suggest that in the absence of dietary cholesterol the intestinal flora of the chicken exert little or no effect on the serum cholesterol level. The effect of the intestinal flora in germ-free chickens fed cholesterol is under investigation.

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Résumé

On démontre qu'il n'y a pas de différence entre le cholestérol du sérum de poulets élevés dans des conditions aseptiques et ceux élevés dans des conditions normales et soumis à un régime sans cholestérol. Les poulets sans bactéries croissent mieux que les poulets normaux.

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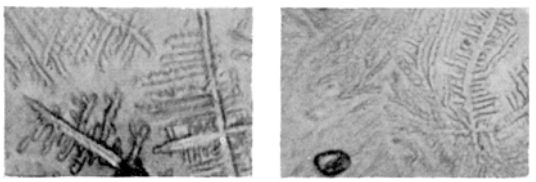
'Arborization' Phenomenon (AP) in Semen

Several years ago PAPANICOLAOU[1] observed a so-called 'arborization' phenomenon (AP) or palm (fern) leaf (PL) reaction in the cervical mucus secretion, which was spread on a slide and allowed to dry. The drying material, particularly if taken at the time of ovulation, crystallizes in an 'arborizatory' pattern. This interesting and clinically significant phenomenon stimulated a series of investigations *in vitro* as well as *in vivo*[1–9]. It has been proved that



a b

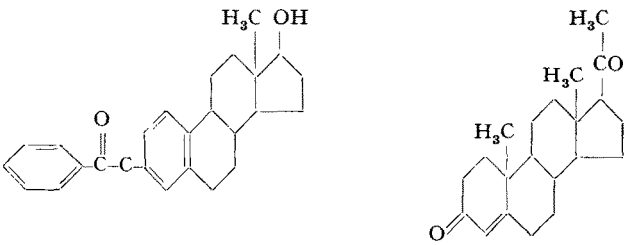
Fig. 1a–b.—Arborization phenomenon in semen. 450 ×.

any protein or carbohydrate substance may produce a PL or PL-like arborization phenomenon if it comes into the contact with electrolytes, such as NaCl, KCl, KBr etc.[2,4]. The occurrence or failure of the AP to appear, even though it still has 'PL-like' possibilities, may yet mask some factors which are beyond previous experimental conclusions.

After storage of semen of three different species (rabbit, bull, and human) one observed the PL phenomenon (Fig. 1a–b) which, in the series of experimentations, appeared more pronounced on the slide in the incubated (at 39°C) material than in the material stored at room temperature (RT) of all three species[9]. In most instances the PL reaction began to appear after about 5–6 days of incubation. The longer the material was incubated the more frequent and pronounced the PL appeared. The phenomenon, however, did not show essentially identical 'arborizatory' pattern in all cases. In most instances, the arborization consisted mainly of straight branches extending in a somewhat quadrangular shape, creating numerous figures, from which smaller side branches of different lenghts crossing and intercrossing distally with various-sized 'buds' of crystals frequently attached to the very ends. In other instances, however, the main lines showed curvations creating in some areas almost 'whorl-like' patterns with side branchings having somewhat indented leafy projections going toward the peripheries. Still in other cases, they appeared more or less pronounced if observed at a moderate microscopic magnification and with a partially closed diaphragm (see Fig. 1a–b).

It is probably true that incubation accelerated the degradation of certain protein products (peptides, tripeptides, polypeptides etc.) or even mono- or polysaccharides[6,10], which experimentally has been proved to have a definite relation with this phenomenon at certain concentrations (usually less than 4–6% a.s.o.). No APor PL reaction, was observed in fresh seminal material, undiluted or diluted at room temperature (about 20°C). For the purpose of better microscopic identification the adding of dilute mixtures of the sodium salt of *p,p'*-dibenzyldiethyldiamino-*p''*-hydroxyphenylcarbinol trisulfonic acid anhydride ($C_{37}H_{34}O_{10}N_2S_3Na_2$) together with nigrosin or sodium salt of tetrabromofluorescein-nigrosin solutions[9], did not seem to interfere with that crystallization pattern.

On the basis of clinical observations, it was found that certain administered steroid substances[3–5, 11, 12] (e.g. Fig. 2a–b).



a Fig. 2a–b b

are closely related with the appearance or non-appearance of AP *in vivo* (PL structures in the material from patients obtained from 5–7th day to the 20–22nd day of cycle – ROLAND[5], CAMPOS DA PAZ[3]). Cervical as well as endometrial secretory activity ('glaire filante') of the uterus reflects in the administration (dosage) of those steroids[3,5]. There is a hope that further clinical as well as laboratory observations, in addition to the results obtained in veterinary field[8], will soon shed more light onto the nature of this interesting phenomenon.

