

Accessory Nuclei and Annulate Lamellae in Hymenopteran Oocytes

ACCESSORY nuclei have been described in the oocytes of Hymenoptera¹⁻³ and some other insect orders. They are present during vitellogenesis, probably originating from the oocyte nucleus³. Structurally they resemble nuclei and their electron-dense inclusions contain RNA, possibly of nuclear origin. They seem to be concerned with the control of the synthesis of albuminous yolk and afterwards perhaps with the formation of the vitelline membrane^{3,4}. In *Nasonia vitripennis* (Walk.) they first appear near the nucleus of the oocyte, and spread from there through the peripheral cytoplasm (Fig. 1).

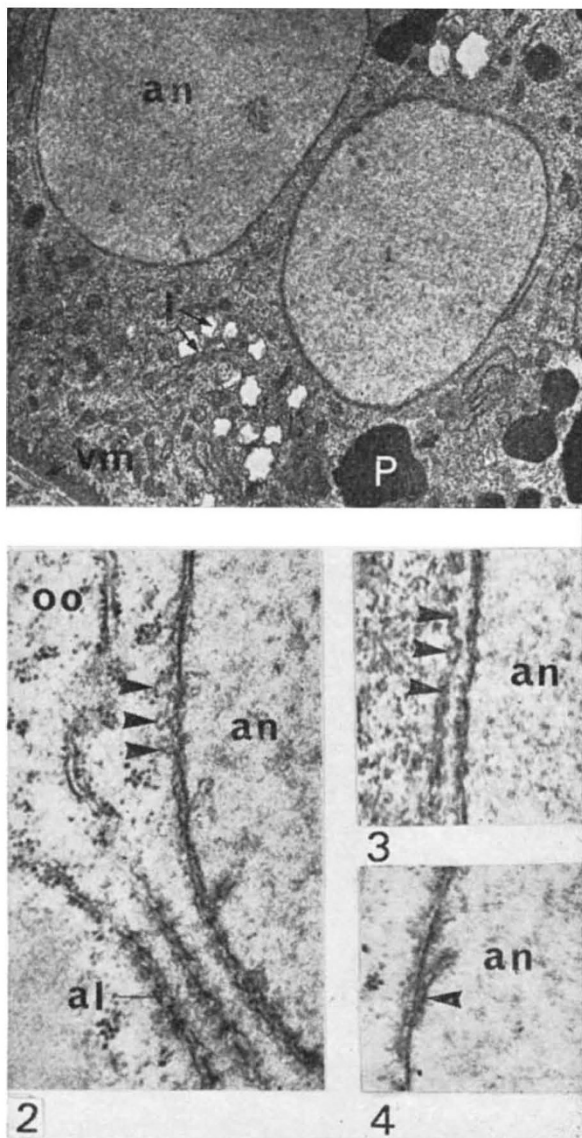


Fig. 1. Section of the periphery of an oocyte of *Nasonia vitripennis* showing accessory nuclei near the vitelline membrane. (Glutaraldehyde $\times 72,000$.) an, Accessory nucleus; P, protein yolk; vm, vitelline membrane; l, lipid.

Fig. 2. Section of accessory nucleus of *Ophion luteus* showing blebbing and annulate lamellae. (Glutaraldehyde $\times 49,500$.) al, Annulate lamellae; oo, ooplasm.

Fig. 3. Section of envelope of accessory nucleus of *N. vitripennis* showing separate and united vesicles. (Glutaraldehyde $\times 36,000$.)

Fig. 4. Section of envelope of accessory nucleus of *O. luteus* showing an intranuclear bud. (Glutaraldehyde $\times 49,500$.)

Swift⁵ described annulate lamellae as pairs of cytoplasmic membranes arranged in parallel stacks and with periodically spaced annuli. They have been recorded in the cytoplasm of vertebrate and invertebrate cells⁵⁻⁸ and in some cells they occur within the nucleoplasm^{5,6}. They are most abundant in rapidly growing and differentiating cells, such as oocytes, embryonic cells, spermatocytes and various kinds of tumour cells^{10,11}. In the salivary glands of *Drosophila*¹², *Necturus* oocytes¹³ and adrenal cortical cells⁸ of the alligator, the annulate lamellae seem to arise through the coalescence of vesicles from the nuclear membrane. Alternatively, they may be delaminated from the nuclear membrane as in the seagull adrenal⁸. Their function is not clear, but they are presumably involved in the transfer of information between the nucleus and the cytoplasm.

