other food constituents were fed at separate times. These results in general confirm the findings of Larsen and Chaikoff and of Cuthbertson, McCutcheon, and Munro under somewhat different experimental conditions.

The experiments described above prove that, like the nitrogen equilibrium of the adult rat, the growth rate of the infantile rat is affected by separation in the time of feeding of protein from the feeding of the remainder of the diet. Our experiments throw no light on the possible mechanism of growth retardation by the temporal separation of feeding constituents of the diet. We plan to investigate whether simultaneous feeding of carbohydrates, fats, vitamins, or salts with the protein is necessary in order to achieve optimal growth. These experiments may help to clarify the contradiction between our results and those obtained by Cuthbertson, *et al.*

References

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Formaldehyde as a Mutagen in *Drosophila*

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Rapoport (*1*) reported that a high lethal mutation rate, chemically induced by the use of formaldehyde, was obtained in *Drosophila melanogaster*. He transferred 48-hr larvae and eggs of wild type *Drosophila* from "standard food" to food containing a water solution of formaldehyde "at sub-lethal concentrations." Directly after emergence, males from these formaldehyde cultures were tested for lethal mutations by the CLB method. The treated flies showed a mutation rate of 5.92% (47 lethals among 794 cultures). Among his 833 control cultures, only 1 lethal, or 0.12%, was recorded.

The work herein reported confirms Rapoport's findings. The food medium used contained cornmeal, molasses, agar-agar, and water and was enriched with a yeast extract. Formalin was added to the test medium after it had cooled, but was still the consistency which permitted the complete mixing of the formalin throughout the medium. The percentage of formalin added was determined using the water content of the medium as the point of reference and was expressed as per cent of formaldehyde. It is not known, however, whether or not the effective concentration of formaldehyde present in the medium was the same as that added to it originally, as some volatilization is certain to have taken place.

It was found that no eclosions were obtained from a medium containing more than 0.25% formaldehyde. Consequently, this may be said to be the highest "sub-lethal concentration" that was used.

Eggs from 1 to 4 hrs old were collected on agar blocks and permitted to hatch out and develop for an additional 48 hrs. Following this period the larvae were transferred to formaldehyde-containing medium. Table 1 gives data on the number of adults obtained in experiments with different concentrations of formaldehyde. Eclosions in untreated controls were close to 100%. It will be noted that a greater percentage of eclosions was obtained from 0.10% and 0.15% formaldehyde than from the two higher concentrations.

TABLE 1

Percentage of Eclosions Obtained With Four Different Concentrations of Formaldehyde

Concentration of formaldehyde (%)	No. of larvae	Percentage of eclosions
0.10	131	70.2
0.15	119	89.9
0.20	122	56.6
0.25	90	53.3

The Muller-5 method of detecting lethals in the X chromosome was used. The newly-hatched formaldehyde-treated males were crossed to Muller-5 females, and the daughters of these matings were individually crossed to Muller-5 males. All flies were raised at 25° C.

A lethal mutation was indicated as having occurred when there were more than four males in the culture, none of which possessed the wild type eye. Progeny tests were run from cultures that contained four or less males, all with the Muller-5 type eye.

Table 2 gives the number and percentage of lethal mutations obtained.

TABLE 2

Number and Percentage of Lethal Mutations

Formaldehyde (%)	No. of cultures	No. of lethals	Lethals (%)
0.10	713	46	6.45
0.15	644	29	4.50
0.20	441	27	6.12
0.25	212	12	5.66
Total treated	2010	114	5.66
Controls* ..	505	1	0.20

* Two doubtful cases not included. (Less than four Muller-5 males obtained from progeny tests.)

There is a very significant difference in the percentage of lethals obtained by the use of formaldehyde and the percentage obtained in the control animals. Moreover, the 5.66% herein obtained is very close to the 5.92% reported by Rapoport. The data presented here do not show a correlation between the concentration of formaldehyde and the induced rate of mutation. More extensive data and tests for the occurrence of lethal clusters will be needed to elucidate this question.

Reference

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