostly IgG, are found in the serum of virgin and parous female rabbits², and women³, and in rabbit vaginal and uterine fluids. Immunoglobulin levels in these fluids, however, all measured in unmated animals, are generally much lower than those in serum^{4,6}. Large numbers of neutrophil leucocytes enter the tissues and fluids of the female tract of many mammals soon after mating⁷. Within 30 min of insemination in the rabbit, such a reaction is elicited in the vaginal wall⁸ and cervix⁹. It would seem likely that if this leucocytic invasion is accompanied by local changes in vascular permeability, there should be an increased transfer of immunoglobulins from serum into fluids of the female tract.

We have investigated the changes in vascular permeability

and in the local leucocytic response, following unilateral insemination of rabbit does after surgical division (figure 1) of the paired female tracts. This preparation allows a comparison to be made, in the same animal, between the responses of inseminated and control parts of the tracts.

Materials and methods. Vaginal division was carried out upon 4 virgin and 8 parous New Zealand White rabbit does. Injections of semen (0.5 ml containing approximately 10⁸ spermatozoa) into 1 pouch, and an equal volume of phosphate buffered saline (Dulbecco 'A', PBS) into the other, were immediately followed by i.v. administration of Pontamine Sky Blue (500 mg in 10 ml PBS). After 2 h, the animals were killed and the vaginal pouches and uteri were each flushed with 0.5 ml of PBS. Localization of blue dye

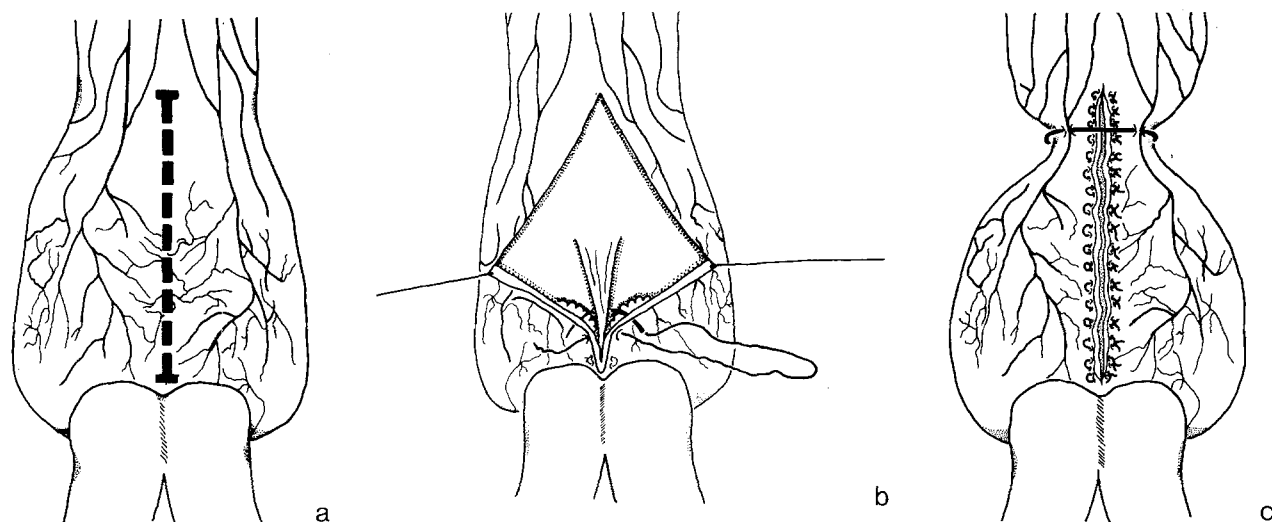


Fig. 1. Surgical division of the vagina. *a* The vagina is exposed and anteverted to reveal the dorsal wall. A midline incision is made after ligation of all vessels crossing the midline, giving complete haemostasis. *b* The cut edges of the vaginal wall are reflected to expose the paired cervixes which are separated anteriorly by a midline septum. Interrupted mattress sutures are placed through vaginal wall-septum-vaginal wall so producing a division between the cervixes. As the suture line continues posteriorly, the septum merges with the ventral wall of the vagina. *c* A ring suture is placed around the vagina avoiding occlusion of blood vessels supplying the anterior vagina and cervixes, to isolate the two vaginal pouches formed. Each pouch will accept up to 2.0 ml of fluid.

in the tissues was qualitatively assessed prior to removal of samples of cervix, vaginal wall and uterus from both sides for histological examination.