In the peripheral ooplasm of *Nasonia vitripennis* the accessory nuclei multiply by a process of amitotic division. Annulate lamellae then arise by the coalescence of vesicles produced by the outer membrane of the accessory nucleus envelope. A similar process has been observed in the ichneumonid, *Ophion luteus* Fab. (Figs. 2 and 3). Sometimes they are also budded into the nucleoplasm (Fig. 4). The annulate lamellae are either in single units or in stacks, and after formation they move away from the accessory nucleus. Within the stacks the lamellae are connected both with each other and with the endoplasmic reticulum (Fig. 2). It is possible that after the first lamella has been formed, the rest of the stack bud off it and not the nuclear membrane (Fig. 2). Near the site of origin of the lamellae vesicles occur between the membranes of the nuclear envelope, which suggests that nuclear material is being extruded presumably to the lamellae. This would support the view that their function is to extend the influence of the nucleus, started by the budding off of the accessory nuclei. This would be very important in a highly active cell, particularly if, as in the oocyte of insects, movement within the cell is impeded by numerous yolk globules. Their proximity to the accessory nuclear membrane supports the view that annulate lamellae are of nuclear origin and not specialized regions of the endoplasmic reticulum^{9,13}. Lipoid yolk first appears in their vicinity and perhaps one of their functions is to contribute to its formation.

P. E. KING
J. G. RICHARDS

Department of Zoology,
University College,
Swansea.

Received February 28; revised March 20, 1968.

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Chromosome Abnormalities and Psychotropic Drugs

DURING a study of chromosome abnormalities in five patients treated with *d*-lysergic acid diethylamide (LSD) we examined thirty patients in a mental hospital and ten hospital staff members as controls. The frequency of gaps was significantly higher in the thirty patients than in the staff members group ($\chi^2 = 6.859$, $P < 0.01$). When we divided the control group of patients into those treated

Table 1. GAPS, BREAKS AND HYPERDIPLOID CELLS IN PATIENTS TREATED WITH LSD OR PSYCHOPHARMACOTROPIC DRUGS, AND IN CONTROLS

	Mean age and S.D.	Cells examined	Total	Gaps Per cent	Total	Breaks Per cent	Hyperdiploid cells Total	Per cent
Five psychiatric patients treated with LSD	36.4 ± 6.47	358	57	15.9	6	1.7	7	2.0
Seventeen psychiatric patients treated with psychotropic drugs	36.9 ± 8.89	510	67	13.1	5	1.0	1	0.2
Thirteen psychiatric patients given no drugs	32.8 ± 6.28	476	33	6.9	0	0	0	0
Ten hospital staff members	27.8 ± 5.04	326	18	5.5	0	0	0	0

with drugs and those not treated with drugs, we found that the frequency of gaps and breaks in the group treated with drugs was significantly higher than in the group of patients not treated with drugs ($\chi^2 = 10.399$, $P < 0.005$, $P(\text{Fisher}) = 0.037$, Table 1).

The difference between the frequency of gaps in the group of patients treated with drugs and the staff members group was significant ($\chi^2 = 12.629$, $P < 0.0005$), but the difference between the frequency of breaks in the two groups was not significant ($P(\text{Fisher}) = 0.084$).

This indicates that some of the psychotropic drugs may induce gaps and breaks to the extent found in patients treated with LSD. We found, however, a significantly higher frequency of hyperdiploid cells in the patients treated with LSD than in the control patients treated with psychotropic drugs ($P(\text{Fisher}) = 0.010$). There was no significant correlation between any specific drug and the frequency of gaps, breaks and hyperdiploid cells in our comparatively small amount of material.

Further work is needed on the toxicity of different psychotropic drugs and other drugs. Any new drug should be tested for toxic effect on chromosomes, in animals and *in vitro*, before they are released for general use.

JOHANNES NIELSEN
URSULA FRIEDRICH
TAKAYUKI TSUBOI

The Cytogenetic Laboratory,
Aarhus State Hospital,
Risskov, Denmark.

