lation (swabs/min). Increasing concentration of gustatory stimuli resulted in increased rates of secretion. The response was linear throughout for sucrose and quinine, but with citric acid and sodium chloride it was linear only with lower concentrations. Mathematical expression of relationship between glandular secretion rate and frequency of stimulation permitted calculation of maximal response and reflex equilibrium constants for the various stimuli employed. Results indicated that at least 3 different gustato-salivary reflex pathways were involved.

1. Pfaffman, C., *J. Comp. Cell. Physiol.*, 1941, v17, 243.
2. Beidler, L. M., *J. Neurophysiol.*, 1953, v16, 595.
3. Beebe-Center, J. G., Waddell, D., *J. Psychol.*, 1948, v26, 517.
4. Curby, W. A., *J. Lab. and Clin. Med.*, 1953, v41, 493.
5. Lineweaver, H., Burk, D., *J. Chem. Soc.*, 1934, v56, 658.

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Urethan Induced Acceleration of Hexobarbital Metabolism.* (25558)

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Reduction and demethylation of certain azo dyes by rat liver microsomes have been increased by administration of such carcinogenic hydrocarbons as 3-methylcholanthrene and 3, 4 benzpyrene(1). The evidence is that induced enzyme synthesis occurs in these cases. Recently, increases in other hepatic microsomal enzyme activities have been obtained with several other classes of chemical compounds(2,3). The present article deals with data which suggest that urethan (ethyl carbamate) may similarly induce increases in activity of certain enzyme systems. Urethan is of particular interest owing to its cancer chemotherapeutic activity and carcinogenic activity.

Materials and methods. Sleeping times were measured in Swiss albino mice (20-35 g). Experimental groups were pretreated with urethan or 3-methylcholanthrene. After a definite time (in most cases 24 hours), these and control groups were given hexobarbital, sodium, and their sleeping times determined as duration of loss of righting reflex. Mice were considered asleep unless they could right themselves 3 times in one minute. Significance of the difference between sleeping times of control and experimental groups of mice was assessed by the "t" test. For determination of effect of urethan on hexobarbital body concentrations, the experimental mice were treated with urethan (1200 mg/kg, i.p.) and followed in 24 hours by hexobarbital sodium (150 mg/kg, i.p.). All drug solutions were of such concentration that .01 ml was given for each 1 gram body weight. Control mice received hexobarbital only. Determination of total body hexobarbital was done one hour after hexobarbital administration by homogenizing each mouse with 4 times its weight of water in a Waring Blender. Four ml of homogenate were extracted with chloroform by method similar to that of Axelrod *et al.*(4), except that the final NaOH extract of chloroform was immediately adjusted to pH 10.3 with $KHCO_3$ and read at 245 mμ in the Beckman DU spectrophotometer.

Results. In Table I pretreatment with urethan shortens hexobarbital sleeping time. Thus, if urethan is given 24 to 48 hours previous to hexobarbital, mean hexobarbital sleeping time is decreased from 41 to 31 minutes. This effect of urethan is largely gone by 72 hours. If the interval is only 12 hours between urethan and hexobarbital administration, sleeping time is prolonged beyond control levels owing possibly to persistence of CNS depressant effect of the urethan.

The most striking effects in shortening

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TABLE I. Urethan on Hexobarbital Sleeping Time (S.T.): Effect of Pretreatment Time.

Pretreatment with urethan*		Mean hexobarbital† S.T. ± S.E., min.	P
Control	(20)‡	40.9 ± 1.7	
12 hr	(20)	86.6 ± 6.3	.001
24	(19)	31.5 ± 1.1	"
48	(20)	31.1 ± 2.5	"
72	(18)	36.5 ± 2.5	.2

* 1200 mg/kg, i.p. † 150 mg/kg, i.p. ‡ No. of mice used.

hexobarbital sleeping time were observed when 1200 mg kg urethan was administered 24 hours previous to hexobarbital (Table II).

In Tables I and II, a difference is noted in control sleeping times which occur with different batches of mice. Thus, controls were always tested with experimental group(s). That different batches of mice frequently yield variable results and therefore necessitate separate controls has been emphasized by others, for example Brown(5).

Since there existed the possibility that the vehicle for drug solution may have had effect, water and oil pretreated (24 hour, i.p.) mice were compared against untreated controls. These pretreatments had no significant effect (water, .01 ml/g, $p = .8$; oil, .01 ml/g, $p = .9$). As seen in Table III, shortening effect on hexobarbital sleeping time was obtained whether urethan was given i.p., s.c., or p.o.

The data for 3-methylcholanthrene indicate that it is qualitatively similar to urethan as regards effects on hexobarbital hypnosis. Pretreatment with 3-methylcholanthrene (30 mg/kg, i.p. in sesame oil; 24 hours) shortened hexobarbital (150 mg/kg, i.p.) sleeping time from 50.3 ± 3.3 to 37.7 ± 1.8 minutes ($p = .01$).

TABLE II. Urethan on Hexobarbital Sleeping Time: Effect of Dose of Urethan.

Urethan dose,* mg/kg, i.p.	Mean hexobarbital† S.T. ± S.E., min.	P
0	65.6 ± 2.0	
150	53.1 ± 3.8	.01
300	56.5 ± 5.2	.10
600	49.0 ± 6.0	.02
1200	39.0 ± 2.3	.001
2400	61.0 ± 5.2	.4

* Administration 24 hr before hexobarbital, 10 mice/group.
† 150 mg/kg, i.p.

