Summary

The amphidiploid hybrid of *Nicotiana glauca* × *N. langsdorffi*, producing tumors spontaneously, contains essentially more of the following free amino acids than the inital species: cystine, histamine, oxyproline, alanine, kynurenine, tryptophane, and a non-identified ninhydrine positive substance. It is supposed that the abnormally high content of free amino acids is connected with the spontaneous formation of tumors.

Genetic and Autoradiographic Studies of Tritiated Thymidine in Testes of *Drosophila melanogaster*[1]

A study on the influence of mating intensity and rate of sperm utilization upon the rate of spermatogenesis in *Drosophila melanogaster* is presently under way. Tritiated thymidine (T–H^3), a specific label for DNA in most biological systems studied (BRACHET[2]), is being used in conjunction with autoradiography to label those premeiotic cells which are synthesizing DNA and to follow the development of these cells to mature sperm. In addition, it is planned to use the incorporability of T–H^3 as an indication of the rate of entrance of cells into the premeiotic DNA synthesizing stages. This preliminary report contains information obtained in the earliest of these experiments.

Additionally, in order to gain some insight into the degree of genetic damage caused by the amounts of isotope which must be incorporated to give satisfactory autoradiographs of testicular cells, studies on the rate of production of sex-linked recessive mutations by this compound have been initiated and preliminary data on this subject, also, are herein reported.

To decrease the amount of non-radioactive nucleic acid precursors ingested so that strong autoradiographs could be obtained after a minimum feeding time, application of the isotope was not carried out in the standard fly culture medium which is rich in yeast. Instead, the isotope-containing medium was made up as follows: 0·1 ml T–H^3 solution (Schwarz Laboratories, Sp. Act. 1·9 c/*mM*) with a total activity of 100 microcuries, 0·5 ml of a 1% agar solution and 0·5 ml of a modified Eagle's tissue culture medium.

Twenty-five 16-h Canton-S larvae not identified as to sex were placed upon the above medium for a period of 4 h and then removed to standard corn meal, yeast, and sugar food. Eight of the 25 larvae died during these 4 h, while only two of 25 control larvae died. Two larval males were fixed immediately after the feeding period; three males were fixed 19 h later (39-h larvae) and the remaining four males were fixed 56 h after removal from the radioactive food (76-h larvae). All larvae were fixed in hot 45% acetic acid and imbedded in parlodion and paraffin according to the Peterfis double imbedding technique. Sections were cut at 6 μ and mounted serially. After dissolution of the paraffin and parlodion, and hydration of the sections by means of a graded ethanol series, the slides were treated with 5% trichloroacetic acid at 3–5°C for 5 min to remove any acid soluble isotope. From this point on, autoradiographs were prepared according to the stripping film technique as published by TAYLOR[3].

At 16 h, the larval testes are composed entirely of spermatogonia with no evidence of any anterior-posterior developmental gradient, except for the difference in size between the apical cells and those slightly larger spermatogonia that have been proliferated from them. The testes of male larvae that were fixed immediately after the 4-h period show activity over the apical region where mitotic proliferation occurs. Also, there are some radioactive nuclei located posteriorly, indicating that very early in spermatocyte development, before the long growth stage, during which the volumes of the nucleus and cytoplasm of primary spermatocytes increase so markedly, the chromosomes are reduplicated in preparation for the meiotic divisions.

19 h following feeding (39-h larvae), there is still some activity present anteriorly but many labeled cells have moved posteriorly (Fig. 1). All nuclei of a cyst are either entirely radioactive, or completely lacking in activity, indicating that the cells of any one cyst are physiologically synchronized, at least in terms of incorporation into DNA.

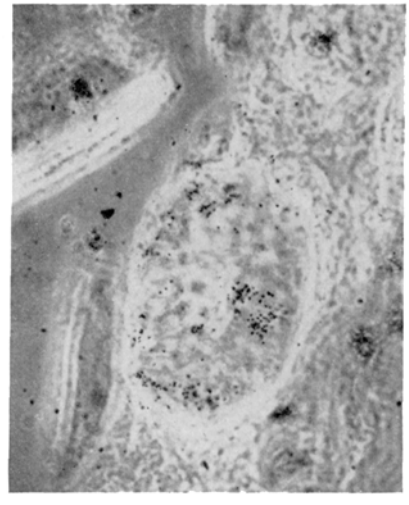

Fig. 1.—39 h larva, 19 h after removal from labeled food. × 850.

56 h after removal from radioactive food (76-h larvae), radioactive primary spermatocytes may be observed in the posterior third of the gonad (Fig. 2 and 3). The radioactivity observed anteriorly derives from interstitial cells located throughout the testis.

While additional work must be carried out to establish the developmental pattern of the testis in relation to the time of DNA synthesis, it may be seen from our preliminary data that spermatocytes reduplicate their chromosomes very early during their life history and move posteriorly as additional cells are proliferated from the apical spermatogonia.

