acute inflammatory response is of greater etiological importance in arthritis. AE and AA should be useful for differentiating the immunosuppressive and anti-inflammatory activities of drugs.

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1. Mackay, I. R., Burnet, F. M., in Autoimmune Diseases: Pathogenesis, Chemistry and Therapy, Charles C Thomas, Spingfield, 1963.

2. Peason, C. M., Wood, F. D., Am. J. Path., 1963, v42, 73.

3. Paterson, P. Y., in: Immunological Diseases, M. Samter, ed., Little, Brown & Co., Boston, 1965, 788.

4. Ward, J. R., Cloud, R. S., Drowitt, E. L., Jones, R. S., Arthritis & Rheumatism, 1964, v7, 654.

5. Ward, J. R., Cloud, R. S., J. Pharmacol. Exp. Therap., 1966, v152, 116.

6. Brandriss, M. W., Smith, J. W., Friedman, R. M., Ann. N. Y. Acad. Sci., 1965, v122, 356.

7. Baum, T., Rosenthale, M. E., Cir. Res., 1966, v18, 118.

8. Baxter, B. L., Rosenthale, M. E., Proc. Soc. Exp. Biol. & Med., 1966, v121, 1075.

9. Newbould, B. B., Brit. J. Pharmacol., 1963, v21, 127.

10. Van Arman, C. G., Begany, A. J., Miller, L. M., Pless, H., J. Pharmacol. Exp. Therap., 1965, v150, 328.

11. Selye, H., in The Physiology and Pathology of Exposure to Stress, Acta, Inc., Montreal, 1950.

12. Waksman, B. H., Peason, C. M., Sharp, J. T., J. Immunol., 1960, v85, 403.

13. Waksman, B. H., Wennersten, C., Int. arch allergy, 1963, v23, 129.

14. Pearson, C. M., Wood, F. D., J. Exp. Med., 1964, v120, 547.

15. Isakovic, K., Waksman, B. H., Proc. Soc. Exp. Biol. & Med., 1965, v119, 676.

16. Winter, C. A., Nuss, G. W., Arthritis & Rheumatism, 1966, v9, 394.

17. Good, R. A., Campbell, B., Good, T., Proc. Soc. Exp. Biol. & Med., 1949, v72, 341.

18. Field, E. J., Miller, H., Arch. Int. Pharmacodyn, 1961, v134, 76.

19. Page, A. R., Condie, R. M., Good, R. A., Am. J. Path., 1962, v40, 519.

20. Nuss, G. W., Winter, C. A., The Pharmacologist, 1965, v7, 181.

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Influence of Age and Dietary Stress on Hexobarbital Activity in Mice.* (32036)

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The activity of the liver enzyme systems concerned with drug detoxication can be influenced by numerous factors including age and dietary intake of the animal. In the newborn mouse the activity of these drug metabolizing enzymes is very low(1). The ability of the liver enzymes to detoxify drugs increases rapidly during the growth period of the animal and reaches a relatively steady state in the well-nourished adult. However, when the adult animal is subjected to starvation or is maintained on a low level of dietary protein, the activity of drug metabolizing enzymes is significantly decreased (2,3). In the present study, using mice from weaning to young adulthood, we have compared the effect of two isocaloric diets, differing only in protein and carbohydrate content, on the development and stability of the liver enzyme system concerned with hexobarbital detoxication.

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Materials and methods. Diet. The two semisynthetic, isocalroic diets used in this study were similar to those we used previously with rats(4) but differed in that starch was substituted for a portion of the carbohydrate in the diet (Table I). The addition of starch allowed for pelleting of the diet. The use of such a pelleted diet considerably minimized urinary and fecal contamination of the food by the mice.