In 3 of these animals, 2 virgin and 1 parous, transfer of immunoglobulin into the tissues and fluids, from the bloodstream, was measured using autologous IgG, separated from serum on DEAE-Sephadex¹⁰ and labelled with ¹²⁵I (50 μ Ci/mg) by the method of Hawker¹¹, and reinjected. After collection of fluids from the vaginal pouches and uteri, the animals were killed and the blood was flushed from the vessels of the reproductive tracts by rapid aortic perfusion with PBS after ligation of the femoral arteries. Samples of cervix, vaginal wall and uterus were taken from each side and placed in 10% formol-saline for γ -counting and subsequent histological examination. During dissection of these tissues, it was noticed that the lymphatics draining this region were deeply coloured with the blue dye: lymph was therefore collected from the thoracic duct of 2 animals to measure the content of ¹²⁵I-IgG.

Results and discussion. 10 of the 12 animals examined showed distinctly greater accumulation of blue dye in the cervix and vaginal wall of the inseminated side, ranging from diffuse patches to strong colouration of the entire area, whereas the other 2 animals showed little or no colour difference between the 2 sides. The cervixes of 9 of these 12 animals were examined for leucocytes: 4 showed far larger numbers (1.5 – $6.3 \times 10^4/\text{mm}^3$) in the cervix of the inseminated side than in the control side (4.7 – $11.2 \times 10^2/\text{mm}^3$) and 5, including all 3 virgins examined, showed a similar leucocyte reaction on each side.

γ -Counting of fluid and tissue samples from animals injected with autologous ¹²⁵I-IgG before insemination produced the following results (figure 2). Significantly higher levels of labelled IgG were always found in the vaginal wall samples from inseminated side ($35 \pm 9\%$ of blood levels) than the control ($13 \pm 7\%$), and this difference was also found in the vaginal fluid samples. In 2 of the 3 animals, a significantly greater level of IgG was seen in the cervix and uterus of the inseminated side, though in the 3rd animal no significant difference was seen for either tissue. Furthermore, lymph samples from the thoracic duct of the 2

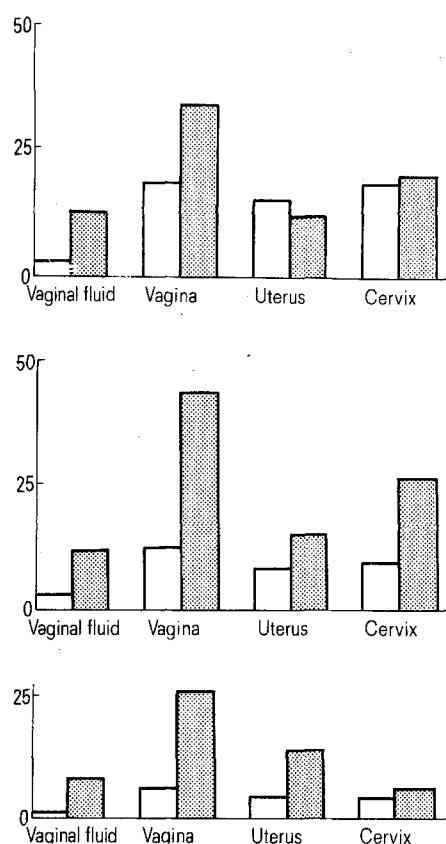


Fig. 2. ¹²⁵I-IgG content of vaginal fluid and tissues in the control (unshaded) and inseminated (shaded) sides of the reproductive tract in three rabbit does. Values are expressed as percentages of blood concentration. Rabbits were injected with 50 μ Ci of ¹²⁵I-labelled autologous IgG i.v. immediately following unilateral insemination. 2 h later, tissue samples were taken after vascular perfusion (to remove blood from vessels), and counted on a NE5505/SR5 (Nuclear Enterprise, Ltd.) system tuned to 0.035 MeV.

animals were shown to contain $70 \pm 4\%$ of blood levels of labelled IgG.

