The distribution of the 212 cases arranged according to the presence or absence of the cell-types in a four-dimensional contingency table is shown in Table I. Since the unrestricted sampling corresponds to Model 1 of LANCASTER[11], the analysis of the associations (first-order interactions) between the pairs of cell-types, and of the higher-order interactions were performed according to that method. All the associations between the pairs of cell types (Table II) were highly significant. No second-order interaction was significant, but the third-order interaction (C-O-S-F) was of possible borderline significance. The interpretation of such higher-order interactions is often difficult, but in this instance the largest contributions to the overall χ^2 were from the boxes in which all four cell-types were either present or absent. The excessive numbers observed in these boxes represent a tendency to an all-or-none phenomenon that is reflected in the third-order interaction.

Table I. 2^4 contingency table showing numbers of benign mammary dysplasias with and without colostrum cells and periductal leucocytes

Colostrum cells present (+)						
SRC[a]	absent	(−)		SRC[a]	present	(+)
5	0	+		+	39	20
12	0	−	Ochrocytes	−	35	7
−	+				−	+
Foam cells				Foam cells		
Colostrum cells absent (−)						
SRC[a]	absent	(−)		SRC[a]	present	(+)
0	0	+		+	2	1
44	1	−	Ochrocytes	−	44	2
−	+				−	+
Foam cells				Foam cells		

[a] SRC = periductal small round cells.

Table II. Significances of associations and higher-order interactions (calculated from data in Table I)

Interaction		χ^2 (d.f.)	
Colostrum cells (C)	– Ochrocytes (O)	63.07	(1)[a]
Colostrum cells	– Small round cells (S)	28.31	(1)[a]
Colostrum cells	– Foam cells (F)	14.53	(1)[a]
Ochrocytes	– Small round cells	22.46	(1)[a]
Ochrocytes	– Foam cells	21.93	(1)[a]
Small round cells	– Foam cells	11.89	(1)[a]
2nd-order interactions:	C-O-S	0.02	(1)[c]
	C-O-F	1.24	(1)[c]
	C-S-F	0.54	(1)[c]
	S-O-F	1.79	(1)[c]
3rd-order interaction:	C-O-S-F	6.18	(1)[b]
Total χ^2		171.96	(11)

[a] $p < 0.001$; [b] $p < 0.025$; [c] not significant.

The findings that colostrum cells in non-neoplastic human breast tissue are closely associated with small-round-cell, ochrocytic and foam-cell infiltrates, and that all these cell-types themselves are mutually associated, is not due to second-order interactions. Nevertheless it is possible that the weak third-order interaction may have contributed to the observed first-order associations. Naturally, mere association is no proof of a common histogenetic origin of these cells. However the similar fluorescent and staining characteristics of colostrum cells and ochrocytes[8,12], and the tendency of colostrum cells, ochrocytes and foam cells to show multinucleate forms[8–10] suggest similar functional characteristics. It should be noted that none of these morphological similarities is likely to have produced a selectional bias in the assessment of the presence or absence of the cell-types.

The mutual associations found here, together with the shared morphological characteristics and active phagocytic capacity of human colostrum cells[13], support the view that most cells in all four categories are of macrophage nature. Possibly colostral secretion itself contains an irritant that attracts the cells into its vicinity. The slight tendency to an all-or-none response would accord with reaction to such a stimulus.

Résumé. L'étude de 212 biopsies de la maladie fibrocystique du sein humain montre des associations fortes et mutuelles entre les cellules du colostrum les petites cellules rondes, les ochrocytes et les cellules écumeuses qui se trouvent dans le tissu conjonctif entourant les canaux du sein. Une analyse statistique multidimensionelle ne fait pas apparaître une interaction de second ordre entre les combinaisons des facteurs. Mais il y a une faible interaction de troisième ordre, dont une explication possible est une réponse inflammatoire des quatre types de cellules à un stimulant irritant du colostrum lui-même.

