EXPERIMENTAL STUDIES ON POLE CELLS AND MIDGUT DIFFERENTIATION IN DIPTERA

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Summary

Highly localized irradiation with ultraviolet of the posterior polar region of eggs of *Drosophila melanogaster* and *Lucilia cuprina* in pre-pole cell and pole cell stages results in reduction in numbers of the cuprophilic cells of the middle midgut as well as in reduction of gonad size and number. Carefully timed eggs were exposed to dosages of ultraviolet (from a source giving about 90 per cent. at wavelength 2536 Å) ranging from 1200 to 2400 μW sec/cm^2 over periods of 2–4 min. Treatments at the time of active pole cell formation were found to be most effective in producing defects of both gut and gonads, thus demonstrating the common origin of the cuprophilic cells of the middle midgut and the germ cells of the gonads.

Detailed quantitative data concerning gonads and midgut cells are presented for *D. melanogaster* for various ages at time of treatment. Separability of the types of defects before and after the time of active pole cell formation suggests the derivation of the cuprophilic cells from the more peripheral and the germ cells from the more internal polar regions. An inverse relation was found between the numbers of calycocytes (cuprophilic cells) in the midgut epithelium and the numbers of free cells in the lumen of the gut in older embryos. Evidence indicates that larvae with severely reduced numbers of cuprophilic cells seldom survive either in *Drosophila* or *Lucilia*. Although the gut epithelium is usually continuous even in complete absence of cuprophilic cells, morphogenesis of the middle midgut is always abnormal in such embryos and larvae fail to hatch. Quantitative data are presented for surviving larvae of *Lucilia*.

A study of control embryos of *D. melanogaster* demonstrated sexual dimorphism with respect to calycocyte number in the midgut epithelium at the time of hatching, the numbers being consistently higher in females than in males, an inverse correlation with gonad size.

I. INTRODUCTION

Modern work on the physiology of the insect gut has demonstrated the presence of remarkable patterns of functional and morphological differentiation (Waterhouse and Day 1952; Waterhouse 1957). In particular, the larval midgut of Diptera provides excellent material for study and in *Lucilia cuprina* (Wied.) it has been demonstrated that, in addition to regional differentiation, there is differentiation among cells within regions (Waterhouse 1945; Waterhouse and Stay 1955). Thus the copper-accumulating cells of *L. cuprina* are distributed in a mosaic fashion

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among the lipoid cells of the middle midgut. In species of *Drosophila*, the cuprophilic cells of the larval midgut usually form a distinct region within which other cell types are either rare or relatively inconspicuous, although occasional instances of a mosaic arrangement may sometimes be found (Poulson and Bowen 1952; Waterhouse and Stay 1955; Poulson, unpublished data). In contrast to *Lucilia* and other related genera, the principal cuprophilic cells of *Drosophila* are morphologically quite distinct from other midgut cells. They are the goblet-like calycocytes described by Strasburger (1932).

The remarkable properties of the cuprophilic cells, together with the fact that their cytological fine structure differs markedly from that of other gut epithelial cells (Waterhouse and Wright 1960), have led us to enquire into their embryological origin and the course of their differentiation.

The rationale of the experiments reported here rests on the observational evidence that only a fraction of the pole cells of the higher Diptera contribute to the germ cells of the gonads (Noack 1901; Rabinowitz 1941; Poulson 1947, 1950; Sonnenblick 1950), the balance, in *Drosophila* at least, becoming associated with the midgut rudiment (Poulson 1947, 1950). The technique employed is an adaptation of that used by Geigy (1931) in his classical demonstration that the germ cells of the gonads of *Drosophila* are derived from the pole cells. However, we have followed the effects of treatment on both the midgut and the gonads in embryos and larvae rather than confining observations to the surviving adults.

This paper provides an account of a number of experiments in which the pole cells and polar regions of the eggs of *L. cuprina* and *D. melanogaster* Meig. were exposed to ultraviolet radiation with the object of interfering with this region and the cells derived from it. A preliminary presentation has been given by Poulson and Waterhouse (1959).

II. Methods

Egg deposition, exposure to ultraviolet radiation, and subsequent incubation were carried out in a room maintained at 25°C and 75–80 per cent. relative humidity, thus minimizing disturbances from temperature and humidity changes.

(*a*) *Cultures and Egg Collection*

Eggs of *D. melanogaster* were collected from young gravid females of the strain Oregon-R-C maintained in this Laboratory. Slides bearing a little cornmeal, molasses, and agar medium baited with honey and yeast were placed in the laying bottles for periods of a few minutes. Eggs of known age were harvested into saline for further use.

A culture of the Australian sheep blowfly, *L. cuprina*, containing many gravid females was offered liver slices and observed until egg laying commenced. Five minutes or less sufficed for the deposition of enough eggs for any one experiment. These were transferred to moist filter paper (since immersion in saline for any period produced adverse effects) until the appropriate time for treatment.

(*b*) *Dechorionation*

Drosophila and *Lucilia* eggs at 30 min or more after laying (depending on how early it was desired to irradiate them) were immersed in a solution of sodium hypochlorite diluted with insect saline to a concentration (3 per cent.) at which dechorionation was accomplished in 1–2 min at 25°C. As this process was nearing completion the solution was drawn off and replaced by five or more successive lots of saline until all traces of hypochlorite had been removed. The eggs were then pipetted on to coloured blotting-paper to await transfer for irradiation. The moist substrate and high room humidity prevented desiccation. As a further precaution the heat rays from the microscope lamp used during manipulation were filtered off with Chance O.N.20 glass.

(*c*) *Irradiation*

The general technique used by Geigy (1931) and Aboim (1945) was adopted, although a number of modifications were introduced in order to increase the uniformity of the conditions of exposure. Dechorionated eggs were transferred with a glass needle to a rectangular coverslip which was very lightly lubricated with 50 per cent. aqueous glycerol to facilitate their manipulation into a straight line. Each egg was so oriented that it lay on its slightly convex ventral surface with the posterior pole at the edge of the coverslip. No difficulty was experienced with the orientation of *Drosophila* eggs. However, in *Lucilia* there was occasional difficulty in distinguishing the posterior pole of the egg prior to appearance of the pole cells. The egg-bearing coverslip was then lightly attached with vaseline to a brass wedge, thereby giving the eggs an inclination of 30° and making the posterior pole visible from vertically above (Figs. 1(*b*), 1(*c*)).

The brass wedge was, in turn, held in a tray about 1 mm deeper than it. The entire assembly was placed on a track which allowed the tray to be moved from under a dissecting microscope at one end to a point directly beneath an ultraviolet lamp at the other (Figs. 1(*a*), 1(*b*)). Two glass microscope slides (3 by 1 in.), rendered opaque with photographic masking paint, were used to form a narrow slit of determined width. After placing the wedge in the tray the first microscope slide was moved into position against a stop. The wedge was then moved with forceps under the microscope by means of the projecting pins (*P*, Fig. 1(*c*)) so that the same measured amount of the posterior pole of each egg protruded from under the opaque slide. The second opaque slide was then adjusted to give a parallel slit of desired width (Fig. 1(*b*)). The effectiveness of this shielding and the width of the slit were tested photographically.

For exposure to ultraviolet radiation the tray was moved along the track to a position directly beneath and 10 cm below an appropriately shielded ultraviolet source and a shutter opened for the desired period. The source* was a coiled quartz tube containing neon, emitting 85–95 per cent. of its radiant energy at 2536 Å and producing, at 10 W, an intensity of 10^4 ergs/cm^2/sec at 10 cm distance (Lea 1946). Exposure times ranged from 1 to 4 min. In most cases a 2-min exposure was employed; in others 3 min; and in one series a 4-min exposure was given to test dosage effects.

* Thermal Syndicate Ltd., London.

In all the experiments reported here the slit width was constant (2 units on the eyepiece micrometer) as was the length of the portion of the posterior pole of the egg exposed (2 units). After exposure, the coverslip with eggs was transferred to a

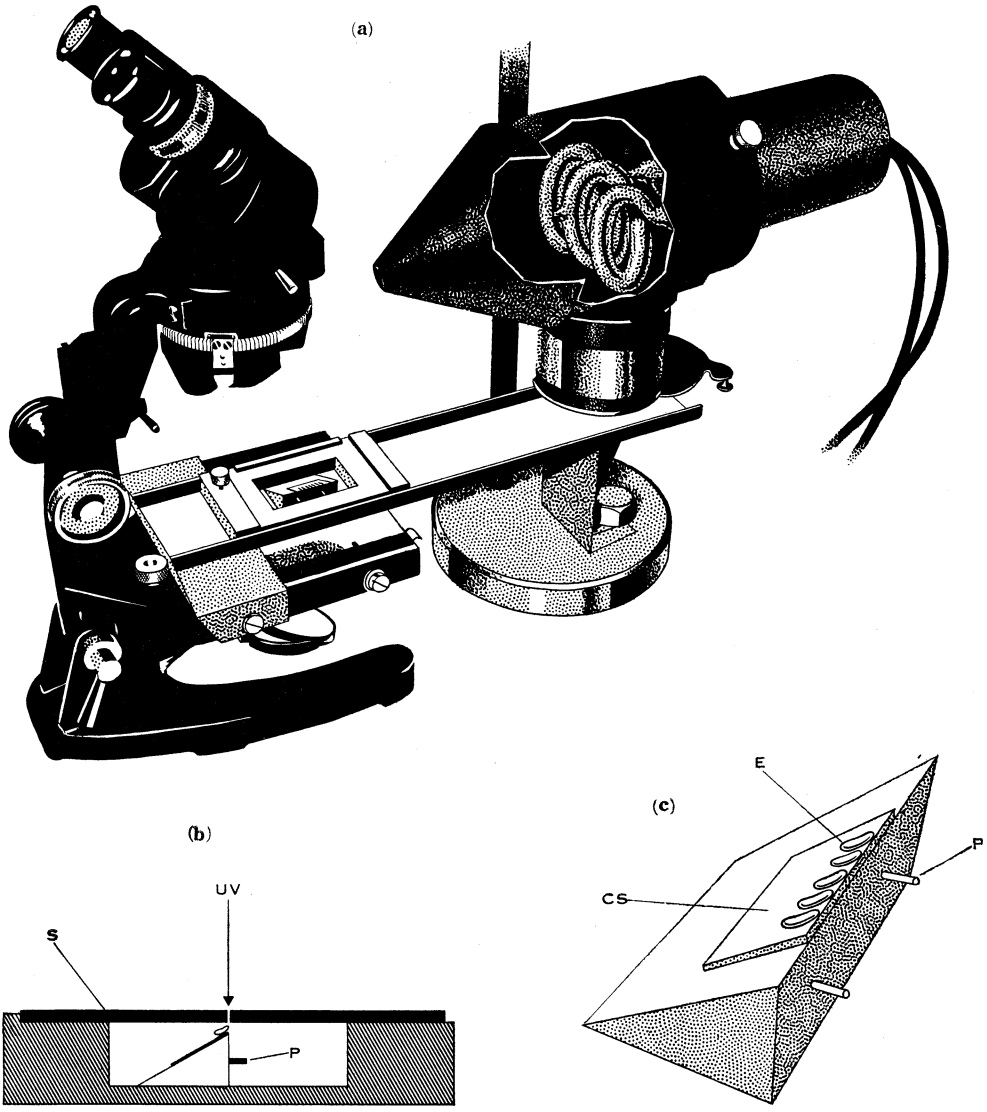

Fig. 1.—Apparatus used for irradiation: (*a*) tray with oriented eggs on holder is set on a track along which it is moved from the binocular microscope to a position directly beneath the source mounted over the track. (*b*) Details of tray with wedge and slit-forming slides (*S*) arranged for irradiation of pole of egg. (*c*) Wedge is moved with aid of pin (*P*); eggs (*E*) oriented on coverslip (*CS*) prior to alignment beneath slit.

