tical analysis by the t test, the difference might be imputed to chance variation.

In Heymans' experiments the action of DFP on the brain was isolated from that of other portions of the body. A similar isolation of the influence of DFP could be achieved only in the experiments on the decapitated heads of newborn rats. The respiratory centers, however, are sensitive to morphine and pentobarbital.

Thus, under the conditions of these experiments DFP did not increase the survival time in acute anoxia, hypoxia, or following pentobarbital and morphine administration.

References

1. BLISS, C. I. *Quart. J. Pharm. Pharmacol.*, 1938, **11**, 192–216.
2. HEYMANS, C. *Abstr. Communications XVII int. physiol. Congr., Oxford,* 1947, 152.

The Importance of the Time Element in Feeding of Growing Rats: Experiments With Delayed Supplementation of Protein

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Recent reports from two laboratories (*1, 3*) indicate that simultaneous feeding of all the essential amino acids is necessary to obtain maximum growth in animals (cf. *5*). As an extension of these studies, it seemed desirable to investigate the general importance of the time factor in feeding with respect to the synthesis of new body substance of infantile rats.

A survey of the literature reveals that the significance of the time factors in nutrition was recognized as early as 1937 by Larsen and Chaikoff, who demonstrated that the degree of N sparing in adult dogs "effected by a single feeding of carbohydrate was related to the time that elapsed between the consumption of the daily meal and the ingestion of the extra carbohydrate." Cuthbertson, McCutcheon, and Munro (*2*) confirmed these results for the adult rat and the adult human subject. However, they reported that, in contrast to the adult animals, the metabolism in the infantile rat was unaffected by the separation in the time of ingestion of the protein and carbohydrate moieties of the diet. Analysis of their data reveals that the growth rates of the animals in the experiments bearing on this point were all abnormally low, suggesting that some general deficiency in the food mixture used may have been the limiting factor in determining the growth rates.

We therefore decided to reinvestigate the problem, using diets which have proved adequate for the promotion of growth of young rats in earlier experiments.

[1] With the technical assistance of Miss Gloria E. Lusk.

Three distinct diets were prepared with the following composition:

Diet A (protein free): corn starch, 3,050 gm (78.3%); rice bran concentrate, 400 gm (10.26%); cottonseed oil, 200 gm (5.13%); U. S. P. salt mixture, 200 gm (5.13%); fish oil (1 gm contains 2,000 I.U. vitamin A and 400 I.U. vitamin D), 50 gm (1.27%); riboflavin, 75 mg; Ca pantothenate, 150 mg; and choline chloride, 2.5 gm.

Diet B (protein fraction): 50% washed casein and 50% fish proteins.

Diet C (combination): 80% Diet A and 20% Diet B.

Two groups of 6 male litter-mate Sprague-Dawley rats were used in these experiments. The experimental group was fed Diet A from 4: 00 P.M. until 7: 30 A.M. of the next day. The quantity of food consumed by each rat was determined, and between 11: 00 A.M. and 1: 00 P.M. sufficient quantities of Diet B were fed to give a total combined diet during the day with 20% protein. During the next day, the control animals were given an amount of Diet C equal to the total of the diets consumed by their corresponding animals on the mixed diet. The controls were permitted 80% of their total ration from 4: 00 P.M. until 7: 30 A.M. and 20% from 11: 00 A.M. to 1: 00 P.M. Weights on all animals were determined daily.

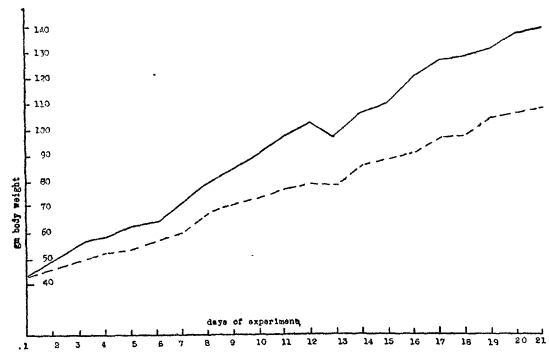

FIG. 1. Average growth curve of rats: ——— on mixed diet; – – – on delayed supplementation of protein.

The experimental animals fed the separate diets failed to grow at a rate comparable to the control group. Within each group the weight curves were remarkably uniform, so that the results are presented in Fig. 1 as the average curves for the two groups.

During the 21-day period of the experiment, the control animals showed an average weight gain of 95 gm, while the experimental animals showed a gain of only 62 gm during the same period. The difference in the effectiveness of the diet when fed in toto as compared to the divided feeding may be judged from the values for the ratio: total consumed feed in grams/gain in body weight in grams. For the control animals the ratio becomes 2.6 ± 0.23, while in the experimental animals with the divided feedings the value is 3.7 ± 0.29 gm.

Experiments with adult rats with protein depletion showed that the weight increase during the period of protein restoration was retarded if the protein and the

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

1. Cannon, P. R., Steffee, C. H., Frazier, L. J., Rowley, D. A., and Stepto, R. C. *Fed. Proc.*, 1947, **6**, 390.
2. Cuthbertson, D. P., McCutcheon, A., and Munro, H. N. *Biochem. J.*, 1940, **34**, 1002.
3. Geiger, E. *J. Nutrition*, 1947, **34**, 97.
4. Larsen, P. S., and Chaikoff, I. L. *J. Nutrition*, 1937, **13**, 287.
5. Schaeffer, A., and Geiger, E. *Proc. Soc. exp. Biol. Med.*, 1947, **66**, 309.

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

1. Rapoport, I. A. *C. R. Acad. Sci. U. R. S. S.*, 1946, **54**, 65–67.