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Uterine Secretion During the Sexual Cycle in the Rat and its Capacity to Disperse Corona Cells in vitro

In recent years, increasing attention has been devoted to the role of the secretions of the genital tract, especially in relation to the fecundation process and particularly concerning the viability and behaviour of the gametes (HOMBURGER and TREGIER[1]) and the implantation of the blastocyte (CLEMENTSON et al.[2]; DICKMAN[3]). Many different kinds of investigation have been undertaken in this field. It has been found that the secretion accumulating in the closed uteri of spayed, oestradiol-primed rats has the ability to disperse the corona cells of the ova in vitro (MEGLIOLI and DESAULLES[4]). Because of the difficulty of collecting enough of this secretion for quantitative analysis and assay, especially from small experimental animals, such as rats and mice, it has proved

Table I. Quantity, viscosity and pH at different stages of the sexual cycle in rat uterine secretion accumulated for 7 days

Stage of sexual cycle based on vaginal smears	No. of animals	Weight of empty uterus (mg)	Weight of uterus secretion (mg)	No. exp./ no. animals	pH of uterine secretion	Viscosity of uterine secretion (cP)
Oestrus	9	269 ± 24	451 ± 57	4/ 9	8.31 ± 0.12	1.71 ± 0.08
Metoestrus	17	206 ± 16[a]	302 ± 40[a]	5/17	8.52 ± 0.04	1.93 ± 0.07
Di-oestrus	10	206 ± 19[a]	327 ± 58	3/10	8.57 ± 0.17	1.92 ± 0.06
Pro-oestrus	15	311 ± 23	420 ± 69	4/15	8.20 ± 0.24	1.83 ± 0.10
Quantity, viscosity and pH at different stages of the sexual cycle in rat uterine secretion accumulated for 14 days						
Oestrus	8	409 ± 41	1092 ± 138	4/ 8	8.01 ± 0.20	2.39 ± 0.20
Metoestrus	8	304 ± 20[a]	701 ± 118[a]	3/ 8	8.28 ± 0.10	2.72 ± 0.35
Di-oestrus	8	289 ± 13[a]	721 ± 117[a]	3/ 8	8.37 ± 0.09	2.66 ± 0.18
Pro-oestrus	8	513 ± 29	1080 ± 153	5/ 8	8.07 ± 0.12	2.42 ± 0.11
Quantity, viscosity and pH at different stages of the sexual cycle in rat uterine secretion accumulated for 21 days						
Oestrus	9	501 ± 41	1694 ± 402	3/ 9	8.20 ± 0.17	2.98 ± 0.39
Metoestrus	15	353 ± 31[b]	839 ± 192[a]	3/15	8.25 ± 0.21	5.40 ± 1.74
Di-oestrus	16	334 ± 15[d]	1196 ± 141	3/16	8.27 ± 0.07	4.48 ± 1.89
Pro-oestrus	9	553 ± 30	1533 ± 230	3/ 9	8.05 ± 0.12	2.82 ± 0.43

Significance (*t*-test): [a]≃ 0.05–0.02; [b]≃ 0.01; [c]≃ 0.001; [d]> 0.001 in relation to oestrus stage.

necessary to close the neck of the uterus, either by ligature (ARMSTRONG[5]; HOMBURGER et al.[1]) or by electrocauterization (MEGLIOLI et al.[6]).

This procedure results in the accumulation of fluid within the uterus, but it is not absolutely certain whether this fluid retains the physiological properties of uterine secretion. In the present study the patterns of the sexual cycle in normal rats were examined after closure of both extremities of the uterus at different stages of the cycle. Some characteristics of the fluid collected and its dispersing property in each phase of the cycle as well as at different times after operation were investigated.

Material and methods. Three series of experiments were performed on intact adult rats of the same breed (Ivanovas, Kisslegg, Germany), weighing approximately 165 g and exhibiting a regular 4-day oestrous cycle.