The most obvious advantage of this particular method is that no external control is necessary since all measurements within any experiment are from the same animal. Its success depends upon the creation of a good seal between the 2 sides, with minimal damage to local tissues in the process.

Pontamine Sky Blue has a high molecular weight and binds avidly to serum proteins. It has been widely used to provide a simple qualitative estimate of increased vascular permeability, since it is only released from the bloodstream under these conditions. Our results convincingly demonstrated an increased vascular permeability in the inseminated side relative to the control in both the vagina and cervix of most of the animals studied, although the degree varied considerably between individuals. Blood flow changes were not monitored in these experiments. Measurement of ^{125}I -IgG levels allowed a quantitative assessment of IgG transfer to the tissues from the bloodstream following insemination. In all cases the vaginal tissues and vaginal fluids contained elevated levels of labelled IgG, showing that transfer was actually taking place. It is likely, in view of the surprisingly high level of labelled IgG found in the lymph draining from the area, that these figures represent a dynamic balance between IgG release from the blood system into the tissues and its removal by drainage into the lymphatic system, and that they drastically underestimate the actual amounts of IgG transferred.

The differences found in the vaginal samples seem conclusive, but in the cervical and uterine tissues, where contact with spermatozoa is more restricted, increased permeability and leucocytosis occurred on both sides to a similar degree. This suggests that functional isolation of the cervix may not be complete. Virgin and parous rabbits did not differ

consistently in their responses: both showed increased vascular permeability and leucocyte invasion of the cervix following insemination.

Thus, we have found that the presence of semen in the rabbit female tract triggers a vascular response which drastically modifies the antibody content in the immediate environment of the spermatozoon. Mating with a vasectomized buck or insemination with seminal plasma⁹ (i.e. no spermatozoa) does not elicit a similar response. Considering the present interest in measurement of immunological response of the female tract to spermatozoa, we must emphasize the necessity of also measuring antibody levels in the female tract after mating, since it is these levels that the spermatozoa actually face.

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Adenylate cyclase in the developing rat cerebral cortex and olfactory bulb

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Summary. Activities of adenylate cyclase, measured either in the absence or presence of sodium fluoride and Triton X-100, are determined in cerebral cortex and olfactory bulb homogenate of rats of 1 to 35 days of postnatal age. Differences in properties of the enzyme in the 2 structures are demonstrated.

The involvement of cyclic AMP in the regulation of cell division, growth and differentiation¹⁻³ through a series of metabolic alterations^{4, 5} has been suggested for various tissues. Moreover, in the nervous system, cAMP may play several roles in the control of neuronal function; it has been involved in inhibition of rapid axonal transport of proteins⁶ and in modulation of membrane potential, i.e. in transduction step in the primary olfactory neurones⁷, in initiation of bursts of action potentials⁸ and in variation in duration and depth of inter-burst hyperpolarization⁹. Furthermore, its role in the control of transmitter synthesis and release from nerve endings has been postulated¹⁰. These effects require ion fluxes through the cell membrane; thus the regulation of these fluxes may be one of the most important actions of cyclic nucleotides.

Regulation of intracellular cAMP concentration is determined by a balance between the activities of 2 enzymes: adenylate cyclase, required for synthesis of cyclic nucleotides from ATP, and phosphodiesterase which catalyzes its

hydrolysis to 5'-AMP; it is also influenced by ATPase activity. Adenylate cyclase is a complex enzyme bound to membrane structures; it is formed by physically separate and independent components (receptor, regulatory sites and catalytic units); these components do not develop as a functional unit but may vary independently during maturation of the cell^{11, 12}. Changes of cyclic nucleotide metabolism could provide valuable information about changes of tissue functions. Brain development is largely postnatal in the rat; cellular growth¹³ and enzyme maturation¹⁴ vary considerably among different regions. The present investigation was undertaken in order to compare the ontogenetic evolution of adenylate cyclase in olfactory bulb and cerebral cortex homogenates; olfactory bulb maturation is characterized by active hyperplasia and moderate cell hypertrophy, the reverse being observed in the cerebral cortex¹⁵.

Material and methods. Experiments are conducted on Wistar rats of our inbred laboratory strain. The offspring are