loosely closed container lined with moist "Kleenex" tissue and incubated at 25°C in the dark to minimize photoreactivation (Altenburg and Altenburg 1952).

(*d*) *Histological Methods*

Eggs and newly hatched larvae were fixed by puncture with a fine glass needle in a formol–alcohol–acetic acid mixture (5 : 15 : 1 v/v) diluted to 50 per cent. with insect saline. After dehydration they were cleared in benzol and embedded in paraffin at 56°C, sectioned at 7 μ, and stained in Harris's haematoxylin. In a few instances sections of *Lucilia* eggs were cut at 9 μ.

The cuprophilic cells of *Lucilia* larvae were made distinguishable for counting by placing the freshly dissected midguts in a solution of neotetrazolium chloride as described by Waterhouse and Stay (1955).

(*e*) *Controls*

These consisted of embryos and larvae carried through all procedures except the irradiation and prepared as described.

III. Experimental Results

The experiments were carried out in three series. In the first the surviving larvae from treated eggs (of both *Lucilia* and *Drosophila*) were raised up to a stage at which the midgut cells could readily be recognized and counted using appropriate procedures. In these instances evidence of some reduction in the numbers of cuprophilic cells in larval guts of *Lucilia* was observed. However, it was at once apparent that these larvae represented only a portion of the treated eggs and that those which failed to hatch or to grow to normal size constitute the really significant material. Sections of these were then examined and evidence of gut and gonad disturbances were found. The data from these are presented along with those from a second series in which all embryos and larvae were fixed at the time of hatching. A third series consisted of embryos fixed at intervals of 30, 60, 90 min, and at 5 and 7 hr following treatment. These were useful in demonstrating the degree of localization and the more immediate effects of the radiation treatment.

Because of its larger size, the egg of *Lucilia* lends itself well to manipulation and allows more precision in localization of treatment; however, the lack of criteria for recognizing the cuprophilic cells in sections of embryos and early larvae makes quantitative work difficult. On the other hand, the principal cuprophilic cells of *Drosophila* (calycocytes) are morphologically distinguishable from other gut cells (Plate 1, Figs. 1–3) well before the time of hatching of the larvae and can be counted with relative ease. Hence we have used the *Drosophila* material for examining the quantitative relations following pole-cell treatment and the *Lucilia* material largely for comparative purposes and for following the localization and immediate effects of treatment.

(*a*) D. melanogaster—*Quantitative Relations*

Sections of treated *Drosophila* eggs fixed at the time of hatching of normals were carefully examined and counts made of the number of calycocytes in the middle midgut and of the number of gonads (and cells per gonad) in embryos for each time of treatment (Table 1). In a portion of the material the numbers of "large" cells (Fig. 2) in the region of the middle midgut just posterior to the calycocytes

were counted (Fig. 5, Table 5). In unhatched larvae the numbers of "free" cells in the lumen of the gut were also counted. The relationships between these and calycocyte numbers are given in Figure 4. The data presented include only embryos, all sections of which were complete and analysable for the different ages at treatment.

TABLE 1

NUMBERS OF CALYCOCYTES AND GONADS IN TREATED EMBRYOS OF D. MELANOGASTER FOR DIFFERENT AGES AT TIME OF TREATMENT

60–75 Min		79–86 Min		89–102 Min		103–119 Min		120–132 Min		124 Min	
Calyco-cyte	Gonad	Calyco-cyte	Gonad	Calyco-cyte	Gonad	Calyco-cyte	Gonad	Calyco-cyte	Gonad	Calyco-cyte	Gonad
74	2	76	0	60	0	65	0	82	1	72	2
73	0	76	2	60	2	62	2	75	2	60	2*
66	2	72	1	56	0	62	2	68	0	58	1
60	2	72	2	48	1	60	1	68	1	45	0
60	2	68	2	46	0	42	1	67	1	40	0
58	0	67	0	46	0	40	0	60	2	34	0
57	2	62	2*	42	0	38	2	60	2	32	2*
56	1	60	0	36	1*	36	0	60	2	23	0
55	1	56	2	32	0	35	1	58	2	22	0
54	2	56	0	32	0	34	2	56	2	14	0
54	2	51	2	32	0	30	0	51	1	0	0
54	0	50	0	30	0	28	0	40	2*		
54	2	48	2*	28	0	25	0	38	0		
52	0	40	2	24	0	20	0	26	0		
50	2	36	1*	24	0	18	0				
48	0	36	2	24	0	16	2				
42	2	14	2	20	0	0	0				
42	2	12	2	16	0	0	0				
38	0	7	0	14	0						
37	0	6	0	10	0						
34	0	0	0	6	0						
30	1	0	0	6	0						
0	0	0	0	3	0						
0	2	0	0	0	0						
0	0			0	0						
0	0			0	2						
				0	0						

* Gonads in which germ cell numbers were much reduced.

(i) *Calycocytes.*—The quantitative data on the effects of ultraviolet treatment on the middle midgut and the gonads are summarized in Tables 2 and 3 according to age at time of treatment. In Table 2 the mean numbers of calycocytes per embryo are given in column 4. Calycocyte numbers are markedly reduced in all groups as compared with the controls in which the mean was $71{\cdot}8 \pm 2{\cdot}7$ with a range of 62–83. The proportions of embryos showing calycocyte reduction are large throughout the series. Altogether 101 embryos out of 120 analysed showed reduction of calycocyte number in the middle midgut.

That these may be considered as the minimal effects on calycocytes will be evident by reference to Table 5 in which the numbers of calycocytes in control

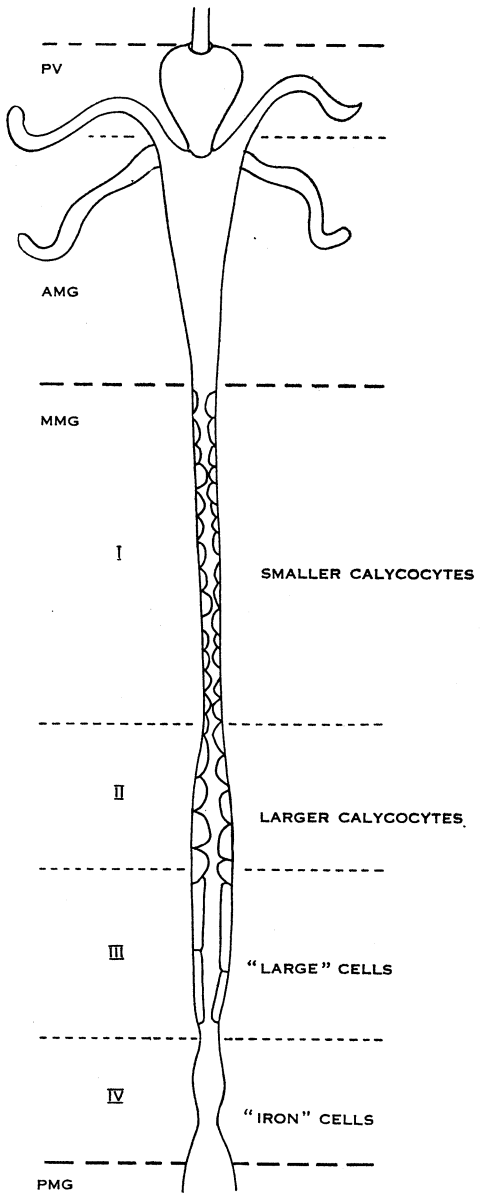

Fig. 2.—Diagram of a portion of the midgut of *D. melanogaster* indicating positions of calycocytes of the middle midgut (*MMG*) relative to the anterior midgut (*AMG*) and the posterior midgut (*PMG*). The "large" cells described in the text form the epithelium in region III between the larger calycocytes of region II and the "iron" cells of region IV.

embryos are presented along with data bearing on the sex of the embryos. In half these embryos the number of calycocytes is between 70 and 83, in the others between

TABLE 2

SUMMARY OF EXPERIMENTAL DATA ON CALYCOCYTE AND GONAD NUMBERS COMPARED WITH CONTROLS IN D. MELANOGASTER

Age at Treatment (min)	Dose (min)	No. of Embryos Analysed	Mean No. of Calycocytes	Range of Calycocyte Number	No. of Embryos with 60 Calycocytes or Less	Mean No. of Gonads per Embryo	No. of Embryos with Gonads Affected	Embryos Herniated (%)
60– 75	3	26	44·1±4·3	0–74	23	0·96	14	3·8
79– 86	3	24	40·2±5·7	0–76	17	1·00	15	8·3
89–102	2, 3	27	25·7±3·7	0–60	27	0·22	25	44·4
103–119	2	18	33·9±4·6	0–65	15	0·72	13	33·3
120–132	2	14	57·8±4·0	26–82	9	1·28	8	0
124	4	11	36·4±6·5	0–72	10	0·63	10	18·1
Controls	0	12	71·8±2·7	62–83	0	2	0	0

62 and 70. By the criteria used, a reduction of calycocyte number in one of the former to the range of the latter would be undetectable although it is highly probable that such must have occurred. The numbers of embryos involved are too small for the sort of statistical analysis which would be required to demonstrate this.