1. Effect of closure of the uterus on the sexual cycle. Vaginal smears were investigated by the exfoliative cytology technique and stained with carbol-fuchsin and methylene blue to determine the stages of the sexual cycle. The tests were repeated every morning for 2 cycles before operation, and only rats displaying a regular cycle were selected. The animals were then divided into groups for each stage. Laparotomy was performed under ether anaesthesia in the afternoon; the distal ends of the uterine

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Table II. Difference in the surface area of ova with corona cells after incubation for 6 h in uterine secretion from intact rats at each stage of the sexual cycle

Stage of cycle	No. of ova	Initial surface (mm^2)	Surface after 6 h incubation (mm^2)	Absolute increase (mm^2)
In secretion accumulated for 7 days after closure of uterus				
Oestrus	9	1452 ± 84	6233 ± 615	4782 ± 598
Metoestrus	9	1177 ± 103	2084 ± 256	907 ± 191[d]
Dioestrus	9	1166 ± 89	1846 ± 232	680 ± 206[d]
Pro-oestrus	9	1302 ± 118	3769 ± 457	2467 ± 444[b]
In secretion accumulated for 14 days after closure of uterus				
Oestrus	9	1137 ± 77	3977 ± 230	2841 ± 182
Metoestrus	9	1367 ± 91	2466 ± 287	1099 ± 268[d]
Dioestrus	9	1343 ± 81	2006 ± 177	663 ± 121[d]
Pro-oestrus	9	1222 ± 62	3330 ± 238	2108 ± 217[a]
In secretion accumulated for 21 days after closure of uterus				
Oestrus	9	1361 ± 82	3943 ± 332	2582 ± 343
Metoestrus	9	1193 ± 78	2257 ± 223	1063 ± 220[c]
Dioestrus	9	1301 ± 100	1730 ± 118	429 ± 98[d]
Pro-oestrus	9	1105 ± 135	3022 ± 207	1917 ± 171

Significance (*t*-test): [a]≃ 0.05–0.02; [b]≃ 0.01; [c]≃ 0.001; [d]> 0.001 in relation to oestrus stage.

horns were closed by electrocauterization, and the ovarian-uterine junction ligated, care being taken to leave the regional blood supply and the ovaries intact. One group of unoperated rats served as controls. Vaginal smears from both the controls and the operated animals were examined daily at about 08.00 h for 5 consecutive cycles. Smears were taken every 12 h (08.00 and 20.00 h) from half of the control animals to determine the approximate duration of each stage. The duration of the cycle was measured form the day of oestrus to the next oestrus.

2. Uterine secretion in the various stages of the sexual cycle at different times after operation. In this second experiment, uterus ligation by the technique described above was performed in a larger number of rats, which were then divided into 3 groups. The uterine fluid was allowed to accumulate for 7 days in the first group, 14 days in the second and 21 days in the third. At these times the animals were sacrified. Vaginal smears were examined just before autopsy, at 08.00 h and the rats in each group were then classified according to the stage of the sexual cycle. The uteri were removed and the uterine fluid collected and pooled separately according to the phases of the sexual cycle in each group. The quantity, viscosity and pH of the secretion were determined, as well as the weight of the empty uterus. Details of the technique adopted have already been described elsewhere (MEGLIOLI et al.[6]). The mean values obtained for the metoestrus, di-oestrus and pro-oestrus groups were compared statistically with those obtained for oestrus.

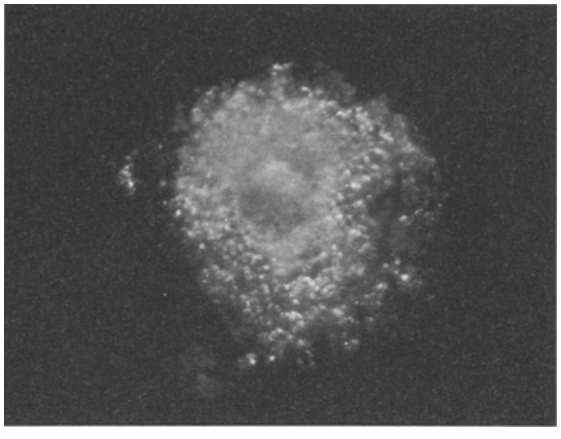

Fig. 1. Rat ovum surrounded by corona cells. ×125.

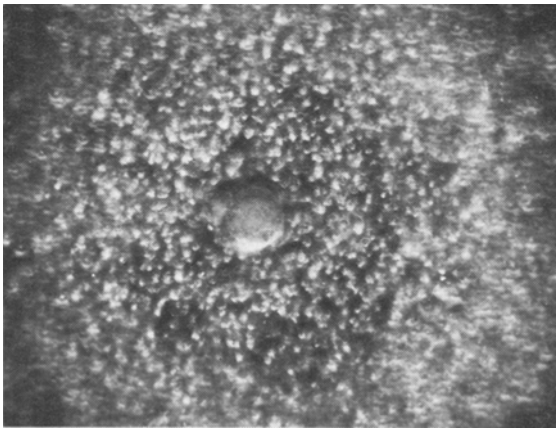

Fig. 2. Ova incubated in uterine secretion from rats in oestrus. Ova denuded. ×125.

