tone, dehydrated and imbedded in paraffin by the method of Gomori (*3*). Sections of 4–6 μ thickness are baked on slides with glycerine egg albumin, then washed in toluene, graded alcohols, and finally in water. They are then incubated for 48 hr at 37° C in one of the following solutions, each at 0.4% strength: pectinase (Nutritional Biochemical Company or Rohm and Haas), pectinol O (Rohm and Haas), pectin esterase (Rohm and Haas), polygalacturonase (*4*), ß-glucuronidase (*1*).

The pH of the solution is adjusted to 4.0 by acetate-acetic acid buffer and checked with a Beckman pH meter. A crystal of thymol is added to each solution to inhibit bacterial growth.

After the period of incubation the slides are washed in running tap water for 5 min. The sections are stained with hematoxylin and eosin, or with the acid orcein stain for elastic tissue, or colored by the periodic acid-Schiff's reagent (PAS) method (*7*), with and without a counterstain of hematoxylin. Usually, each set of sections has been stained or colored by the three methods.

There is a loss of PAS-positive materials—mucin, glycogen, reticulin of spleen and lymph node, ground substance of cartilage, hyaline, etc.—with pectinase solutions. With pectinol O the removal is not so complete as with pectinase although qualitatively similar. Polygalacturonase removes about the same amount as pectinase. Pectin esterase does not remove PAS-positive material but enhances the coloration. ß-Glucuronidase does not remove PAS-positive material. Diastase in 1% solution at pH 6.8 removes everything, and usually the section from the slide with 48-hr incubation at 37° C. Pectinase solution does not remove nuclear material or elastic tissue.

Two effects of pectinase solutions are to be differentiated—one morphological and the other histochemical. The removal of hyaline, while leaving nuclear material and elastica, allows something like a microdissection on the slide. Elastic fibers of blood vessels become traceable in their finest ramifications. The hyaline in the glomeruli in Kimmelstiel-Wilson intercapillary glomerulosclerosis is seen to contain nuclear material. It can be completely removed, as can tubular basement membrane, whereas glomerular basement membrane is preserved.

The evidence that PAS-positive materials may be carbohydrates is enhanced by their removal by pectinase and especially by polygalacturonase. The data are not taken to be conclusive for the reasons mentioned earlier —complexity of enzyme and substrate. A pure enzyme is difficult to prove and a pure substrate is difficult to find, especially in nature. The chemical information should be considered conditional until pure enzymes, electrophoretically homogeneous and crystalline, have been used in a large series.

Techniques and results will be described in full in later publications. For the present, morphological information of definite value can be derived from the action of pectinase solutions on acetone-fixed human tissues. In the future, enzymes of the pectinase group may give chemical information about tissue structures composed of or containing the appropriate substrate.

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The Action of Radioactive Phosphorus in Drosophila[1]

Johanna Blumel[2, 3]

Genetics Laboratory, The University of Texas, Austin

Since Muller's (*2*) classical discovery that x-rays produce mutations in *Drosophila melanogaster,* much attention has been directed to the study of this action. More recently ultraviolet light, radium, and chemicals have also been employed to induce mutations. Law (*1*) attempted to influence the lethal mutation rate of *Drosophila melanogaster* by the use of radioactive phosphorus. However, no lethals were found after injecting various concentrations of radioactive Na_2HPO_4 into 4-day-old larvae of the Oregon-R strain. In the present investigation, radioactive P^{32} was used to study the action of beta rays on *Drosophila melanogaster* and *Drosophila virilis.*

The stock of *Drosophila virilis* Sturtevant used is a lethal-free and fertile strain from Pasadena. The Muller-5 of *D. melanogaster* used has an X-chromosome marked by the dominant gene Bar (B), the recessive gene apricot (w^a), and the scute (sc^8) inversion.

In each case, pairs of mature flies from stock bottles were placed in shell vials containing a radioactive medium. This culture medium was prepared by adding approximately 3.2 ml of radioactive H_3PO_4 (containing about 1.54 mc/ml at the time of its use) to 300 ml of the standard *Drosophila* culture medium. The radioactivity of the original volume was determined with a Geiger counter, and was found to be 265,000 cpm/ml. The medium was distributed among 50 vials, each containing approximately 6 ml. Twenty-five vials were used to test *D. melanogaster,* and 25 for *D. virilis.*

Twelve days after exposure, the distribution of radioactivity in the various tissues of *D. virilis* and *D. melanogaster* was determined. The results are summarized in Table 1. The determinations on distributions of radioactivity on *D. virilis* were made on the original flies, the treated larvae, and the brains, gonads, and salivary glands dissected from treated larvae. No treated adult *D. virilis*

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[2] Present address, St. Joseph's Infirmary School of Nursing, Houston, Texas.