For the measurement of the mutagenic effect of T–H^3, the same sample of food used in the previous experiment was mixed with 1·1 ml of the standard fruit fly medium: agar, 0·8%; sugar, 5%; corn meal, 6%; yeast, 1·5%; water, 86%.

[1] Supported in part by Grant G-6097 of the National Science Foundation.

[2] J. BRACHET, Exp. Cell Res. Suppl. *6*, 78 (1959).

[3] J. H. TAYLOR in *Physical Techniques in Biological Research*, Vol. 3 (Ed. G. Oster and A. W. POLLISTER, Academic Press, N. Y. 1956), p. 545.

Fig. 2.—76-h larva, 56 h after removal from food. Radioactivity of nuclei of one cyst illustrated. × 850

16 h after hatching, 50 Canton-S larvae were placed on this food and, as pupae appeared, they were removed and placed in a vial with standard food. Although the 16-h larvae, which were placed upon the radioactive food, hatched from eggs that were laid over a 2-h period, there was a spread of two days in pupation time. Furthermore, there was a spread of four days in the eclosion of adult males from these pupae. In the case of a group of 50 control larvae, pupation occurred over a period of two days and the eclosion of adults from these pupae over a period of three days.

Accordingly, the following groups were derived:

Group	*Days after pupation*	*Number of ♂♂*
I	5	6
II	6	5
III	6	5
IV	7 and 8	5

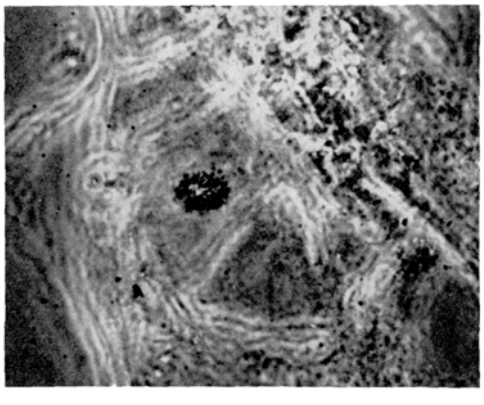

Fig. 3.—Same larva as in Figure 2. Heavily labeled nucleus of posterior spermatocyte illustrated. The large size of cells and nuclei at this time prevent the visualization of labeled nuclei of any one cyst in a single section. × 850

On the day following their appearance, the adult males were mated to Muller-5 females for the purpose of determining the sex-linked recessive mutation rate. The males of Groups I, III, and IV were mated individually to three females, each for a period of three days, and were then provided with a second group of three females each, and so on, for a total of four broods. Males of Group II were mated in mass culture of three females per male and four broods obtained.

The Table summarizes the data derived from the four separate groups. Unquestionably, the presence of the tritium produces a mutagenic effect. With the exception of Group II, the numbers are generally small and the attendant sampling error correspondingly large, so that a detailed analysis is unwarranted. However, it may be seen that in all cases the highest mutation rate is present in the second brood.

Table

	Brood	Mating period	Non-lethal chromosomes	Lethal chromosomes	% lethal chromosomes
Group I . . .	1	1–4 days	126	1	0·78
	2	4–6 days	440	6	1·35
	3	6–9 days	81	1	1·22
	4	9–12 days	120	1	0·82
Group II . .	1	1–3 days	226	2	0·88
	2	3–6 days	448	18	3·90
	3	6–9 days	365	2	0·54
	4	9–12 days	465	3	0·64
Group III . .	1	1–3 days	175	4	2·23
	2	3–6 days	78	3	3·70
	3	6–9 days	47	1	2·08
	4	9–12 days	50	0	—
Group IV . .	1	1–3 days	102	2	1·92
	2	3–6 days	52	2	3·70
	3	6–7 days	34	0	—
	4	7–12 days	87	3	3·33
Controls . . . (mass matings)	1	1–3 days	1,701	1	0·06
	2	3–6 days	1,646	4	0·24
	3	6–9 days	935	0	—

It is still to be determined whether this is a case of differential sensitivity, or merely whether the population of labeled sperm has been diluted by unlabeled sperm which had reduplicated their chromosomes, either before the T–H³ was presented or after the labeled pool had been depleted. The question of clusters of mutations also remains unresolved. However, the observed mutations are well distributed among the individual males whose progenies have been tested. For example, in Group I, Brood 2, the six mutations occur in four different males, two of which carried two mutations each. Furthermore, where more than one mutation has been scored for any single brood, the mutant chromosomes have not been restricted to any individual male.

PLAUT[4] discusses the question concerning the possible biological damage to chromosomes that have incorporated T–H³ and points out the need for experimental work to

[4] W. PLAUT, Labor. Invest. *8*, 286 (1959).

examine this question. PAINTER, DREW, and HILL[5], TAYLOR[6] and WIMBER[7] all present data which indicate that endogenous radiation can cause damage to the chromosomes. This brief report indicates the possible intrachromosomal damage that may follow the incorporation of radioactive compound into the chromosomes.