3. Capacity of uterine secretion to disperse corona cells in vitro. Ova surrounded by corona cells from a rat which had recently ovulated were placed in small chambers filled with uterine secretion collected at each of the various phases of the sexual cycle and incubated at 37 °C (for details of the technique adopted see MEGLIOLI and DESAULLES[4]). Immediately thereafter and then every 2 h the projected surface areas of the ova surrounded by the corona cells were measured planimetrically. The difference between the surface areas before and after 6 h incubation was noted. Additional experiments were performed under the same conditions with uterine secretion that had been left to accumulate for 14 and 21 days.

Results. 1. Sexual cycle after closure of the uterus. In more than 80% of the control rats the 5 cycles observed remained regular. The average duration of the cycle, based on vaginal cytology, was about 96 h; oestrus lasts approximately 12–18 h, metoestrus 48–52 h, di-oestrus 10–12 h and pro-oestrus 18–22 h.

By contrast, in most of the animals operated in the afternoon of the days of oestrus, metoestrus or di-oestrus, disturbance of the cycle was noted during the 2 first cycles. Oestrus took place normally in the first cycle in the rats operated on the day of pro-oestrus, but in all these animals the duration of the second cycle was altered. In all the groups of rats, the cycle began to become regular again about 8 days after operation, i.e. approximately during the third cycle, the normal rhythm being fully re-established in about 80% of the rats in the fourth or fifth cycle.

2. Uterine secretion in the various phases of the cycle at different times after closure of the uterus (Table I). The increase in mean uterine weight observed in the groups of animals autopsied on the 7th, 14th and 21st day after operation must be considered in relation not only to the gain in body weight, but also to the operative procedure and the quantity of uterine secretion accumulated, which increased according to the length of time following operation. The pH of the secretion was similar in the 3 groups and its viscosity showed a tendency to increase proportionally to the time of accumulation. Comparison of these parameters within each group sacrified at different times revealed the following pattern: The quantity of fluid was decreased in the animals sacrified at metoestrus and di-oestrus in comparison with that obtained at oestrus and pro-oestrus. In relation to the amount measured at oestrus, the decrease found at metoestrus was significantly different.

The pH of the fluid was alkaline and, like the viscosity, appeared to increase at metoestrus – di-oestrus more than during pro-oestrus or oestrus, but not significantly so.

The weight of the empty uterus showed a significant reduction in the metoestrus – di-oestrus stage in all 3 groups of animals. The uteri of animals killed at pro-oestrus were slightly but not significantly heavier than those of animals killed in oestrus.

3. Ova incubated in uterine secretion from each stage of the sexual cycle (Table II). After 4 h incubation in uterine secretion from rats in oestrus, the 'nebula' of corona cells surrounding the ovum (Figure 1) was found to have expanded. The resultant pattern was significantly different from that observed after incubation in secretion collected at the other phases of the cycle. The surface area of the ovum was twice to three times the initial area and, at the end of the test, the corona cells were completely detached from the ovum; this was especially noticeable after incubation with secretion from rats in oestrus accumulated for 7 days (Figure 2), the activity of which was greater than that of secretion accumulated for 14 and 21 days. This difference could be correlated with

the quantity of secretion accumulated. The effect of the secretion on the corona cells might depend on the concentration of the dispersion factor.

The secretion obtained from rats in the metoestrus and di-oestrus phases did not dissolve the gel cementing the follicular cells; the surface areas found in these cases were smaller. The secretion from rats in pro-oestrus increased the surface area of the ovum to nearly twice the initial area, but the corona cells remained attached to the ovum. The patterns of corona-cell-dispersing activity produced by the uterine secretion from rats in each of the various phases of the sexual cycle were similar, regardless of the lenght of time for which it has been accumulated.