Animals and their care. The CF-1 male

TABLE I. Composition of Diets.*

	Control %	Experimental %
Principal components		
Casein, vitamin-free†	27.0	8.0
Starch	11.6	15.0
Cane sugar	52.4	68.0
Corn oil	5.0	5.0
Salt mix, U.S.P. XIV	4.0	4.0

* Prepared by General Biochemicals, Inc., supplemented with adequate vitamins.
† 89%–91% protein.

mice used in this study were obtained from Carworth Farms, Inc. on the 21st day after birth. The mice were randomly divided upon arrival and offered one of the two diets and water *ad libitum*. The mice were housed 20 per wire-grid cage in air conditioned animal quarters.

Hexobarbital. The interval between loss and return of the righting reflex was used as the index for hexobarbital sleeping time. The mice were discarded after each experiment so that fresh mice were used for each determination of sleeping time and for the *in vitro* investigation of drug metabolism. For the metabolic studies, the mice were sacrificed and bled, and the weights of the individual livers were recorded. The rate of hexobarbital metabolism was determined in the 9000 $\times$ *g* supernatant fraction of pooled liver homogenates according to the method of Cooper and Brodie(5).

Results. Growth. Mice maintained on the 27% casein diet (control) grew at approximately the same rate as this strain does when fed a commercial diet. As shown in Fig. 1, the weight of the mice maintained on the 8% casein diet (experimental) increased more slowly and after 7 weeks reached approximately one-half the weight of the mice maintained on the control diet. Mice have been maintained on these semisynthetic diets for as long as 6 months without any gross abnormality other than the weight difference being noted. Necropsy findings in the experimental group during the first 4 weeks of feeding revealed a virtual absence of gross body fat in contrast to that noted in the control group. After 6 weeks or more of feeding, fat deposits were evident in the omentum and other tissues of both groups.

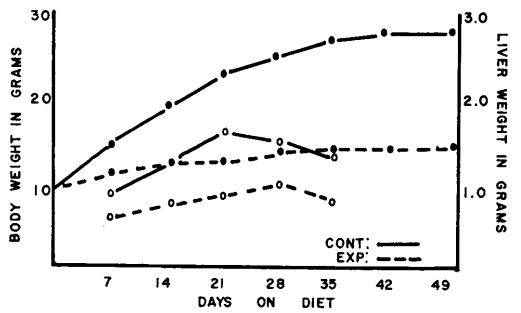

FIG. 1. Rates of growth of CF-1 mice fed control (27% casein) or experimental (8% casein) diet. Body weights (closed circles) are the means of 50 mice. Liver weights (open circles) are the means of 15-20 livers.

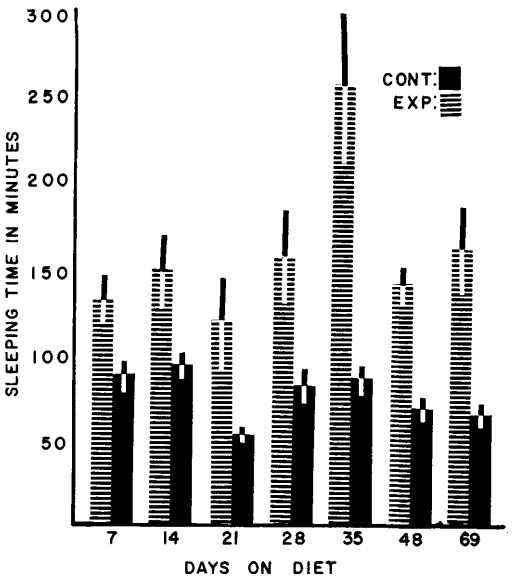

FIG. 2. Hexobarbital sleeping time in CF-1 mice fed control (27% casein) or experimental (8% casein) diets. Mean $\pm$ standard error of mean. 10 mice per group.