(ii) *Gonads.*—The effects on the gonads are also strikingly shown in Tables 1, 2, and 3. Most common is the complete absence of the gonads and germ cells. It was usually not possible in such instances to determine whether a gonad sheath was present and it seems probable, as discussed below, that the formation of a sheath is dependent on the presence of at least one pole cell in the appropriate part of the mesoderm. Cases of single gonads (unilaterals) were less frequent, there being 15 compared with 66 totally lacking gonads. However, these 81 do not represent the whole effect for in a number of instances germ cell numbers in gonads were much reduced. Five clear cases of this were recorded (marked with an asterisk in Table 1) among embryos with two gonads. Of these, four were among embryos with reduced calycocyte numbers, the other was in an embryo with normal calycocyte number. In these cases the number of cells per gonad was either 2 or 3 compared with the normal range (6–8 per gonad in females and 10–12 per gonad in males up to 18 hr; and higher numbers after the onset of pre-hatching mitoses—cf. Table 5). In only one group was the mean number of gonads per embryo above 1·00.

(iii) *Calycocyte–Gonad Correlation.*—While correlation between calycocyte and gonad reduction is apparent in Table 2 this is dramatically brought out in Table 3 in which the numbers and proportions of embryos affected (at different ages of treatment) with respect to *both calycocytes and gonads, calycocytes alone, gonads alone, and neither* are compared. This table was prepared from the data of Table 1 by summing up as affected for the different ages of treatment, all embryos with fewer than 60 calycocytes, and all with one or no gonad, together with the five cases of reduction of cell number in gonads described above. Thus, although calycocyte reduction is greater, the gonad effects parallel the calycocyte effects. The proportions of embryos in which calycocytes alone are affected are small. The proportion of unaffected embryos is generally less than 10 per cent. and we suspect that these escaped irradiation by virtue of being slightly misplaced in relation to the exposure slit.

To demonstrate the degree of correlation of the calycocyte and gonad effects, the mean numbers of missing calycocytes and gonads were determined from Table 2 for the different age groups and plotted to give Figure 3.

(iv) *Calycocytes and Free Cells.*—A feature of a large proportion of treated embryos was the presence in the lumen of the midgut of numbers of free cells clearly distinguishable from yolk cells (*F*, Plate 2, Figs. 2 and 3). These cells were generally very similar in size to pole cells and early calycocytes and clearly represented cells which have failed to enter the midgut epithelium. As Table 5 shows, such free cells are rarely present in normal embryos. The emptying of the gut in newly hatched larvae soon removes all traces of these as well as the yolk remnants. Counts of free cells could only be made in embryos or unhatched larvae. The results for the different ages at time of treatment are presented in Figure 4 in relation to the numbers of calycocytes present. An inverse relation between numbers of free cells

TABLE 3

RELATIONS BETWEEN CALYCOCYTE AND GONAD EFFECTS IN D. MELANOGASTER EMBRYOS OF DIFFERENT AGES AT TREATMENT

Cells Affected	60–75 Min		79–86 Min		89–102 Min		103–119 Min		120–132 Min		124 Min		Totals	
	No.	Fraction	No.	Fraction	No.	Fraction	No.	Fraction	No.	Fraction	No.	Fraction	No.	Fraction
Calycocytes and gonads	13	0·50	11	0·45	25	0·93	12	0·67	4	0·29	10	0·91	75	0·63
Calycocytes only	10	0·38	6	0·25	2	0·07	3	0·17	5	0·35	0	0	26	0·22
Gonads only	1	0·04	4	0·17	0	0	1	0·05	4	0·29	0	0	10	0·08
Neither affected	2	0·08	3	0·13	0	0	2	0·11	1	0·07	1	0·09	9	0·07
Totals	26	1·00	24	1·00	27	1·00	18	1·00	14	1·00	11	1·00	120	1·00

and calycocytes is apparent in all instances and is marked where older embryos (Figs. 4*C*, 4*D*) were treated. In the younger group (60–75 min together with 79–86 min) the relation appears roughly hyperbolic. During the period of pole cell formation (89–102 min) there is a wider scattering of points (Fig. 4*B*). In the next two groups, in which pole cell mitoses are numerous (103–119 min and 120–132 min), the linear relation becomes marked. In Figure 4*D* the 124-min age group of (double dose) embryos are included along with those receiving only the 2-min dose. Taken alone these show a clear inverse linear relation.

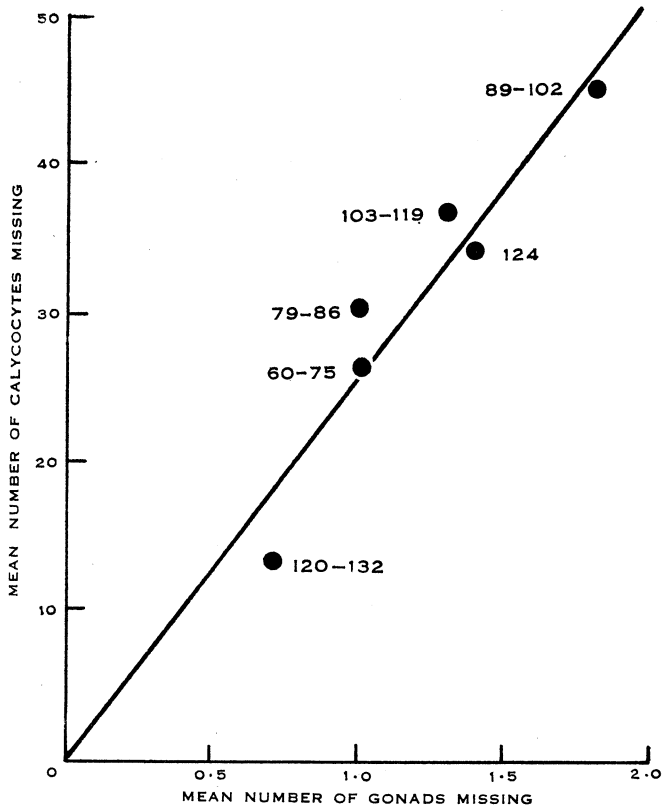

Fig. 3.—Relationship between numbers of missing calycocytes and numbers of missing gonads in embryos from eggs treated at the different ages (in minutes) indicated.

(v) *Effects of Age at Time of Treatment.*—To consider the effects of age at time of treatment it is necessary to describe briefly the basis for the age groupings used in Tables 1–3. The embryos analysed from the youngest group derive from eggs treated at 60, 62, 67, 70, or 75 min after laying respectively. This is before the time of migration of nuclei into the polar region and well before the formation of any pole cells (Rabinowitz 1941, Sonnenblick 1950). The treatments at these *early* pre-pole cell stages represent irradiations of the polar plasm alone.

In the second group (79–86 min) the treatments were given at 79, 83, 85, or 86 min respectively. Although nuclear movement into the polar region begins there is no pole cell formation during this period. Treatments at this *late* pre-pole cell stage also represent irradiations primarily of polar plasm rather than nuclei.

The third group (89–102 min) comprises embryos in which the movement of nuclei into the polar plasm has begun and there is active budding of pole cells. In these treatments, which were at 89, 91, or 102 min, many nuclei were in the region exposed to radiation.

In the next interval (103–119 min) pole cell formation is largely completed but pole cell mitoses continue. Most of the embryos in this group were close to 110 min of age when irradiated.

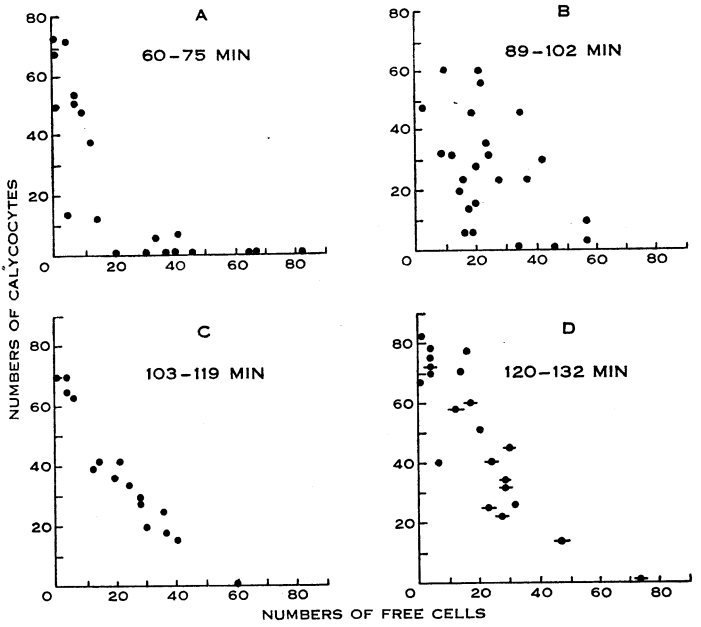

Fig. 4.—Relationship between numbers of calycocytes in the gut epithelium and the numbers of free cells in the lumen of the gut in embryos from the times of treatment indicated. These correspond to early pre-pole cell (*A*), late pre-pole cell (*B*), pole cell formation (*C*), and pole cell multiplication (*D*) stages respectively. —●— in *D* indicates the 124-min eggs which received a 4-min irradiation.

The older embryos are presented in two separate groups because some of them (124 min) were exposed to a double dose (4 min) compared to others (120 and 132 min) which received the 2-min dose. Pole cell mitoses continue in this period, near the end of which some of the inner pole cells begin the movement toward the underlying blastema layer. Shortly this becomes (156 min) a full-fledged migration to the interior of the egg.

From Tables 2 and 3 it is apparent that reduction of calycocytes is strong in the earlier periods, reaches its maximum during the period of pole cell formation

(89–102 min), and subsequently drops off markedly. For gonads the effects are seen to be least in the early period, but to reach their maximum simultaneously with the calycocyte effects in the third period. They drop in the two later periods, but not as low as at the earliest time. From Table 3 which presents the proportions of embryos showing both calycocyte and gonad effects, those showing only calycocyte effects, those showing only gonad effects, and those unaffected in either way, the total calycocyte effects and total gonad effects are readily obtained. The relationships of these are graphically presented in the summarizing figure (Fig. 6). The interpretation of these is dealt with in Section IV.

