serum of most other mammals do not contain a specific lipase similar to pancreatic lipase. The hydrolysis of olein described in this paper is affected by another type of enzyme. For convenience the term lipase has been employed in this paper.

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Variation in Response of Various Mouse Strains to Hexobarbital (Evipal). (22039)

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The use of inbred strains of mice, as an established experimental procedure in areas of medical research other than cancer, has been less extensive for various reasons, particularly because of the lack of information concerning characteristics of these established strains other than tumor type and incidences. Various investigators have determined some of the other characteristics these strains exhibit. Russell, Newfeld, and Higgins(1) have investigated the normal blood picture in 18 inbred mouse strains; Law, Morrow, and Greenspan (2) have investigated the liver glucuronidase activity in inbred strains; Tuba(3) has determined serum tributyrinase levels in 3 inbred strains; and Wragg and Spiers(4) have demonstrated inbred strain differences in response to adrenal cortical hormones.

The data herein reported is additional information on strain differences in mice, and is concerned with the response to a barbiturate drug, hexobarbital (Evipal), as measured by sleeping time.

Materials and methods. The various strains tested, shown in Table I, came from the production colonies of the National Institutes of Health. Initially, the investigation was concerned only with closely inbred strains, but because of the existence of some doubt about the uniformity of inbred strains in research of this sort(5), it was decided to extend the observations to additional strains in order to test other genetic situations. The first 10 strains listed in the table have been closely inbred by brother x sister matings, so they represent a genetic condition in which maximum homozygosity is to be expected. The CFW strain has been mated brother x sister in a large closed colony with no effort made to maintain close relationships between matings, so that many family lines are maintained parallel in each animal generation. Genetically, such a population will no doubt exhibit considerable homozygosity, but is expected to be more variable than the more closely inbred strains. The non-inbred Swiss strain has been random

TABLE I. Mean Sleeping Time in Minutes for Various Strains of Mice Injected with 125 mg per Kilo Body Weight of Evipal. All animals were males, 70-80 days old.*

Strains	No. of animals	Mean sleeping time	Stand. dev.
A/LN	25	48.44	4.17
DBA/2JN	64	46.83	4.22
A/HeN	25	41.25	1.92
BALB/cAnN	63	41.01	1.99
C57L/HeN	29	32.52	3.22
C57BL/6JN	37	32.26	5.71
C57BR/cdJN	25	31.93	5.51
C3HfB/HeN	30	22.02	2.99
BRSUNT/N	26	18.34	3.34
SWR/HeN	38	18.08	4.15
CFW	32	46.43	11.75
Swiss (non-inbred)	47	43.39	14.51

* The author wishes to thank Dr. Marvin Schneiderman, National Cancer Institute, for his advice on statistical procedures.

mated within a large closed colony, and hence represents considerable heterozygosity. All animals were 70-80 days old when tested, and only males were used. The barbiturate, Evipal, was injected intraperitoneally at a dose level of 125 mg per kilo of body weight. Sleeping time was measured in minutes from the time the injection was made until the righting reflex was restored. The test for significant differences between sleeping time means was made according to the method of Baten(6).

Results. Table I shows the results of sleeping time determinations in various strains of mice. As the data show, strain differences in response to the drug are evident. The A/L and DBA/2 strains sleep the longest with no significant difference existing between the 2 sleeping times. Likewise, the A/He and BALB/c strains fall into a second group with no difference between the 2, but both differing significantly from the A/L and DBA/2 strains. The 3 C57 strains fall into a third group with no difference between the 3, but differing significantly from the first 4 strains. The C3HfB is similar in sleeping time to the BRSUNT and SWR, but differs significantly from these 2 strains. The CFW and Swiss have long mean sleeping times, comparable with the A/L and DBA/2 strains.

The uniformity of the distributions of the various strains is of particular interest. An examination of the standard deviations will readily show that the distributions of the inbred strains are considerably less variable than those of the CFW strain, and it in turn is less variable than the non-inbred Swiss. This latter strain was quite variable, ranging from one animal that was completely unaffected to 2 animals that slept 97 minutes. Thus, 3 different levels of genetic homogeneity have exhibited 3 different levels of variability, with the closely inbred strains showing minimum variation at this particular injection level. Whether this will hold true for other injection levels is not known. However, in this regard, preliminary trials with injection levels of 25 mg, 50 mg, and 75 mg per kilo of body weight showed considerable variation in response by all the strains. More complete information on this point is not yet available.