A. F. GRINIUS

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Zusammenfassung

Das Auftreten der sogenannten «AP»- oder «PL»-Kristallisationsreaktion in gelagerten Samen (Sperma) von drei Spezies (Kaninchen, Bulle und Mensch) wird beschrieben und mikrophotographisch demonstriert. Es wird versucht, eine Interpretation und Korrelation zwischen einigen der früheren wichtigsten Laboratoriums- und klinischen Beobachtungen zu geben.

Morphogenesis of Melanotic Tumours (pseudotumours) and its Genetical control, in three Wild Stocks of *D. melanogaster*

Melanotic masses are already visible during the 3rd larval stage, in the wild stocks tu-A_2, tu-B_3 and melanotic e 144. Their percentages in the adult stage are: A_2, 74·0% ± 3·296; B_3, 100%; melanotic e 144, 68·5% ± 2·659. The phenotype is almost the same in all stocks, and is fully viable.

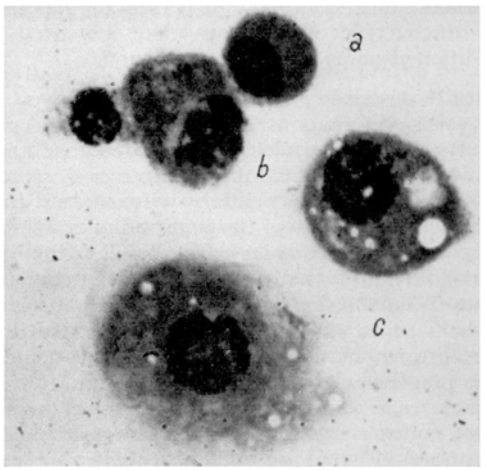

Fig. 1.—Haemolymph cells: *a* small basophilic cell; *b* medium-sized basophilic cells; *c* large cell (× 1500).

Morphogenesis progresses along the following steps, which are basically the same in all three stocks, with some minor differences:

(1) Release of the cells from the lymph gland, either as migration from the gland (tu-A_2, melanotic e 144) or as detachment of gland lobes (tu-B_3);

(2) Differentiation of a number of typical cells (large cells, see Figure 1) outside the gland, showing frequencies from 3% up to 10% or more;

(3) Formation of clumps of large cells, which are very thin and, lately, are embedded in melanine.

In two tumorless stocks $\left(\text{Varese and } \frac{+}{+}\ \frac{\text{CyL}}{\text{Pm}}\ \frac{\text{H}}{\text{SbMe}'}\right)$ the percentage of the large cells is lower than 3%, and (especially in Varese) the gland remains intact. A genetical analysis (replacing whole chromosomes by means of marked tumourless stocks) was brought about, which gave these results: the three morphogenetic steps mentioned above are under control of different parts of the genome. The three major chromosomes control (although with different intensity) both the differentiation of the large cells, and the behaviour of the lymph gland (no localization within the chromosome has been attempted); melanization is only controlled by the second chromosome (a short portion close to the left end in A_2, and another close to the right end in B_3).

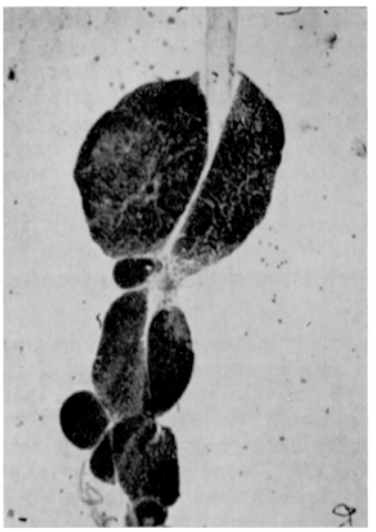

Fig. 2.—Entire lymph gland of Varese stock (× 100).

At least in melanotic e 144 it has been proved that the genes acting on melanization are different from those localized on the same chromosome, but acting on the other morphogenetic steps. Several loci with similar effect (polygenes) are located in the second chromosome (tu-B_3), to control melanine production.

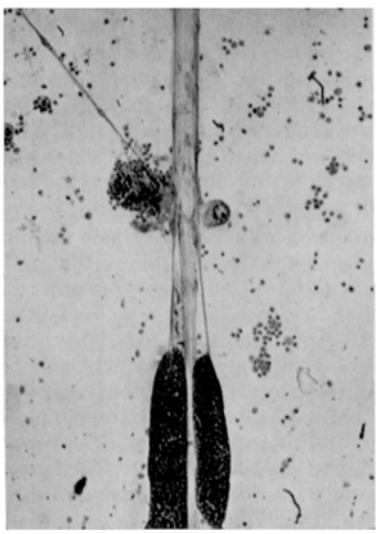

Fig. 3.—Entire lymph gland of A_2 stock (× 100).

To conclude, in the stocks studied so far, the melanotic masses are the end result of a complicated developmental procedure, corresponding to a similarly complicated system of multichromosomal factors.

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Institute of Genetics, University of Milan Italy, September 9, 1958.