(vi) *Dosage Effects.*—Three different dosage levels were employed as represented by exposure times of 2, 3, and 4 min (Table 2). All the eggs of the first two groups were given 3-min exposures. The third group contained both 2- and 3-min exposures but, as no statistically significant difference was evident, they have been pooled.

TABLE 4

EFFECTS OF ULTRAVIOLET DOSAGE ON CALYCOCYTES AND GONADS IN D. MELANOGASTER

Dose (min)	Age at Time of Treatment (min)	Number of Embryos Analysed	Mean No. Calycocytes Missing	Mean No. Gonads Missing	Proportion Embryos Affected
2	120–132	14	13·3	0·72	0·29
4	124	11	34·7	1·37	0·91

Except for those given a 4-min exposure at 124 min all the remaining eggs received a 2-min treatment.

In terms of reduction in calycocyte number (Table 4) the effect of doubling of the dose at 124 min as compared with 120-132 min is nearly triple. The same is true when proportions of affected embryos are considered. However, in the case of the gonads the mean number of missing gonads is almost exactly doubled with the double dose. Thus the effects of the higher dose are disproportionately greater on calycocytes than on gonads at the only time for which adequate dosage data are available. An interpretation of this is offered in Section IV.

(vii) *Calycocyte Number and Sex.*—Tables 2 and 5 show that calycocyte numbers in the series of controls ranged between 62 and 83. It is evident from Table 5 that embryos with the lower numbers of calycocytes are characterized by large gonads whereas those with higher number have small gonads. Gonad size has been the sole means of distinguishing sex in larvae of *D. melanogaster* (Kerkis 1931) and also in embryos (Poulson 1937; Sonnenblick 1941, 1950). Large gonads are characteristic of males, small gonads of females. Although the control series available here is not extensive, the correlation between calycocyte numbers and gonad size is unequivocal. The embryo with 70 calycocytes was damaged in the region of the

gonads and size could not be established with certainty. Although the sexual dimorphism with respect to calycocyte number is not likely to be of much practical use in separation of the sexes it is the only non-gonadial sex difference so far detected in embryos and early larvae. Its existence makes it clear that not all of the calycocyte reduction produced by the ultraviolet treatments is apparent.

(viii) *Large Cells of the Lower Middle Midgut.*—During the examination of the embryos of the experimental series it was observed that the region of the middle midgut below the calycocytes is characterized by cells much larger than cells of

TABLE 5

CALYCOCYTES, "LARGE" CELLS OF MIDGUT, AND GERM CELLS IN D. MELANOGASTER CONTROLS AT 22 HR

No.	Condition	No. of Calycocytes	No. of "Large" Cells	No. of Cells Free in Gut Lumen	Gonads	
					Size	Av. No. of Germ Cells
1	Unhatched	62	15	0	Large	30
2	Unhatched	62	16	0	Very large	24
3	Unhatched	62	14	1	Large	14
4	Hatched	63	15	0	Large	20
5	Unhatched	66	14	0	Very large	20
6	Unhatched	70	16	0	—*	—
7	Unhatched	72	16	0	Small	8
8	Hatched	79	14	0	Small	10
9	Hatched	79	16	0	Small	10
10	Hatched	82	15	0	Small	16
11	Unhatched	82	16	0	Small	12
12	Unhatched	83	18	0	Small	—*

* Damaged during fixation.

any region of the midgut possessing correspondingly large nuclei. It was at first thought that these large cells might have been derived from pole cells or other cells whose division had been inhibited by the ultraviolet treatment. Counts of the large cells demonstrated them to be a persistent feature and remarkably uniform in number not only in the treated embryos, but also in the controls where their numbers ranged from 14–18 (Table 5). A compilation of the data for embryos in which large cells as well as calycocytes were counted is given in Figure 5. This includes data from control as well as treated embryos. Reduction in number of large cells is seen to be relatively infrequent and not correlated with reduction in calycocyte numbers.

These large cells give rise to the very flat epithelial cells of the lower middle midgut described by Strasburger (1932) for the larva. They appear to be derived from the tip of the posterior midgut invagination and their constancy in the experimental materials is strong evidence for localization of the ultraviolet treatment to the pole cells.

(*b*) D. melanogaster—*Other Effects*

Effects other than those which have been dealt with above, but less susceptible to quantitative treatments were found. In all instances of severe reduction in calycocyte number the midgut region failed to undergo the normal form change and remained sac-like (Plate 1, Fig. 4; Plate 2, Figs. 1–3). In those instances in which calycocytes were recognizable in the epithelium they were contiguous (Plate 1, Fig. 4; Plate 2, Fig. 1). Moreover, with the exception of one embryo which suffered extensive posterior damage, the structure of the gut epithelium was completely continuous throughout, demonstrating successful union of anterior and posterior midgut rudiments. This was also true in those embryos which suffered some dorsal herniation. Herniation was infrequent among the early treated eggs (Table 2), although about 30–40 per cent. was found among those of the middle periods (89–102 and 103–119 min). None was present among later-treated embryos except for about 18 per cent. among those receiving the double dose at 124 min. Aside from the mechanical displacements arising from herniation and the gut and gonad disturbances these embryos were essentially normal.

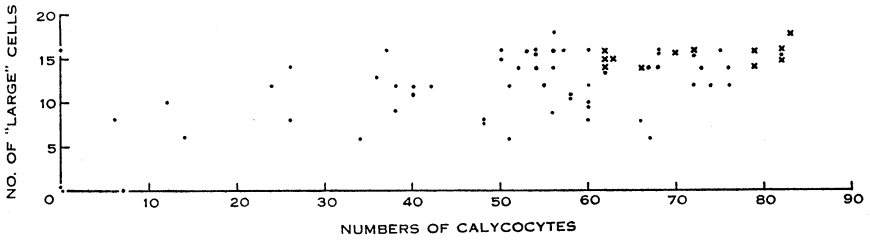

Fig. 5.—Relationship of "large" cell numbers to calycocyte numbers in treated (●) and control (×) embryos.

A few cases of extensive posterior damage were found. In these it was clear that rather more than the pole cell region was exposed to the ultraviolet and damaged pole cells remained in a yolky mass at the posterior of the egg (Plate 2, Fig. 4). In the embryo figured the gut is incomplete and the peritrophic membrane is pushed out from above into the external yolk mass.

An interesting and unusual abnormality, found in a few embryos only, consisted of inverted malpighian tubules growing into the lumen of the gut (Plate 2, Fig. 5) instead of into the haemocoele. In the instance figured all four of the malpighian tubules can be followed. No instances of complete absence of malpighian tubules were encountered except in the few cases of extreme posterior damage.

(*c*) L. cuprina

The effects of the treatments of pole cells on the midgut of *L. cuprina* parallel very closely those described for *D. melanogaster*. The principal quantitative data are derived from counts of cuprophilic cells in the midguts of larvae which survived the treatment and, on being fed liver, attained the third instar. Cuprophilic cells were counted with the aid of the neotetrazolium procedure (Waterhouse and Stay 1955) by which they are readily distinguished from the adjacent lipophilic cells.

The data, summarized in Table 6, show a statistically significant reduction in cuprophilic cells in these surviving larvae. If the range in the controls is an expression of sex dimorphism then the effect may be rather greater than the table indicates. By itself this table is not very impressive, but taken together with data from sectioned material of unhatched larvae and embryos from the same and other series of treated eggs it is certainly quite significant. These showed conditions closely resembling those found in *D. melanogaster*. Characteristic features were the presence of considerable numbers of free cells in the gut lumen (Plate 4, Figs. 2, 3, and 4), the failure of the middle midgut to change from sac to tube (Plate 4, Figs. 3, 4, and 5), and the presence of free pole cells in the posterior yolk (Plate 4, Fig. 4) in more seriously damaged embryos.

TABLE 6

L. CUPRINA: NUMBERS OF CUPROPHILIC CELLS IN THE MIDDLE MIDGUT OF THIRD-INSTAR LARVAE FOLLOWING ULTRAVIOLET TREATMENT IN THE EGG STAGE

Age (min)	Dose (min)	No. of Larvae	Mean No. of Cuprophilic Cells	Range
65–73	2	10	137·6±5·5	105–167
77–87	2	18	137·1±7·1	78–195
120	2	5	118·0±7·7	94–135
Controls	0	23	152·7±5·5	99–210

L. cuprina was most useful in determining the localization of the treatment in the polar region and in following the immediate effects of treatment. In one series of experiments eggs were treated during the period of pole cell budding and division, 71–79 min after oviposition, and embryos were fixed at intervals of 30, 60, and 90 min as well as at time of hatching of normals. The degree of localization of the ultraviolet in the polar region is strikingly clear in Plate 3, Figures 1, 2, and 3, in which the limits of the treated region are clearly demarcated.

The effects of the treatment of the pole cells are apparent in these same figures: cessation of mitosis and growth, vacuolization, clumping of chromatin, and some fragmentation of cytoplasm. At 1 hr following treatment there is in addition an increased basophilia of cytoplasm in the unfragmented pole cells (Plate 3, Fig. 4; Plate 4, Fig. 1) which enables them to be recognized more readily than in untreated controls.

Gonads were also strongly affected by the treatment. Although the data from sectioned material is not extensive enough for tabulation the following statements can be made about the effects on gonads. In the early-treated material (67–81 min, budding of pole cells, and mitosis) there were four gonads among 16 embryos, a mean of 0·25 per embryo, while among later-treated eggs (95–107 min, beginning

of blastoderm formation) there were 13 gonads in 14 embryos, a mean number of 0·93. In these instances the dosage was the same, 2 min. Control embryos fixed at the same times all contained two gonads each. In about half (three) of the controls the average number of germ cells per gonad was 24–28 whereas in the remainder (four) it was 13–16 per gonad. These counts were made before the time of onset of mitosis in later stages. Thus the sexual dimorphism in gonad size has its basis, as in *Drosophila*, in smaller or larger numbers of initial germ cells which enter the gonads.

In comparison with *D. melanogaster* the rate of embryonic development of *L. cuprina* under the conditions maintained during these experiments (25°C and 75–80 per cent. humidity) is considerably greater and larvae hatch is approximately 14 hr from oviposition. No pre-pole cell stages were treated in *L. cuprina* and no blastoderm stages were treated in *D. melanogaster*.

IV. Discussion

The results described above, and summarized for *Drosophila* in Figure 6, are sufficiently clear to require little elaboration. However, they have an important bearing on problems relating to the determination and fate of the pole cells.