Larger scale experiments in which precise quantities of T-H^3 are injected into larvae will be carried out to examine more critically the mutagenic properties of the tritium atom after its incorporation into the chromosomes.

W. D. KAPLAN and J. E. SISKEN

Departments of Genetics and Experimental Pathology, City of Hope Medical Center, Duarte (Calif.), August 20, 1959.

Zusammenfassung

Die Aufnahme von H^3-Thymidin in 16 h alte männliche Larven von *Drosophila melanogaster* wurde autoradiographisch verfolgt und die mutagene Wirkung des einverleibten H^3-Thymidin durch die Bestimmung der Häufigkeit der geschlechtsgebundenen rezessiven Mutationen gemessen. Es zeigte sich, dass das der männlichen Larve der *Drosophila melanogaster* verabreichte H^3-Thymidin eine bedeutende Häufigkeit der Mutationen verursacht.

[5] R. B. PAINTER, R. M. DREW, and W. L. HILL, Science *127*, 1244 (1958).
[6] J. H. TAYLOR, Genetics *43*, 515 (1958).
[7] D. W. WIMBER, Proc. Nat. Acad. Sci., Wash. *45*, 839 (1959).

Hormonal Control of Mating Behavior in an Insect[1]

Mating behavior in insects was not demonstrated until very recently to be influenced by endocrine glands. At one time it was thought that the activity of the gonads might be involved in this behavior, but it was shown that, for example, in *Lymantria*[2], *Gryllus*[3], and *Leucophaea*[4] gonadectomy in males as well as in females does not impair mating activity. Confirmatory results are also given here (Table). Furthermore, in females of *Diploptera*[5,6] mating occurs shortly after emergence, when the gonads are still inactive. Recently, it was observed that after the implantation of nymphal prothoracic glands into adult females of *Leucophaea* only a small percentage of these females mated[7]. This experiment was repeated, and again only 4 of 16 (25%) experimental animals accepted a male within 4 weeks after emergence. Apparently, the implantation of active prothoracic glands changes the normal response of adult females to courting males.

It remained to be seen whether this effect was direct or indirect, since implants of active prothoracic glands in adult females of *Leucophaea* inhibit the activity of the corpora allata. To test this point, females of *Leucophaea* were allatectomized one day after emergence and then were kept with normal males. In the experimental as well as control series 2 couples were placed together in each finger bowl, so that each female had a chance to mate with either of the 2 males.

In the normal control series, 90% of the females had accepted a male within 26 days after emergence as seen by the presence of a spermatophore in the bursa copulatrix (Table). By contrast, only about 30% of the allatectomized females accepted a male within the same period. Sham operated females mated as readily as normal animals (Table). From this it seems that mating in females of *Leucophaea* depends on the presence and activity of the corpora allata. This conclusion is supported by the observation that, in *Leucophaea*, mating normally occurs at a time when the corpora allata show histological signs of beginning activity, i. e. an increase in cytoplasmic content. Thus, the effect of the implantation of active prothoracic glands on the female response to courting males can be accounted for by the inhibition of the corpora allata[7].

Mating in normal and experimentally treated *Leucophaea*

	No. of animals observed	No. of animals mated	
		within 26 days after emergence	within 12 days after re-impl. of corpora allata
1. Normal females	156	141 = 90 %	—
2. Castrated females	16	15 = 94 %	—
3. Normal females mated with castrated males	58	55 = 95 %	—
4. Allatectomized females	36	11 = 30·5 %	—
5. Sham allatectomized females	16	14 = 87 %	—
6. Allatectomized females that received four active corpora allata 26–30 days after emergence	11	—	9 = 82%

This conclusion is further substantiated by the following experiment: Eleven allatectomized females were kept with normal males for 26–30 days during which period they did not mate. Each of these 11 females then received implants of 4 active corpora allata taken from last instar nymphs 3–5 days after their molt. Corpora allata of such donors are known to be active, since after implantation into adult females they bring about egg maturation. Five days after the implantation of the corpora allata, 6 females had already accepted a male, and on the 12th day after the implantation a total of 9 (82%) of the experimental animals had mated (Table). In the 2 animals that had not mated when the experiment was discontinued on the twentieth day after re-implantation, the implanted corpora allata must have been quite inactive because the oocytes of these animals contained very little yolk. This experiment with re-implantation of the corpora allata demonstrates that in *Leucophaea* active corpora allata are involved in the responsiveness of females to courting males.

[1] Supported by U.S.P.H.S. Grant C-3413 administered by Dr. B. SCHARRER.
[2] J. TH. OUDEMANS, Zool. Jb. Syst. *12*, 71 (1899).
[3] J. REGEN, Zool. Anz. *35*, 427 (1910).
[4] B. SCHARRER, Personal communication.
[5] L. M. ROTH and E. R. WILLIS, Psyche *62*, 55 (1955).
[6] F. ENGELMANN, Biol. Bull., Woods Hole *116*, 406 (1959).
[7] F. ENGELMANN, Z. vgl. Physiol. *41*, 456 (1959).