Hexobarbital sleeping time. The sleeping time observed following the intraperitoneal injection of 100 mg/kg of hexobarbital is given in Fig. 2. Mice maintained on the 8% casein diet slept longer than those fed the 27% casein diet at all periods tested. The minimum sleeping time occurred in both groups after 3 weeks of feeding (age 42 days). This is the approximate age at which these mice reach sexual maturity. The marked rise in sleeping time shown in this study on the 35th day of the experimental diet occurred between 28 and 36 days in other studies

TABLE II. Hexobarbital Metabolism by 9000 × *g* Liver Supernatants.

Days on diet	Control, μM/g/hr	Experimental, μM/g/hr	P value
7	.656 ± .002*	.439 ± .018	<.01
14	.720 ± .012	.489 ± .012	<.01
22	.936 ± .024	.736 ± .011	<.01
29	.958 ± .021	.570 ± .021	<.01
36	.858 ± .015	.442 ± .013	<.01

* Mean of 4 determinations ± S.D. Each flask contained 2 ml of supernatant derived from the pooled livers of mice maintained on a 27% casein (control) or 8% casein (experimental) diet plus 1 μM hexobarbital, 0.26 μM TPN, 50 μM nicotinamide, 25 μM $MgCl_2$ and 0.1 M phosphate buffer to final volume of 4 ml. One hour incubation at 37°C.

with CF-1 mice. When CD-1 mice from the Charles River Breeding Laboratories were used in similar studies, a marked rise in hexobarbital sleeping time occurred after 18 days of experimental feeding.

Hexobarbital metabolism. As can be seen in Table II, the liver supernatants of mice maintained on the experimental diet metabolized significantly less hexobarbital than those of mice maintained on the control diet at all periods. Both groups showed an increase in hexobarbital metabolizing activity for the first three weeks on the diets, *i.e.*, from age 28 to 42 days. A significant decrease in drug metabolizing activity was evident during the fourth and fifth week in the supernatants from the experimental group.

In similar studies, it was noted that the enzymatic activity in the liver supernatants from either group could be increased by addition of higher levels of cofactors to the incubation mixtures. The concentrations of cofactors necessary to obtain maximum *in vitro* hexobarbital metabolizing activity varied depending on the length of time that the mice had been fed the experimental diets. Thus early in the dietary period concentrations as high as 0.952 μM of TPN, 11.53 μM of glucose-6-phosphate and 0.2 unit of glucose-6-phosphate dehydrogenase in a final volume of 4 ml were necessary to obtain maximum activity whereas after several weeks on the diet maximum activity was obtained with lower concentrations of TPN and glucose-6-phosphate. Data obtained from studies in which maximum activity was determined are presented in Fig. 3. The maximum activity noted in the experimental liver supernatants is graphed as the percent of maximum control activity. At the end of one week of feeding the protein deficient diet, the experimental supernatants could still metabolize 90% as much hexobarbital as the controls. However, with further feeding, the drug metabolizing activity in the experimental supernatants progressively decreased as compared to control supernatants. In a limited number of studies which have been completed after 2 months of feeding the protein deficient diet, the drug metabolizing activity in the experimental group has not been found to fall below 50% of the control value.

Discussion. Our data indicate that the drug metabolizing enzymes in the liver increase in activity from weaning to early maturity in mice that have been fed either the control or the protein deficient diet. The increase in activity occurs during a period of rapid growth of the animal when the liver is also increasing in size. Hence, the demand for synthesis of drug metabolizing enzymes can be met even when the animal is undergoing severe dietary stress. Since it was possible at the 7-day period to achieve 90% of the control rate of metabolism in the experimental supernatants by the addition of sufficient cofactors, it is evident that there is little difference in the enzyme level at this time. Our studies, as well as those of others(6), indicate that shortly after animals have been subjected to a dietary stress the level of glucose-6-phosphate is low.