From the time that the term "pole cells" was applied to them by Weismann (1863) the significance of these cells aroused interest. Once Metschnikoff (1866) had traced them to the gonads in *Miastor* they provided the most striking and diagrammatic example of the separation of the germ line from the soma and were so employed by Weismann (1885) in his classical exposition. Later studies, primarily on lower Diptera, confirmed those of Metschnikoff and called attention to characteristic inclusions in the polar plasm (Ritter 1890; Kahle 1908; Hasper 1911), usually granular, which were always incorporated in the pole cells and the derived germ cells and which from then on were referred to as germ cell determinants (Hegner 1908, 1914). With the passing of the hey-day of organ-forming substances and particularly since the work of Huettner (1923) on *D. melanogaster*, in which the whole of the early work on pole cells and their relation to the gonads was reviewed, the term polar granules has been employed to refer to these inclusions. Still, the aura of determinism surrounding the particles has been persistent and will probably continue until their real function is better understood. The recent studies of Counce (1959) on several *Drosophila* species suggest an early breakthrough in this respect.

Huettner (1923) recognized, and his students Rabinowitz (1941) and Sonnenblick (1941, 1950) demonstrated that, despite the presence in them of polar granules, only a fraction of the pole cells in *D. melanogaster* reach the gonads. Both Huettner and Rabinowitz interpreted the early entry of a group of the pole cells to the interior of the egg just before blastoderm formation as the source of secondary yolk cells and considered the conspicuous group of pole cells which enter the posterior midgut invagination the source of the germ cells of the gonads. As some of these pole cells were observed in between cells of the midgut rudiment they were interpreted as passing through the gut wall in their migration to the gonads (Aboim 1945; Sonnenblick 1941, 1950) whereas others remained lost in the gut. The interpretation that, instead of being lost, the majority of pole cells become incorporated

as part of the midgut epithelium was put forward on the basis of descriptive embryology (Poulson 1947). Subsequently it appeared increasingly unlikely that passage

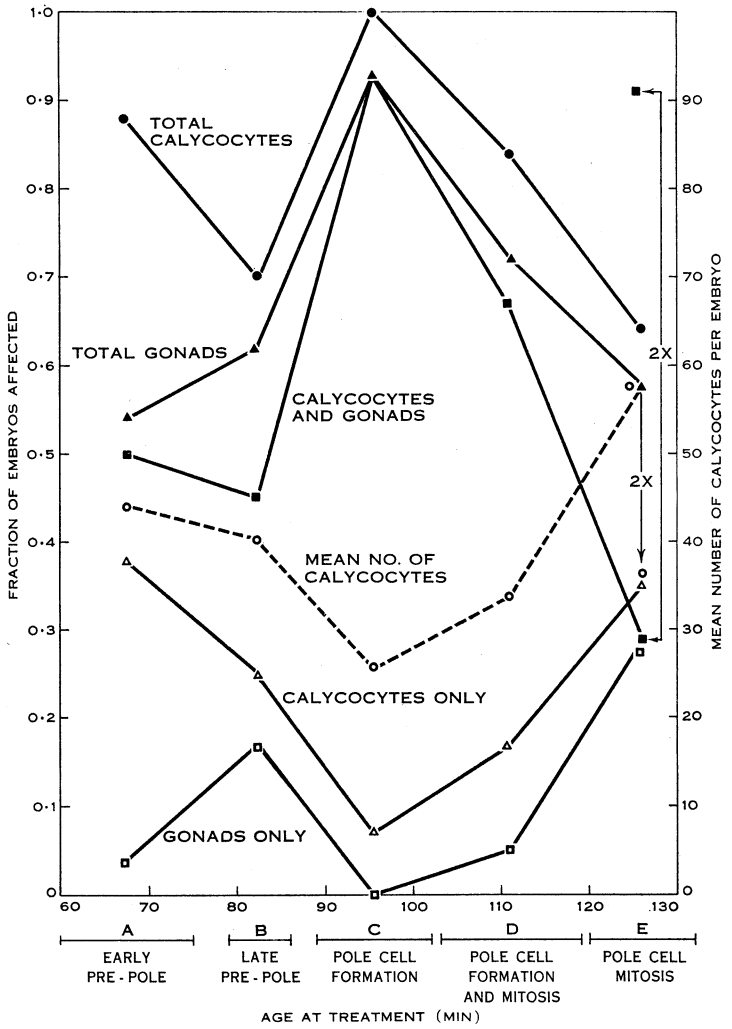

Fig. 6.—Graphic summary of effects of treatment at different ages. □ Embryos with gonads only affected; △ embryos with calycocytes only affected; ■ embryos with calycocytes and gonads affected; ▲ total gonad effects; ● total calycocyte effects. Broken line unites mean numbers of calycocytes per embryo for different times of treatment. Effects of the double dose on calycocyte number and on fraction of embryos affected is indicated at the right (2 ×). *A*, the period 60–75 min is early pre-pole cell before entry of any nuclei into polar plasm; *B*, the period 79–86 min is pre-pole cell with nuclei beginning to enter polar plasm; *C*, the period 89–102 min is that of active pole cell formation; *D*, the period 103–119 min includes pole cell formation and mitosis; *E*, the period 120–132 min is chiefly one of pole cell mitosis.

of pole cells through the midgut represented the path to the gonads and much more probable that the early migration (pre-blastoderm) pole cells provided the source

of the germ cells of the gonads (Poulson 1950). Evidence from several lethal mutants in *D. melanogaster* strongly supports this view (Counce and Ede 1957; Poulson, unpublished data), as does a consideration of the evidence from the comparative embryology of Diptera.

In the lower forms (Nematocera) in which the pole cells have been traced directly to the gonads (*Chironomus* (Ritter 1890; Hasper 1911), *Miastor* (Metschnikoff 1866; Kahle 1908), *Sciara* (DuBois 1932; Butt 1934), *Simulium* (Gambrell 1933)) the total number of pole cells is small. These migrate to the interior before or during the blastoderm stage, and there is no association with the posterior midgut rudiment. Development of the egg is usually slow occupying several days or more.

Among the higher forms (Brachycera) which have been studied embryologically (*Calliphora* (Noack 1901; Pauli 1927), *L. sericata* (Fish 1947), *L. cuprina*, this paper, *Melophagus* (Lassman 1936), *Musca domestica* (Escherisch 1900; Reith 1925; Pauli 1927)) many pole cells are formed and there is an early inward migration similar to that observed in *Drosophila* as well as association of the majority of pole cells with the posterior midgut rudiment. Development in the egg is rapid and usually completed in less than 24 hr. An apparent exception to this may be *Phormia regina* in which according to Auten (1934) the early migrating pole cells go to the gonad and no pole cells appear to be associated with the posterior midgut. This case requires further investigation.

The only experimental evidence prior to that reported here which bears on the time of separation of the germ cells from the remaining pole cells is that of Geigy (1931). Geigy treated eggs of two different stages, "blastema" which corresponds to the later stages of pole cell formation and multiplication, and "blastoderm" at which time the first entry of pole cells is nearing completion. Of the 30 adults which he obtained from treatment in the blastema stage, 16 were wholly castrated (no gonads) and one partially castrated (one gonad), a mean of 0·9 gonads per fly. From treatment of blastoderm stage he obtained 13 adults of which five were partially castrated (one gonad) and none totally castrated (a mean of 1·6 gonads per fly). Although this difference might be attributed to shielding of some cells by others at a stage when they are more numerous, or as a drop in sensitivity to irradiation, it is most probably a reflection of the fact that a large fraction of the germinal pole cells have already passed to the interior of the egg. Moreover, mortality was very high in both stages, 0·63 at blastema and 0·91 at blastoderm, indicating either inadequate localization of treatment or persistent damage to cells other than germinal in their developmental fate. Since Geigy examined embryos only for immediate damage to pole cells and we did not attempt to raise any of our survivors to adulthood it is difficult to make closer comparison. The developmental study by Aboim (1945) gives no frequency data.

Our data (Fig. 6, total gonads) show that gonad effects are low when pre-pole cell stages are treated, that they rise as pole cell budding approaches, are at their maximum during budding, and decline in the later periods. Geigy's blastema stage probably corresponds to our 103–132-min groups, and his blastoderm stage is later than any used in our experiments with *Drosophila* although comparable to the later stages treated in *Lucilia*.

Unfortunately, the fact that our embryos and larvae were fixed at the time of hatching rather than before the onset of mitotic activity in the gonads between 16 and 18 hr makes it impossible for us to assess the gonad effects directly in terms of numbers of germ cells, except in a few extreme instances. Hence all the data have been expressed in terms of number of gonads per embryo. All the available data support the interpretation that the germ cells in *Drosophila* and in *Lucilia* are derived from the early migrating pole cells.

In the agonadic embryos very careful search for evidence of germ cells and empty gonad sheaths was made. While it is conceivable that such escaped attention, it is unlikely. In no instance of complete absence of germ cells were traces of gonad sheath found. In those exceptional cases where only two or three germ cells were present per gonad the sheath cells closely enveloped them. There was no evidence in any of these of a large sheath deficient in germ cells. It appears overwhelmingly likely that the sheath arises in response to inductive action of the germ cells which, in normal circumstances, reach the lateral mesoderm and aggregate. Other evidence for inductive action of germ cells was found by Counce and Selman (1955) in eggs subjected to sonic treatment and by Counce and Ede (1957) in the lethal mutant, *nas*^{4} of *D. melanogaster*. Thus the agametic gonads found by Geigy (1931) and studied in detail by Aboim (1945) most probably arose through the action of less severely damaged pole cells reaching the embryonic mesoderm. Such cells might be fully capable of inductive action without capacity for further division. Evidences of damaged germ cells in gonads of treated embryos were found in *Lucilia* as well as in *Drosophila*. The inductive potency of dead or dying cells is a common phenomenon in embryogenesis.

Among the unsolved problems are: what determines which pole cells engage in early migration and how many do so in a given embryo? It may be that the first signs of sex differentiation are to be found in the numbers of early migrating pole cells at $2\frac{1}{2}$ hr, about 7 hr before the appearance of the definitively ensheathed gonads at $9\frac{1}{2}$–10 hr as described by Sonnenblick (1941, 1950). It is suggestive that Rabinowitz (1941) reported variable numbers moving into the posterior midgut invagination. Seen in the light of our data on different modal numbers of calycocytes in the two sexes these points are clearly worth further experimental study.