Received March 25, 1968.

Results of Consecutive Matings of Female *Anopheles gambiae* Species B with Fertile and Sterile Males

THE *Anopheles gambiae* complex consists of five closely related species; two salt water ones, *A. melas* and *A. merus*, and three fresh water ones, provisionally designated *A. B* and *C*. When males from species *A* or *B* are crossed with females of *A. melas* or *A. merus* the F_1 generation consists almost entirely of sterile males. It has been proposed that the release of such sterile males be used to control members of this species complex¹. It has already been established that these males compete successfully against normal males when both are caged together with virgin females¹. I have carried out experiments to investigate whether a female, once mated with a sterile male, could lay fertile eggs if she subsequently mated with a fertile male, or if a female, once mated with a fertile male, could be sterilized by mating with a sterile male. Previous authors, seeking evidence of multiple insemination in *Anopheles* spp. with the aid of marker genes, found very little indication of it^{2,3}.

Mosquitoes used were females and fertile males from a species *B* colony from Kano, Nigeria, and sterile males produced by crossing the Kano males with *Anopheles melas* females from a strain from Liberia. Fifty-six females, which had been caged with fertile males and had laid fertile eggs, were re-fed, hand-mated⁴ each to a separate sterile male, and allowed to lay again. In fifty-four cases, the succeeding batches of eggs were again fertile. In only two cases did the eggs fail to hatch. Seventy-five females caged with sterile males laid eggs which failed to hatch. Virgin females of species *B* seldom

oviposit¹ and it can be assumed that most of these females had mated with the sterile males. On re-feeding, and after being hand-mated to fertile males, all seventy-five females again laid eggs which failed to hatch. Thirty of these females oviposited again, twelve of these laid a fourth batch of eggs and two laid a fifth batch. All were sterile.

These results are in agreement with those of George⁵, who found in *Aedes aegypti* that the first mating is the most important.

This work was supported by the World Health Organization.

JOAN H. BRYAN

Ross Institute of Tropical Hygiene,
London School of Hygiene and Tropical Medicine,
London.

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Dark Reactivation of Damage induced by Ultraviolet Light in Mammalian Cells *in vitro*

INDUCTION of pyrimidine dimers in cellular DNA by irradiation with ultraviolet light and the molecular processes involved in the repair of this damage (photo-reactivation¹ and dark reactivation²⁻³) are established in bacteria, bacteriophage and transforming DNA. Investigations of the reactivation of ultraviolet-induced damage have been extended from micro-organisms to cover cultured mammalian cell lines⁴⁻⁶.

We wished to determine the difference in sensitivities of cultured mammalian cell lines to X-rays and ultraviolet light to find out whether the reactivation processes after damage by X-rays and ultraviolet light are identical, and to see whether mechanisms can be demonstrated which split or excise ultraviolet-induced thymine dimers. Mouse fibroblastic L cells, porcine kidney stable (PS) cells, and mouse Ehrlich ascites tumour cells maintained *in vitro* were used.

For determinations of colony forming ability, cells of each line were plated in 60 mm glass Petri dishes containing 70 per cent TC-199 medium plus 10 per cent 'Bacto' tryptose phosphate broth (Difco) supplemented with 20 per cent bovine serum. The dishes were incubated at 37° C for 10 to 14 days in a humidified CO₂ incubator which was flushed with a mixture of 5 per cent CO₂ and 95 per cent air. For X-irradiation, 5 ml. samples of cells were suspended in short 'Pyrex' test tubes (inner diameter 10 mm, length 100 mm), and irradiated with various doses at a rate in air of 64 r./min as measured with a Victoreen condenser chamber. After each 1 ml. of the unirradiated or irradiated cell suspensions had been distributed into 60 mm glass Petri dishes and supplemented with 4 ml. of culture medium, the dishes were placed in a CO₂ incubator. For ultraviolet irradiation, 1 ml. samples of cell suspensions were distributed into 60 mm glass Petri dishes, and irradiated with various doses at the predominant wavelength of 260 mμ from a 15 W germicidal lamp, at a dose rate of about 20 ergs/mm²/s at the surface of the dishes.