Discussion. The significance of the differences in mean sleeping time between the various strains tested is not apparent at this time. Whether this indicates differences in sensitivity to hexobarbital, or differences in the rate of biotransformation is yet to be determined. Quinn, Axelrod, and Brodie(7) have found an inverse relationship between the rate of biotransformation of hexobarbital and the duration of its hypnotic effect in a number of species. Perhaps a similar relationship exists in these various strains of mice. The fact remains, however, that strains do differ in their response to this drug, and these differences are consistent and uniform for the strain. Therefore, a genetic mechanism is probably involved.

Strain differences in response to various drugs could be of considerable importance in routine pharmacological testing of drugs, or in basic studies of drug metabolism systems. It is quite likely that one approach to the study of metabolic pathways in small mammals can be made by the use of genetically uniform animals in combination with pharmacological technics.

The demonstration of strain differences in response to such a physiological condition is interesting from a genetic standpoint, for it supports the concept of the uniformity of response of closely inbred strains, a concept that has been the backbone of the creation and use of inbred strains for many years. Furthermore, the demonstration that the uniformity of response corresponds to the theoretical state of homozygosity, supports the concept even more strongly. However, in this regard, recent work by McLaren and Michie(6) using pentobarbital sodium (Nembutal), indicated the inbred strain tested was just as variable as an F1 hybrid of that strain and as a non-inbred strain. The physiological action of these 2 chemically similar drugs has always been considered similar, so whether this discrepancy in results is due to an actual difference in action, or difference in dose levels, or differences in the animals used cannot be determined at this time. Certainly additional work on drug metabolism in the various inbred and non-inbred strains of mice is indicated.

Cooper and Brodie(8) have recently demon-

strated an enzyme system localized in the microsomal fraction of liver cells, which oxidizes hexobarbital to keto-hexobarbital. The investigation of the activity of this enzyme system in these strains is now underway.

Summary. 1. Significant differences in sleeping time have been demonstrated between various strains of mice, inbred and non-inbred, when injected with 125 mg per kilo body weight of hexobarbital. 2. Three different levels of genetic homozygosity were represented in the various strains tested. The most closely inbred strains (A/L, DBA/2, A/He, BALB/c, C57L, C57BL/6, C57BR/cd, C3HfB, BRSUNT, SWR) showed the least variation, the less closely inbred strain (CFW) showed more variation, and the non-inbred Swiss strain showed the most variation.

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Blood ACTH Levels Following Subcutaneous Injections.* (22040)

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The adrenocorticotropic hormone (ACTH) is known to disappear rapidly from the circulating blood. Van Dyke *et al.*(1) have estimated the average life of the ACTH molecule in the circulation to be approximately 17 minutes, and half-life values of about 5 minutes have been estimated for ACTH administered intravenously to intact rats(2,3). The half-life of endogenous ACTH in the adrenalectomized rat has been reported to be approximately one minute(4). A rapid rate of disappearance of ACTH from the circulation is also indicated by clinical studies. Intravenous infusion of ACTH over a prolonged period brings about a much greater adrenal response than the single injection of a comparable dose of the hormone(5). It becomes of interest, therefore, to establish the rate at which exogenous ACTH disappears from the circulation following a single extravascular administration.

In these studies a large dose of ACTH was administered subcutaneously to hypophysectomized rats and blood ACTH levels were estimated at various intervals after treatment.

Method. Male hypophysectomized rats were obtained from the Hormone Assay Laboratories, Chicago, and used within a 24-hour post-operative interval. An ACTH standard dose response curve was prepared for this study based on accumulated data representing

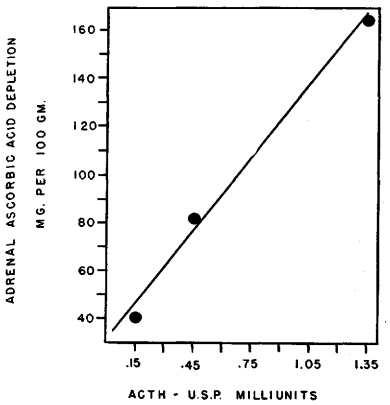

FIG. 1. ACTH standard dose-response curve. Each point represents the mean response of 25 hypophysectomized rats 1 hr after intrav. injection with U.S.P. ACTH Reference Standard.

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