Correlation of deficiency of cuprophilic cells with damage to pole cells might be interpreted as a direct relation: pole cells becoming differentiated into cuprophilic cells; or as an indirect relation: pole cells inducing cuprophilic differentiation in other cells of the middle midgut. While each hypothesis has its merits, the former is better supported by our quantitative data for *Drosophila*, i.e. the inverse relation between calycocyte and free-cell numbers, together with sex dimorphism in numbers of calycocytes.

The high proportion of calycocyte effects in *Drosophila* from treatments prior to the entry of nuclei into the polar plasm is suggestive of localization in the outer or more cortical polar plasm. The relatively low proportion of gonad effects from these early treatments indicates either recovery, avoidance by the entering nuclei of the more severely treated peripheral regions, or preferential formation of germinal cells. At the early time the polar granules form a somewhat dense (truncated

conical) arrangement (Counce 1959) which might provide effective shielding of the deeper polar plasm. These polar granules in *D. melanogaster* are rich in ribonucleic acids as has been shown by ribonuclease treatment (Poulson and Hilse, unpublished data) which removes them, along with other basophilic granules, from the cytoplasm. This is in agreement with the findings of Mulnard (1954) for the bruchid *Acanthoscelides obtectus* Say and the more recent reports of Bhuiyan and Shafiq (1959) on *Musca vicina* Macq. and Nicklas (1959) on *Miastor* sp. In the latter Nicklas found evidence for some protein as well as nucleic acid in the granules.

Since approximately 90 per cent. of the radiation from the source was of wavelength 2536 Å the principal absorbing materials were probably nucleic acids. Before the entry of nuclei to the polar plasm these must have been the cytoplasmic nucleic acids (ribonucleic acids) of the polar granules. However, it must not be forgotten that other materials (e.g. cell proteins) also absorb this wavelength. The shielding effect of the outer on the inner polar granules must become less effective as the granules become distributed among the initial pole cells during the process of budding and during the ensuing mitoses. Nuclear absorption becomes a factor from this time on and, with further mitoses and increasing numbers of pole cells, the inner cells are increasingly shielded by the more external pole cells. These points are of importance in interpreting the gonad and gut effects and the changes in their proportions at different times of treatment.

The correlation of gonad and calycocyte effect is most nearly complete at the time of pole cell budding (89–102 min). All embryos with affected gonads have affected calycocytes and the gonad and calycocyte reductions in terms of mean numbers per embryo are greatest. Only a small fraction of embryos with affected calycocytes showed no measurable gonad damage (Table 3, Fig. 6). In the subsequent periods there is a marked drop in all effects and an increased independence of gonad and calycocyte effects. That this may be a consequence of increased shielding as well as of physical separation when the future germ cells begin their inward migration is suggested by the dosage effect (Tables 2 and 4; Fig. 6). Only by treatment at an even later stage when the first migration is complete would it be possible to distinguish between these.

The decrease in the degree of calycocyte reduction from its maximum (at 89–102 min) to the minimum observed (120–132 min) appears to be a reflection of increasing pole cell numbers during this period. That pole cell mitoses are nearly completed by 132 min is indicated by the nearly linear inverse relation between calycocyte and free cell numbers (Fig. 4*D*) at the two dosage levels employed.

The dosages employed require a brief comment. From the observations on *Lucilia* eggs at short intervals following treatment (Plate 3, Figs. 1–3) and from later stages of both *Lucilia* and *Drosophila* it is clear that the dosage levels used were such as to allow the majority of treated cells to remain intact. But for this the reciprocal relations between calycocytes and free cells (Fig. 4) could not have been established.

It is of interest to compare these dosages with those which have been employed in experiments on mutagenesis in *D. melanogaster* (Altenburg, Altenburg, and Baker 1952; Muller *et al.* 1954). The dosage rate employed by the former was 100 μW

sec/cm²/min. Such a dose for 8 min (800 μW sec/cm²) was the lowest giving a sex-linked lethal mutation rate ($1 \cdot 4 \pm 0 \cdot 5$ per cent.) readily detectable. Doubling the dose gave an increase to $7 \cdot 9 \pm 2 \cdot 5$ per cent., but further increase in dose gave little further increase. Our basic treatment of 2 min represents a dose of about 1200 μW sec/cm² while the double dose was about 2400 μW sec/cm². Thus one must conclude that dosages in the range used in experiments on mutagenesis result in a very high degree of damage to pole cells (and to other unshielded areas of the egg). The failure to obtain increased mutation rates at higher doses (Muller *et al.* 1954) is scarcely surprising.

Our data have another bearing on the mutagenic work on pole cells. This is the importance of the age of the embryo at which treatment is given. Clearly exposure at later ages will give lower rates, particularly if increased dosage is brought about by increasing exposure time rather than by increasing intensity, a fact previously appreciated by Muller *et al.* (1954) on other grounds. Since the "polar cap" stages employed in the mutagenic studies were no younger than 120–135 min after fertilization and our studies indicate that the germ cells are already beginning their inward migration during this period it is evident that in the later polar caps there will be relatively few germinal pole cells susceptible to radiation for they will be well within the egg. It is desirable therefore to add to the set of suggestions for future work on mutagenesis in pole cells (Muller *et al.* 1954) the recommendation that earlier stages (90–100 min), at which germinal pole cells are maximally exposed, be employed.

In our experiments the effects of doubling the dosage in the later period was much greater on the reduction of the calycocytes than on the gonads. It appears from the essentially maximal effects of the 2-min dose for eggs of 89–102 min that this is a near optimal dose for experimental studies of the type undertaken here.

The bearing of these experiments on the genesis and structure of the larval middle midgut is considerable. The relationship between the non-germinal pole cells and the cuprophilic cells in *Lucilia* and the calycocytes in *Drosophila* is clearly demonstrated. One of the puzzling differences between *Lucilia* and *Drosophila* has been the apparent absence in the latter of the striking mosaic arrangement of cuprophilic and lipoid cells of the former, although Waterhouse and Stay (1955) reported that histochemical tests revealed that two cell types did, in fact, occur in *Drosophila* larvae. Another is the absence of the goblet cell type in *Lucilia* and related genera as compared with drosophilids. While the present studies cast very little light on the latter point they do clarify the former. A careful study of the preparations, control as well as experimental, shows that the calycocytes of *Drosophila* embryos are interspersed among other cells with which they clearly alternate in certain of the lower sections of the middle midgut (Plate 1, Figs. 2 and 3). We refer to the other cell type as *interstitial* although the experimental evidence is all in favour of the insertion of the calycocytes between the original cells of the posterior midgut rudiment at its junction with the anterior midgut rudiment. Evidences of failure to become incorporated into the epithelium are clearly present in Plate 2, Figures 1 and 3. Occasional free cells in the lumen show vacuoles suggestive of stages in the formation of the goblet of the calycocyte. The mosaic arrangement

of calycocytes and interstitial cells in the young larva bears a close resemblance then to the mosaic condition of the later larval stages of *Lucilia*. Since treated embryos of *Lucilia* show essentially the same condition of free cells and incompletely differentiated middle midgut (Plate 4, Figs. 2 and 3) the mosaic arrangement appears to originate in both as a consequence of the manner in which the pole cells enter the primitive midgut epithelium. As the larva of *Drosophila* grows, the calycocytes, large to begin with, increase disproportionately to the interstitial cells which become relatively inconspicuous in the later instars so that the original appearance of a mosaic disappears.

Although the calycocytes have been referred to here as goblet-like because of their shape, they appear to bear little resemblance to the rather differently shaped goblet cells of larval Lepidoptera (Waterhouse 1952). In fact, cells closely similar to calycocytes have not been reported outside the Drosophilidae.

The presence in the embryonic midgut of *Drosophila* of a previously unrecognized cell type, the "large" cell is clearly demonstrated in this material. These cells, characteristic of the lower middle midgut below the calycocytes, remained remarkably constant in number in the treated as well as in the controls (Fig. 5), indicating that the cell group from which they take their origin was rarely subject to the radiation employed in these experiments and that their origin in the embryo is quite independent of the calycocytes. In the later larva these become the very large flat cells of the lower middle midgut epithelium described by Strasburger (1932).

In a few of the embryos of *Drosophila* the presence of "inverted" malpighian tubes was encountered (Plate 2, Fig. 5). All four tubules were found to be directed into the lumen of the midgut, demonstrating that morphogenesis of these organs can prceed even in such an abnormal spatial orientation. No case of missing malpighian tubules or other evidence of direct radiation damage to malpighian tubules was found. These observations and the comparative rarity of other abnormalities (except in the cases of extreme posterior damage) indicate a high degree of localization of the radiation treatment in these experiments. It is probable that much of the herniation encountered was non-specific in origin as Goldman and Setlow (1956) observed in their work on whole egg irradiation.

The failure of larvae with greatly reduced numbers of cuprophilic cells (*Lucilia*) or calycocytes (*Drosophila*) to hatch or to grow and metamorphose (most die within 24 hr of hatching if they hatch at all) suggests that these cells are essential to larval life in these two organisms. Further investigations of their structure and functions are clearly desirable.

In other animal groups relationships between endodermal rudiments and the primordial germ cells have been the subject of considerable study and speculation. In the vertebrates in particular a good deal of controversy has revolved around the question of the origin of the germ cells. The subject has been reviewed by Nieuwkoop (1949) and Burns (1955). The evidence indicates an intimate relationship between the earliest germ cells and endoderm. Recently Mintz (1959) has traced the germ cells of the mouse step by step from the yolk sac endoderm into the mesoderm where the definitive gonad is differentiated. The parallel to the situation in *Drosophila*

and *Lucilia* is thus remarkably close. While it is far from clear, either in vertebrates or insects, precisely what factors operate in deciding whether a given cell becomes a germ cell or remains as part of the endoderm, further studies on insect material offer considerable promise for the solution of this basic problem.