Discussion. From the vaginal smears it may be concluded that surgical closure of both extremities of the uterus caused a disturbance of the sexual cycle of the rat, such as might be produced by any surgical stress; later, however, regular cycles recommenced, indicating that the ovaries continued to function normally.

In the second part of this experiment, it was demonstrated that the ovarian function involves modifications, not only in the vaginal epithelium and in the weight of the uterus (ASTWOOD[7]; HEAD et al.[8]) but also in the uterine fluid. These variations were particularly clear-cut after 21 days, when the regularity of the sexual cycle was already fully reestablished.

It has been suggested (BLANDAU[9]) that the fluid observed in the uterus of the normal rat at pro-oestrus – oestrus escapes after oestrus because the effects of progesterone produced by the ovaries at this time relax the neck of the uterus. Our results showed that the accumulation of fluid during this period is significantly diminished since the cervix is closed by electrocauterization and the fluid cannot escape, it appears likely that in metoestrus resorption of the aqueous content of the fluid also takes place, which may explain the increase in its viscosity at this time.

Finally, the last experiment demonstrates that not only the physical properties but also the chemical composition of the uterine secretion of the rat undergoes some modification from one stage of the sexual cycle to the next. Complete dispersion of the corona cells in vitro was only observed after incubation in accumulated secretion extracted on the day of oestrus, suggesting that this phenomenon might be oestrogen-dependent.

All these observations seem to correlate with the modifications induced at the different stages of the sexual cycle by endogenous steroid sex hormones (BROWN-GRANT et al.[10]; BARRACLOUGH[11]). The fact that a similar pattern was observed in each phase of the sexual cycle when the uterine fluid was allowed to accumulate for different periods (7, 14 and 21 days) shows that these modifications in uterine fluid are regular, constant and cyclic, in conformity with a hypothalamic-hypophyseal-gonadal rhythm. The fluid accumulating in the closed uterus is not really a physiological uterine secretion, but it seems to retain in solution the secretion of the endometrial glands.

Summary. It was found that closure of the uterus disturbed the first 2 cycles after the operation; thereafter the normal cycle was resumed. The quantity of uterine fluid was increased at pro-oestrus and oestrus and reduced at met-oestrus and di-oestrus. Slight inverse changes in viscosity were observed. There was no significant difference in the pH. The corona-cell dispersing factor seems to be an oestrogen-dependent constituent of uterine secretion.

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Appearance of Sex Hormone Receptors in Frog (*Rana esculenta*) Tadpole Skin During Metamorphosis

The problem of mechanism of action of sex hormones has been receiving ever increasing attention in recent years. A good deal of reserach has already demonstrated the importance of nuclear and cytoplasmic 'receptors' in numerous target organs.

The gonadal sex hormone secretion begins at different stages of vertebrate development and varies according to the species. Generally their appearance coincides or precedes the differentiation of secondary sex characters (SSC). Few studies were carried out on the appearance of the sex hormone receptors in these organs.

DELRIO and d'ISTRIA[1] have recently demonstrated the presence of an androgen receptor in the $105{,}000 \times g$ supernatant not only in the thumb pad (a male SCC) of *Rana esculenta*, but also in the skin taken from other parts of the body (legs, dorsal, ventral and lower jaw; d'ISTRIA et al.[2]).

This work on the appearance of receptors has been done on the skin of the entire body of tadpoles and not on the thumb pads, since the anterior legs grow later in the course of metamorphosis of frog.

Rana tadpoles in the stages 24, 27, 28, 29, 31, 32 and 33 (according to the table of WITSCHI[3]) were procured from the surroundings of Naples. Tadpoles of stages 31–33 could easily be separated according to sex by observing their gonads under a binocular. Skin taken from these was homogenized in *Tris*-HCl buffer pH 7.4 and centrifuged at $600 \times g$ for 10 min; the supernatant was recentrifuged at $105{,}000 \times g$ for 1 h in a I.E.C. B–60 centrifuge. H^3-testosterone (S.A. 84 Ci/m*M*) and H^3-estradiol-17β (S.A. 85 Ci/m*M*) from Radiochemical Center, Amersham, England were used. For further details of the method used we refer to the works by FANG and LIAO[4], ADACHI and KANO[5] and DELRIO and d'ISTRIA[1].

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