though the most intense auditory, visual, or tactile stimuli may produce a motor reaction in an animal during a prolonged hypnotic state, these stimuli no longer cause the animal to right itself (Fig. 2, left). Sometimes the guinea pig makes a prolonged attempt to right itself by exhibiting disorganized rhythmic movements of the extremities. He may succeed only in displacing his body on the table as if he were submitted to an antagonistic drive to "stick" to the supporting surface.

There is a functional gradation in the intensity of sitmulation necessary to discontinue a prolonged hypnotic state. Thus, the appearance of another guinea pig in the visual field is more effective than an artificial visual stimulus. A slight noise made by the opening of the cage door may be more effective than a loud auditory stimulus. However, frustration of righting attempts in the presence of the most potent stimuli decreases their effectiveness. A well-trained, hungry animal may remain in a prolonged hypnotic state for hours, even though he is surrounded by a great amount of food.

In an animal trained in a lateral hypnogenic position, stimuli may be more effective on one side than on the other. For example, in an animal showing left lateral preference, visual stimuli applied to the left eye are more effective.

Changes in the general behavior of trained animals are difficult to appraise, as even normal guinea pigs, when fatigued or frightened, often show prolonged periods of immobilization. However, some trained animals may particularly easily present the hypnotic state in an upright position. Thus, these animals may show characteristic exophthalmos in a sitting position and may remain undisturbed by stimuli when put in an upright position, their forelegs supported by a stand (Fig. 2, right). There is a decrease in spontaneous motor activity in the trained animals. Furthermore, these animals are more often found in a prone position in their cages than the control animals. However, the total appraisal of changes in general behavior requires more prolonged studies.

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Anoxic Survival and Diisopropyl Fluorophosphate (DFP)

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Heymans (*2*) has found that the use of diisopropyl fluorophosphate (DFP) prolongs the survival period of medullary centers subjected to a complete arrest of circulation. Should this increased resistance to anoxia observed in the isolated head also apply to the intact organism, then DFP might be valuable in minimizing the effects of anoxia.

We have performed a series of experiments in an attempt to apply Heymans' observation under a variety of conditions. Fifteen male rats were injected subcutaneously with 1.5–2.0 mg of DFP/kg, and 15 uninjected animals were subjected to the hypoxia produced by the inhalation of 3.9% O_2 in 96.1% N_2. The mean survival time of the DFP-injected rats was 14.78 ± 2.28[1] min. The controls' survival period was significantly longer, the mean being 23.41 ± 2.87 min. Thus, DFP, instead of conferring protection, apparently produces the opposite result. In this and subsequent experiments the dosage of DFP was not great enough to produce lethal effects in the time of observation. The observed mortality cannot be attributed to DFP toxicity.

Next, the duration of the survival period of the decapitated head of newborn rats was determined, the gasping of the head being taken as the criterion of the length of survival. Two mg of DFP/kg was injected subcutaneously into newborn rats 15–50 min. before decapitation. The 19 controls continued gasping for an average of 19.9 min. The heads of the 19 DFP-injected rats gasped for 19.0 min.—not a significant difference.

The influence of DFP on lethality caused by excessive doses of pentobarbital revealed that the average survival time for 7 control rats was 12.7 min, while 3 rats injected subcutaneously with 1.5 mg of DFP/kg 15 min. before receiving pentobarbital survived 4.3 min. The shortest period in any of the controls was longer than the longest period of survival in any of the injected rats.

Two rabbits, one of which had previously received an injection of 0.3 mg of DFP/kg in a carotid artery, were subjected to the inhalation of 3.9% oxygen in nitrogen. The control animal survived 37.5 min; the injected, 26.5 The control animal survived 37.5 min; the injected, 26.5.

TABLE 1

INFLUENCE OF DFP (1–2 mg/kg SUBCUTANEOUSLY) ON MORPHINE LETHALITY

Morphine dose (mg/gm)	Controls		DFP injected	
	No.	No. surviving 24 hrs	No.	No. surviving 24 hrs
.2512	6	4	9	9
.3981	20	8	23	15
.5012	14	0	14	3
.6000	10	1	10	3

Only with morphine did we have a suggestion of a possible beneficial effect. Thirty-one mice receiving 0.8–1.0 mg/gm of morphine sulfate intraperitoneally survived 14.31 ± 1.60 min, while 26 animals exposed to 2.0–3.0 mg of DFP/kg subcutaneously in addition to the morphine exhibited a mean duration of survival of 17.89 ± 2.32 min. The difference between the means is not statistically significant.

With smaller doses of morphine some of the animals survived more than 24 hrs, at which time the experiment was terminated (see Table 1).

Although the data in Table 1 show an apparent protection in the DFP-injected animals, when the LD_{50} was calculated by the method of Bliss (*1*), the values were 0.3237 ± 0.0388 mg/gm for the controls and 0.4472 ± 0.0258 mg/gm for the DFP-injected animals. On statis-

[1] Standard error of the mean.

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.

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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.

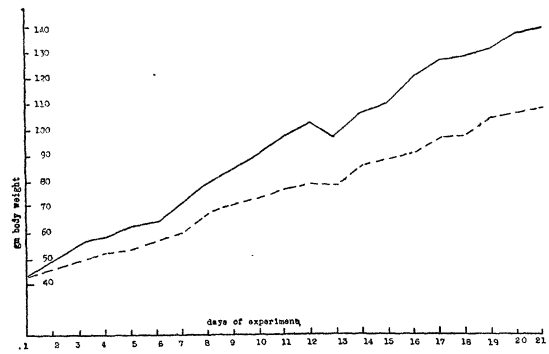

FIG. 1. Average growth curve of rats: ——— on mixed diet; – – – on delayed supplementation of protein.

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