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EXPLANATION OF PLATES 1–4

PLATES 1 AND 2

All figures are of *D. melanogaster* embryos fixed in formol–alcohol–acetic acid at 24 hr after laying, sectioned at 7 μ, and stained with Harris's haematoxylin. The magnification for all figures is in Figure 2 for Plate 1 and in Figure 4 for Plate 2. Embryos in Plate 1, Figures 1, 3, and 4, and Plate 2, Figures 1, 2, and 4, were irradiated for 2 min, in Plate 1, Figure 2, for 3 min, and in Plate 1, Figure 4, for 4 min. In Plate 1, Figures 1, 2, and 3 show no effects of treatment and are comparable with control embryos. *C*, calycocytes; *F*, cells free in lumen; *I*, interstitial cells; *IMT*, involuted malpighian tubules; *L*, "large" cells; *MMG*, middle midgut epithelium; *PM*, peritrophic membrane; *YF*, free cell in yolk herniated from gut

PLATE 1

Fig. 1.—Longitudinal section. Calycocyte region in embryo with 67 calycocytes. Irradiated 132 min after laying.

Fig. 2.—Longitudinal section showing calycocytes and interstitial cells. Irradiated 67–70 min after laying.

Fig. 3.—Transverse section of posterior calycocyte region showing calycocytes and interstitial cells. Irradiated 120 min after laying.

Fig. 4.—Calycocytes and interstitial cells in embryo irradiated at 102 min developmental age.

PLATE 2

Fig. 1.—Free cells in gut lumen. This embryo had 22 free cells and 56 calycocytes and had been irradiated at 102 min developmental age.

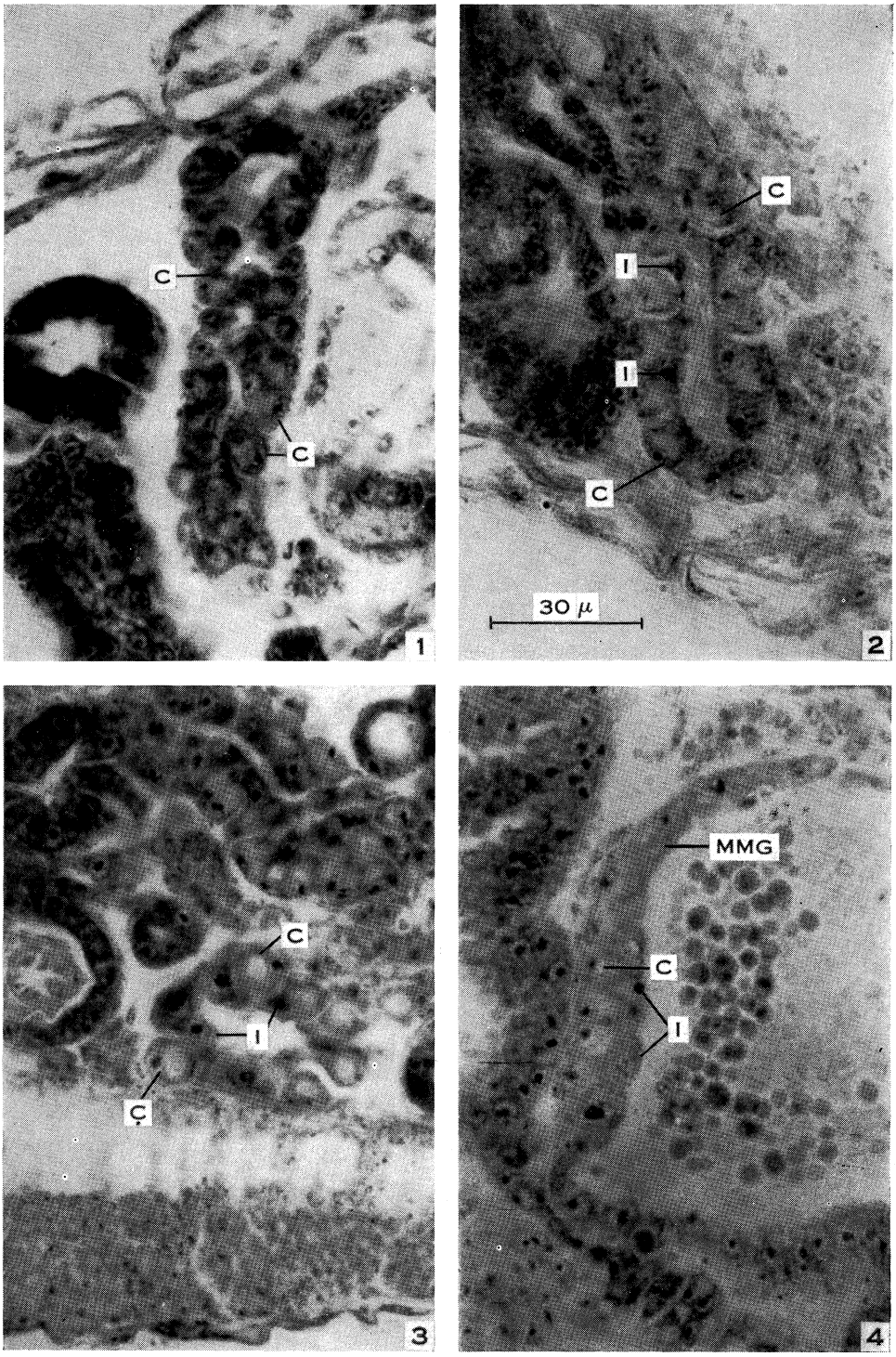
Fig. 2.—Free cells in yolk in embryo with few (less than 20) calycocytes. Irradiated at 90 min after oviposition.

Fig. 3.—Free cells and partially digested yolk in gut lumen. Irradiated 124 min after laying.

Fig. 4.—Free cell in yolk herniated from gut. The peritrophic membrane is also visible. Irradiated 102 min after laying.

Fig. 5.—Invaginated malpighian tubules from embryo irradiated for 2 min at 102 min developmental age.

POLE CELLS AND MIDGUT DIFFERENTIATION IN DIPTERA



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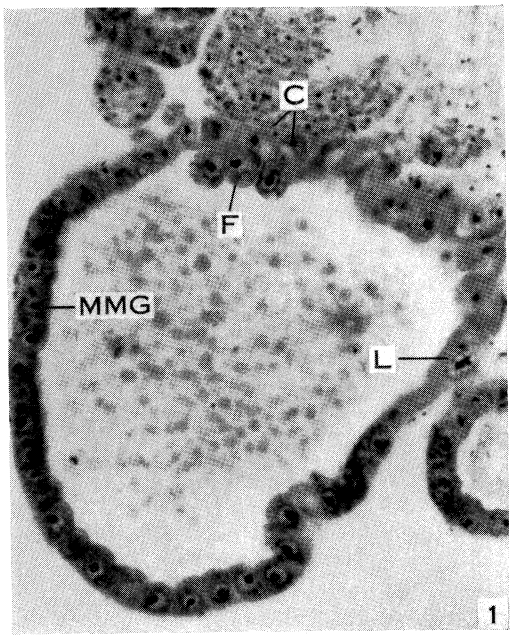