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TABLE 1

DISTRIBUTION OF RADIOACTIVITY IN TISSUES OF *Drosophila virilis* AND *Drosophila melanogaster*

D. virilis	No.	Net total count	Average count per tissue
Larval gonads	23	1374 cpm	59.7 cpm
Larval brains	30	3021 "	100.7 "
Larval salivary glands	30	7801 "	260.0 "
Entire larvae	5	11684 "	2336.8 "
Original ♂	5	13860 "	2772.0 "
Original ♀	5	18873 "	3776.6 "
D. melanogaster			
Entire larvae	5	5732 cpm	1146.4 cpm
Treated ♂	5	4335 "	867.0 "
Treated ♀	5	11044 "	2208.8 "

Net values corrected for background, but not for radioactive decay occurring when these samples were measured.

of the generation raised on the radioactive medium was tested for radioactivity, since only a small number hatched. On the other hand, the hatch of *D. melanogaster* was normal, and here the adults, as well as the larvae, of the generation reared on the radioactive medium, were checked for radioactivity.

All 25 vials of *D. virilis* were fertile and produced the average number of pupae. However, only 39 females and 25 males hatched. Without exception, these imagines were morphologically abnormal. This abnormality pertained mostly to the eyes, legs, abdomen, wings, and genitalia. Twenty-one females and 17 males survived to be tested for fertility to untreated flies. Of these only seven females were fertile.

The low hatch was obviously caused by lack of ability of the treated imagines to emerge. Dissections of unhatched pupae showed fully formed flies with similar or more extreme abnormalities than those just described.

The number of adult offspring from the seven treated *D. virilis* females was very low as shown in Table 2.

Table 2 also shows the number of flies that were again mated to untreated *D. virilis* males and virgin *D. virilis* females. The progeny from these pair matings was about normal in number for *D. virilis*, and enough flies from each tube were inbred so that the progeny from ten tubes could be examined for visible mutations.

The *D. melanogaster* flies were not tested for mutations. Of the treated flies tested for fertility, 78 out of 130 females and 56 out of 109 males were fertile in pair matings to nonirradiated flies. These produced the normal number of progeny.

Beta rays proved to be an excellent source of irradiation for *Drosophila virilis*. The mutations obtained from this treatment are as follows: an eye color, either apricot or an allele of apricot (sex-linked); a wing character, cut or cutlike (sex-linked); scute; extra scutellar bristles; a wing character with unusual venation; another wing character (sterile) in which the wings were folded and rotated 90°; extremely abnormal knobby eyes (both males and females also sterile). Several different mutations of the same general type produced flies with extended wings

TABLE 2

	1		2		3		4		5		6		7	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
No. adult offspring	15	24	2	5	1	3	2	3	4	4	3	2	6	4
No. mated	15	20	1	2	0	3	2	3	4	4	3	2	6	4

with added effects causing sterility. Among the progeny of yet another tube was a male with one apricotlike eye and the other eye a mosaic of areas respectively normal and apricotlike; when mated, no progeny was obtained.

Perhaps the most unusual and interesting mutation found was an aristapedialike character. The ten mutant flies examined (five males and five females) had leglike aristae, extended wings, crippled legs, and all bristles reduced to the size of hairs. They were nonviable and died soon after emergence. The mutant is retained by crossing the heterozygotes. Cytological examination of the salivary gland chromosomes of such heterozygotes show an inversion in the second chromosome. Whether or not this rearrangement is independent of the mutation has not yet been determined. The spineless-aristapedia locus in *D. melanogaster* is located in the right arm of chromosome 3, which is analogous to chromosome 2 in *D. virilis*. This coincidence of mutation and rearrangement in the same chromosome suggests that there is a connection between the mutation and the rearrangement.

The present investigation indicates that radioactive P^{32} not only produces mutations in *Drosophila virilis*, but also chromosomal rearrangements. The tolerance to such irradiation during development is high.

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Chlorophyll Formation in Potato Tubers as Affected by Temperature and Time[1]

Ejnar C. Larsen

University of Idaho, Moscow[2]

In studies of chlorophyll formation, tubers of the potato (*Solanum tuberosum* L.) offer certain advantages as experimental material over the etiolated seedlings of different plants which have been employed commonly in the past for this purpose. The development of chlorophyll in the tubers seems to be dependent on temperature and time in the same general fashion as in etiolated seedlings. However, the rate of development of chlorophyll in potato tubers is slow, the tubers are not dependent on photosynthesis and can thus be kept alive for a long time at the low light intensities required for this kind of study, and by using potato tubers it is possible to avoid the complicating effect of growth of the tissue in which chlorophyll formation is occurring.

In the study here reported concerning the effects of temperature and time on chlorophyll formation, tubers of

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[2] Present address: Odense, Denmark.