The hexobarbital body concentrations in urethan-treated mice were lower than controls receiving hexobarbital alone. For the 9 urethan treated mice, the mean hexobarbital concentration was 36.4 ± 4.8 mg/kg as compared to a mean of 64.9 ± 7.3 mg/kg for the 12 control mice ($p = .01$). These data correlate well with data on the shortening of sleeping time produced by the urethan.

Discussion. The effects described suggest that urethan has the ability to increase in certain enzymes necessary for hexobarbital biotransformation. That is, the shortening of hexobarbital effect by urethan may be due to increased rate of hexobarbital biotransformation. It is unlikely that enhanced excretion of hexobarbital is responsible for the lowered hexobarbital levels. Except for initial urination and defecation at time of hexobarbital administration, little if any further urination or defecation occurs during the one hour period. Since the whole mouse is homogenized, any hexobarbital present in the bladder or gastrointestinal tract would be included in the total hexobarbital level. Since it is known that liver is the primary organ for hexobarbital metabolism(6) this effect of urethan is probably mediated in the liver. Since 3-methylcholanthrene increases liver microsomal enzymes(1,3), studies of mutual interactions of urethan, 3 methylcholanthrene and hexobarbital are indicated as interesting areas for further work.

TABLE III. Urethan on Hexobarbital Sleeping Time: Effect of Route of Administration of Urethan.

Pretreatment*		Mean hexobarbital†	P
Water, i.p.	(7)‡	68.5 ± 4.3	
Urethan, i.p.	(10)	45.8 ± 4.8	.01
Water, s.c.	(8)	66.7 ± 3.4	
Urethan, s.c.	(10)	41.5 ± 4.2	.001
Water, p.o.	(11)	63.5 ± 3.5	
Urethan, p.o.	(9)	40.9 ± 5.9	.01

* Administered 24 hr before hexobarbital. Water, .01 ml/g; urethan, 1200 mg/kg.
† 150 mg/kg, i.p.
‡ No. of mice used.

Summary. Urethan pretreatment shortens hexobarbital sleeping time and lowers hexobarbital body levels in mice. These results suggest that urethan increases rate of bio-

transformation of hexobarbital in mice.

1. Miller, E. C., Miller, J. A., *Ann. Rev. Biochem.*, 1959, v28, 295.

2. Remmer, H., *Arch. exp. Path. Pharmak.*, 1959, v235, 279.

3. Conney, A. H., Davidson, E., Glasgow, C., Burns, J. J., *Pharmacologist*, 1959, v1, 64.

4. Axelrod, J., Reichenthal, J., Brodie, B. B., *J. Pharm. Exp. Ther.*, 1954, v112, 49.

5. Brown, B. B., *Ann. N. Y. Acad. Sci.*, 1957, v66, 677.

6. Cooper, J. R., Brodie, B. B., *J. Pharm. Exp. Ther.*, 1955, v114, 409.

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Serum Proteins of Rats after Partial Hepatectomy.* (25559)

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The assumption that the remarkable increase in mitotic activity associated with some stages of restoration of liver tissue after partial hepatectomy of rats is mediated by humoral factors has been supported by findings obtained after injection of serum from hepatectomized animals(1-3) and by results of parabiosis experiments(4-6). Changes in plasma and serum proteins of rats after partial hepatectomy have been investigated(7-11). During our studies on effects of injections of serum and serum fractions on mitotic activity and liver regeneration (to be presented later), we found it desirable to obtain base values correlating changes in mitotic activity with those of serum proteins following partial hepatectomy.

Methods. Rats of inbred Lewis strain, with average age of 68 days, were used. Weight of male animals was 212 ± 43 g, of females 182 ± 23 g. Blood specimens were obtained by cardiac puncture with needles and syringes coated with "Siliclad," to minimize hemolysis. Serum paper electrophoresis was carried out in Spinco model R instrument using 0.09 M veronal buffer at pH 8.65. Runs were carried out at room temperature with serum quantities of 0.013 to 0.015 ml and continued until satisfactory resolution of components was achieved. Average distance between distant ends of gamma globulin and albumin approximated 13 cm, requiring, as a rule, application of a potential difference of 120 volts for approximately 21 hours. Total protein was determined by digestion of an aliquot of serum and subsequent Nesslerization(12). At time of sacrifice, suitable tissue blocks were fixed in buffered formalin from which 6 μ thick sections were stained with Harris hematoxylin-eosin. In these slides mitotic activity was estimated by the method of Chalkley(13). Nuclei of liver cells were used as points of reference, and mitoses encountered as "hits" from scanning 500 nuclei were recorded for each section of liver tissue.

Results. In addition to 10 controls, 100 animals, evenly divided between males and females, were placed into 10 groups of 10 animals (5 males and 5 females), tested at one of 10 time intervals, the earliest being 6 hours after operation, the others as indicated in Fig. 1. Six animals of each group were subjected to partial hepatectomy according to procedure of Higgins and Anderson(14) while the remaining 4 were subjected to sham operations, *i.e.*, laparotomy and exposure of liver followed by closure of wound. Operations were carried out under ether anesthesia. With few exceptions surgery as well as sacrifice took place between 9 and 11 a.m., to avoid any fluctuations due to diurnal changes of mitotic activity(15). Animals were sacrificed at stated time intervals immediately after blood was obtained by cardiac puncture. Total body weights and weights of livers were recorded. After coagulation of blood specimens, serums were obtained by centrifugation for 5 minutes

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