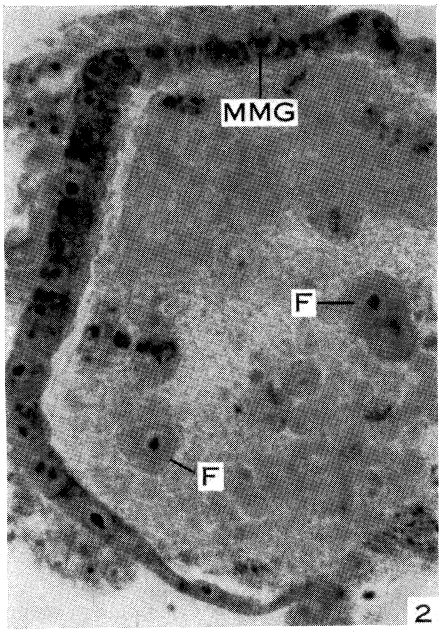

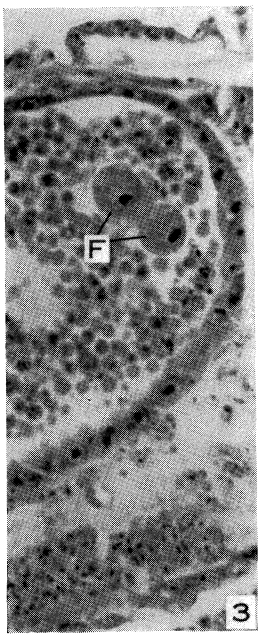

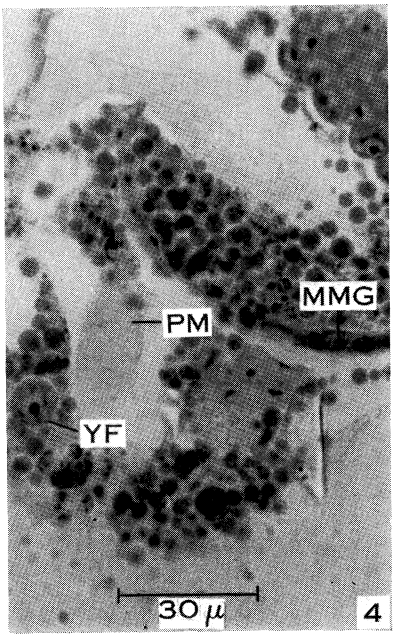

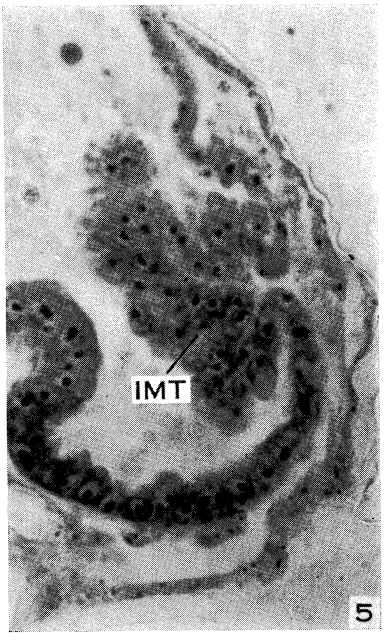

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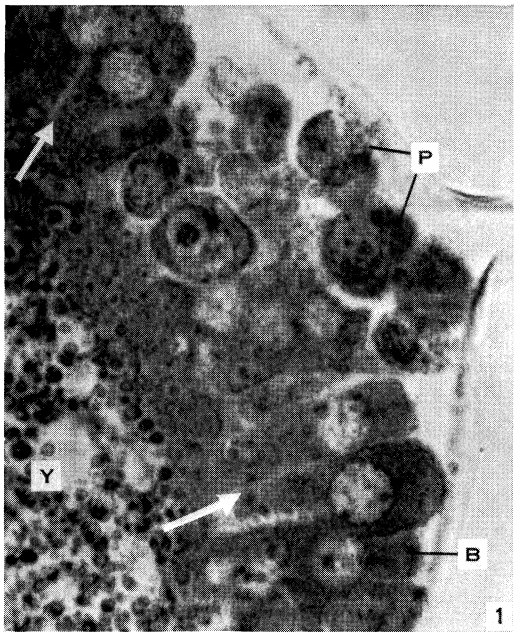
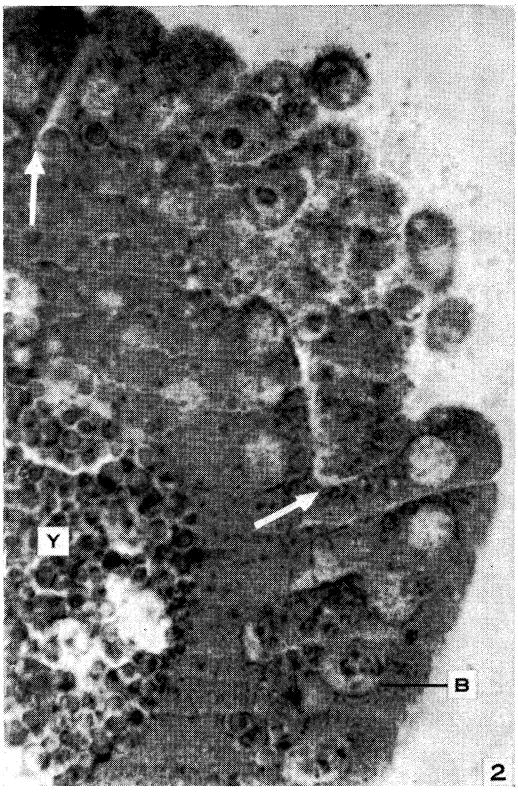
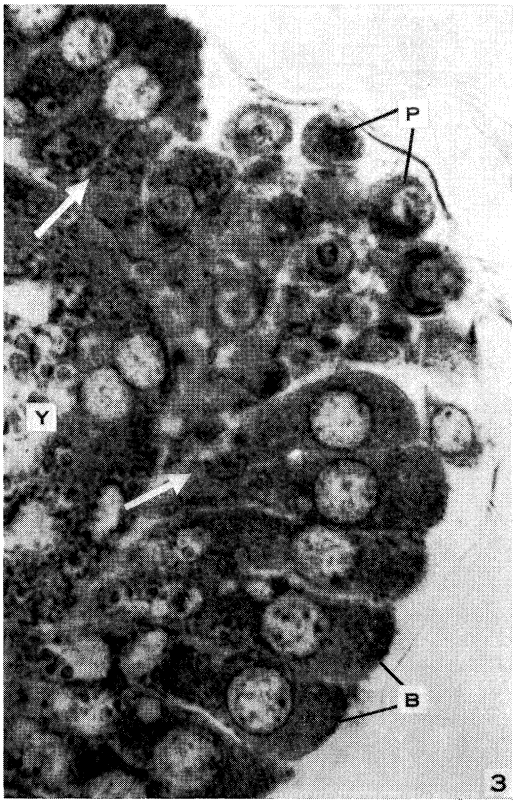
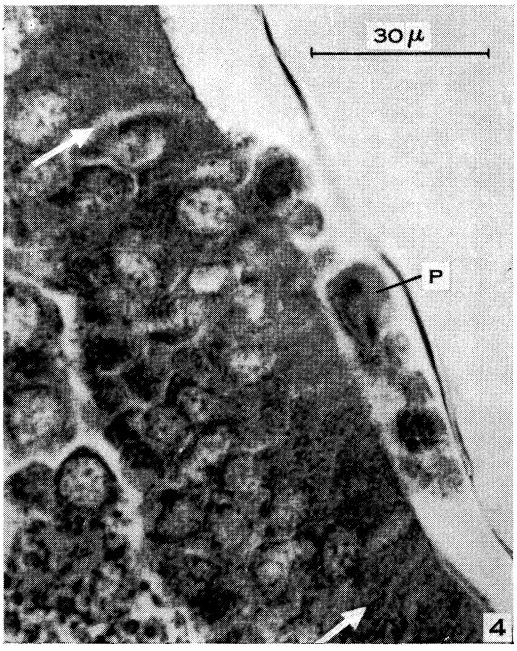
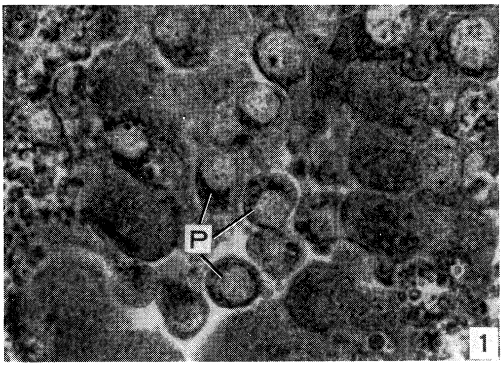


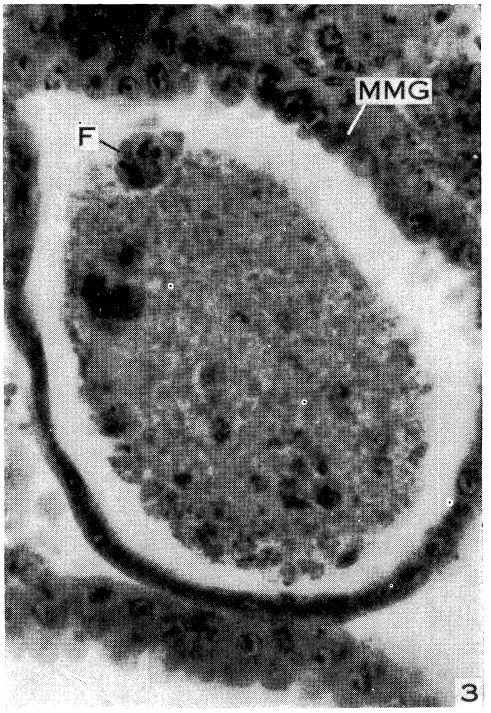


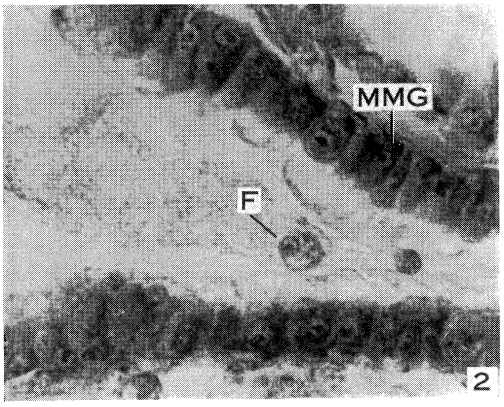


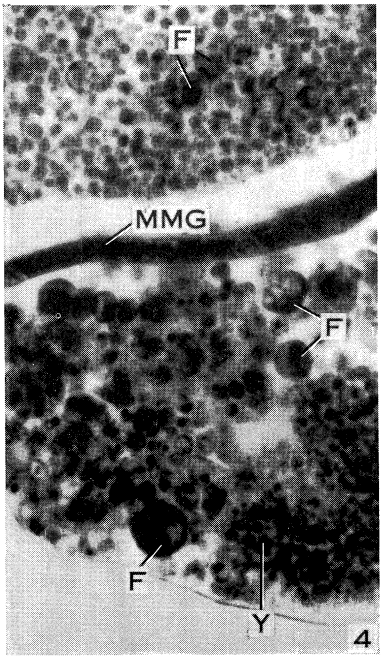


POLE CELLS AND MIDGUT DIFFERENTIATION IN DIPTERA



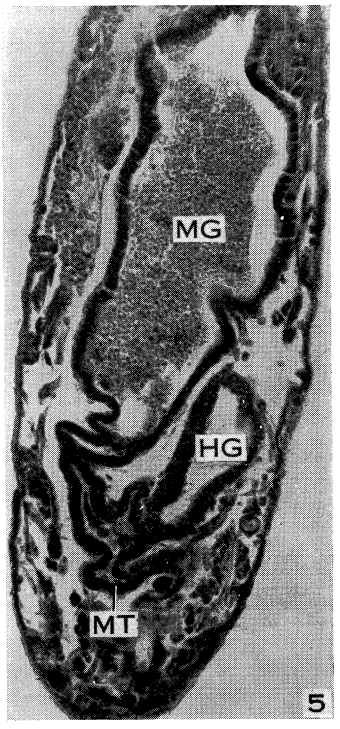






FIGS. 1-4
30 μ

FIG. 5
100 μ



Plates 3 and 4

All figures are of *L. cuprina* embryos fixed in formol–alcohol–acetic acid, sectioned at 9 μ, and stained with Harris's haematoxylin. Embryos in Plate 3, Figures 1–4, and Plate 4, Figures 1, 2, and 4 were irradiated for 2 min, in Plate 4, Figures 3 and 5, for 4 min. Scale of magnification for Plate 3 given in Figure 4. *B*, blastoderm; *F*, cells free in lumen; *HG*, hindgut; *MG*, midgut; *MMG*, middle midgut epithelium; *MT*, malpighian tubules; *P*, pole cells; *Y*, yolk

Plate 3

Figs. 1–3.—Longitudinal sections showing limited, but progressively smaller zones of ultraviolet damage (indicated by arrows) at the posterior poles of three eggs. Figures 1 and 3 irradiated at 71–73 min and Figure 2 at 77–79 min after laying. Fixed at 92 min.

Fig. 4.—Treated pole cells near posterior pole of egg. Irradiated 71–73 min after laying. Fixed at 132 min.

Plate 4

Fig. 1.—Pole cells in posterior midgut invagination. Irradiated 71–73 min after laying. Fixed at 132 min.

Fig. 2.—Free cells in gut lumen. Irradiated 65–67 min after laying. Fixed at 24 hr.

Fig. 3.—Free cells in gut lumen. Irradiated 73–77 min after laying. Fixed at 24 hr.

Fig. 4.—Free cells and partially digested yolk in gut lumen (above). Posteriorly herniated material containing free cells can be seen in the lower half of the photograph. Irradiated 65–67 min after laying. Fixed at 24 hr.

Fig. 5.—Horizontal section of embryo fixed at 24 hr, showing general details of midgut, hindgut, and entry of malpighian tubules. Irradiated 65–69 min after laying.