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Idaho Experiment Station:
Claude Wakeland, (Entomology), Parma
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At the present time E. J. Newcomer is chairman of the project and Anthony Spuler is secretary.

D. F. FISHER,
E. L. GREEN,
E. J. NEWCOMER, *Chairman*

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR CUTTING GLASS TUBING

A THIRD method for cutting heavy glass tubing may be added to those described by Seemann, SCIENCE, No. 1726, and Tolmachoff, SCIENCE, No. 1733.

A piece of stout string about two feet long is wound once and a half around the tube. The two ends are allowed to hang down on opposite sides of the tube. The tube is held in a wooden vise, clamped on a desk with a wooden clamp, or held by a fellow worker, so that the edge of the desk acts as a guide for the string at the point where the tube is to be cut. The two ends of the string are grasped firmly, one end in each hand. The hands are pumped rapidly up and down, keeping the string tightly pulled around the tube. This is continued a short time until the tube and string are hot enough so that the string begins to smoke. Cold water from a beaker is quickly poured on the hot tube causing a clean break. The entire process may be completed in two or three minutes.

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AN INEFFECTUAL ATTEMPT TO DEMONSTRATE THE VACUOME OF CERTAIN PLANT CELLS

IN an investigation concerning the nature of the plant-vacuole the writer had occasion to attempt a silver impregnation of the vacuome of various kinds of cells. The Golgi method of Da Fano was used because it is recommended by Guilliermond. This method involves the following steps:

(1) Fixation of the tissues in Da Fano fluid, a solution of cobalt nitrate in dilute neutral formalin. This fluid has a pH of 6.7.

(2) Impregnation of the tissues with a silver salt solution, silver nitrate.

(3) Reduction of the pieces of tissue thus treated by means of a modified photographic developer, Cajal solution.

(4) The customary procedure of dehydration, infiltration with paraffin, embedding, sectioning, mounting and counterstaining the tissues.

Root-tips of four-day-old wheat, barley and pea seedlings were used. Guilliermond recommends the use of root-tips of barley and pea seedlings.

By means of the Da Fano method, using the concentrations of Da Fano, Cajal and silver nitrate solutions recommended by Da Fano, the writer attempted to demonstrate the vacuome of the cells of the above tissues. In four of the experiments, the concentration of the solutions used was increased and the time of exposure of the tissues to the solutions was increased. Material on 153 slides, each containing from eight to ten sections, was subjected to the Da Fano procedure and examined carefully. In all cases examination of the sections failed to reveal the existence of a silver impregnated vacuome described and sketched by Guilliermond. In a few sections of wheat-root-tips examined, round black granules appeared in the vacuoles of 80 per cent. of the cells of the meristem. These granules resembled very closely in general appearance, distribution and occurrence the granules described by Guilliermond.

Changes in hydrogen-ion concentration of the fixative used, cobalt nitrate in dilute formalin, were tried. This modification has not been recorded by Guilliermond. The solution was brought to pH 2.4, 3.0, 4.6, 7.0 and 8.0 by the use of potassium acid phthalate and potassium dihydrogen phosphate buffer mixtures. The root-tips fixed in these solutions were then submitted to the remaining steps in the Da Fano procedure. Examination of sixty-two slides, twenty-three of barley containing the sections of ten root-tips and twenty-seven of wheat, containing sections of eight root-tips, revealed empty vacuolar spaces. Nuclei of cells of material fixed in solutions of pH 7.0 and 8.0 contained more granules of reduced silver than did those of material fixed in more acid solutions. The cytoplasm of the former cells also contained a much heavier deposit of silver than did that of the latter cells.

The results herein recorded indicate the extreme capriciousness of the method recommended by Guilliermond. The success of the method seems to be a matter largely of chance or depends upon factors which are as yet unknown.

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SPECIAL ARTICLES

THE EFFECT OF X-RAYS IN PRODUCING RETURN GENE MUTATIONS[1]

MOST of the natural mutations known in Drosophila are to the recessive condition. With the exceptions of the reversions from bar eye to full eye and possible return mutations at the white-eye locus there is but scant evidence that this is a reversible reaction.

Muller found that gene mutations produced by X-rays are, in general, in the same direction and of the same nature as those occurring spontaneously in the fruit fly. In the numerous mutations arising in his recent experiments as a result of irradiation Muller has only two cases of return mutations—both involving the same factor locus, *scute*. This raises the problem of why it is more difficult to find mutations in one direction than in another.

Mutations by X-rays are also fortuitous or chance occurrences at the present time. The operator may be likened to a hunter shooting birdshot into a flock of ducks. As the hunter "accepts with natural piety" what comes down, so the investigator shooting X-rays into a flock of genes accepts what is given. For it is impossible to aim at any particular gene at the present time.

However, in spite of the infrequency of return mutations to the normal condition and the impossibility of controlling results, it appeared to the writer that an experiment carried out on sufficiently large scale might give the mutation rate of mutant genes to normal and the relative frequency with which certain specific genes are hit.

The problem then was: Return mutations at specific loci due to the action of X-rays.

The males used in the experiment carried five mutant genes in their X or sex chromosome; those for yellow body, white eye, forked bristles, bar eye and Beadex wings. The first three of these are recessive to normal, the other two are dominants. Part of these males were exposed to X-rays, using a dosage of 50 K. V.; 5 M. A. M.; 15 cm from the target and forty-eight minutes' exposure (known in Muller's Lab. as the T-4 treatment). The remainder were treated for twice this length of time (T-8). During treatment the flies were placed in gelatin capsules punctured by a fine needle to admit air.

Immediately following treatment these males were mated to virgin double-X yellow females. These females are peculiar in that the two sex chromosomes are attached at the right hand end and go together into the same gamete, which is equivalent to 100 per cent. non-disjunction. The double-X yellow females also carry a male Y-chromosome. In such a cross the sons get their X-chromosome from their fathers and their Y-chromosome from their mothers, a reversal of the usual procedure in this species. There are several advantages in using a stock made up in this way. Practically all mutations occurring in the sex chromosome of the treated fathers show up in the first generation of sons, whether recessive or dominant, as they are not covered by normal allelomorphs in the Y-chromosome.

One thousand such virgins were mated to irradiated males, two pairs to a bottle. This gave five hundred bottles and after seven days the parents were transferred to new bottles and remained there for seven days more. This gave one thousand bottles of offspring among which to look for changes in the five specific loci described above. Such heavy dosages of X-rays as were used in this experiment decrease productivity to a marked extent. The writer has shown elsewhere that following dosages of the magnitude used here only 12 per cent. of the eggs laid complete their life history.

The following table gives the count of the young hatching in the one thousand mating bottles. The average young per bottle was only 10.7 per cent. Many bottles contained no offspring at all.

	Males	Females	
T–4	3,796	4,811	
T–8	866	1,243	
	4,662	6,054	10,716

Gene mutations apparently are produced by a dosage which is just under that rendering complete sterility.

While an experiment involving one thousand mating bottles is not exactly small in scale the poor viability of rayed males reduces the offspring to a point where extensive results could hardly be expected. This defect is being remedied by a repetition of the work on an even larger scale. However, the results secured at the bar gene locus throw considerable light on a mooted question and seem worthy of record at the present time.

[1] This work was done in the Zoological Laboratory of the University of Texas during a recent sabbatical leave of absence from Washington University. My appreciation for the many courtesies received is hereby expressed to Professor J. T. Patterson and Professor H. J. Muller.