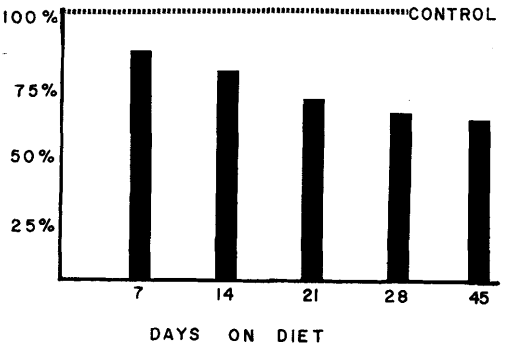

FIG. 3. Maximum hexobarbital metabolizing activity of liver supernatants (9000 × *g*) obtained by addition of optimum concentrations of cofactors. Experimental activity expressed as percent of control activity.

It is likely that the difference in sleeping time noted at the end of 7 days feeding of the experimental diet may result primarily from a difference in activity of the TPNH generating system and not from a difference in the level of drug metabolizing enzyme.

The marked rise in sleeping time noted during the fourth to fifth week may be attributed to a number of factors. The level of drug metabolizing enzyme in the liver must be lower than normal at this time since it was not possible to stimulate metabolism in the experimental supernatants above 75% of control value by the addition of excess cofactors. Our measurements of liver weights indicated that the livers of the control group had reached adult weight at the end of the third week while those of the experimental group were still actively growing. Thus it is likely that the TPNH level in the liver of the experimental animal was lower than in the control since in a rapidly growing liver in a young animal the level of TPNH is decreased (7). Therefore, it appears that the drug is slowly metabolized in the experimental mice during the fourth to fifth week because the level of drug metabolizing enzyme and the effective level of the TPNH generating system are both low at this time. Another factor which undoubtedly contributed significantly to the prolonged sleeping time noted in the experimental group during this period was the noticeable lack of body fat in these mice. Hexobarbital is a fat soluble compound which will equilibrate with the body fat in the controls and therefore the concentration of drug in the brain should be lower in these mice than in the experimental mice who have less body fat.

With prolonged feeding of the protein deficient diet, the hexobarbital sleeping time declines from the elevated level noted during the fourth to fifth week but remains significantly above control values. The decrease in sleeping time may be due to the accumulation of body fat in the experimental mice and to an increased activity of the TPNH generating system since the liver is no longer actively growing. From the sixth to the tenth week on the experimental diets the hexobarbital sleeping time does not change significantly with further feeding of the protein deficient diet. It is evident that the young adult mouse adjusts to the 8% casein diet by this time and further decreases in the level of the drug metabolizing enzymes do not occur.

Conclusion. 1. Mice maintained on an 8% casein diet are capable of synthesizing drug metabolizing enzymes during the period of rapid body growth. 2. Mice maintained on this diet exhibit a prolonged hexobarbital sleeping time at all periods after one week of experimental feeding. 3. The increased sleeping time seen during the earlier period of experimental feeding may result from a less active TPNH generating system than is present in controls. 4. The increased sleeping time seen during the fourth to fifth week on the 8% casein diet probably results from an actual decrease in the level of drug metabolizing enzyme and in cofactor level, as well as from a lower level of body fat in these animals.

1. Jondorf, W. R., Maickel, R. P., Brodie, B. B., Biochem. Pharmacol., 1958, v1, 352.

2. Dixon, R. L., Shultice, R. W., Fouts, J. R., Proc. Soc. Exp. Biol. & Med., 1960, v103, 333.

3. Kato, R., Chiesara, E., Vassanelli, P., Biochem. Pharmacol., 1962, v11, 211.

4. Horn, R. S., Manthei, R. W., J. Pharmacol. Exp. Therap., 1965, v147, 385.

5. Cooper, J. R., Brodie, B. B., ibid., 1955, v114, 409.

6. Roth, J. S., Bukovsky, J., ibid., 1961, v131, 275.

7. Kunz, W., Schaude, G., Schmid, W., Siess, M., Proc. of European Soc. for Study of Drug Tox., 1966, vVII, 113.

Received January 20, 1967. P.S.E.B.M., 1967, v125.