It is of interest that this ratio of synthesis of phospholipides to nucleoproteins is constant in the liver, irrespective of dietary fat or protein content and is not influenced by the presence or absence of fat in the liver(1). Campbell and Kosterlitz(15) have demonstrated in the rat that the ratio of liver protein nitrogen to phospholipides remains approximately constant on various levels of dietary proteins. In the present studies, it has been found that with administration of the pituitary fat mobilization hormone (beef thyrotropin) there was a 51% increase of mobilization of the fat in the liver in 6 hours. This increase in total lipides was statistically significant ($P < 0.01$). However, there was no change in the phospholipide synthesis. Pituitary fat mobilization hormone apparently does effect nucleoprotein synthesis, for there was an increase in the relative specific activities of the nucleoproteins.

The growth hormone, as shown in Table I, fails to affect the total lipide concentration in the liver, but stimulates both phospholipide and nucleoprotein synthesis ($P < 0.01$). This effect of growth hormone on lipide phosphorylation is in agreement with that of Geschwind *et al.*(5).

Summary. 1. Thiourea diminished phospholipide and nucleoprotein synthesis in the liver of rats. 2. The fat mobilization fraction of the pituitary gave a 51% increase in total lipides in the liver of rats in 6 hours. However, there was no change in the synthesis of phospholipides, but a statistical increase in the synthesis of nucleoproteins. 3. The Growth Hormone increased both phospholipide and nucleoprotein synthesis in the liver of rats.

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Further Studies of Metabolic Removal of Alkyl Groups from Nitrogen in Barbituric Acid Derivatives.* (20556)

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In an earlier study(1) of the metabolic fate of N-substituted derivatives of barbital, it was found that when N-methyl barbital or N-ethyl barbital is administered to dogs, a considerable amount of barbital is excreted in the urine. A trace of pure barbital was recovered from the urine of one dog receiving N-*n*-propyl barbital; from another one none could be isolated. The isopropyl, allyl, *n*-butyl, and phenyl derivatives of barbital

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were not converted to barbital in sufficient amounts to allow isolation. By means of an analytical method for the determination of the methylated and demethylated compounds in plasma, a quantitative study of the conversion of N-methyl barbital (metharbital, Gemonil) to barbital has already been carried out(2). In the present study methods have been developed for the determination in plasma of N-ethyl or N-*n*-propyl barbital in the presence of barbital. These methods have been applied to an investigation of the physiological disposition of those 2 derivatives of barbital. An elucidation of the difference between the fates of the propyl derivative and of the 2 lower homologues was considered of particular theoretical interest. Also included in this report is a study of the rates at which the 2 methyl groups are removed from N,N'-dimethyl barbital. The conversion of this compound to barbital had previously been demonstrated (3), and the method for determination of monomethyl barbital and barbital in plasma (2) has now been used to follow the course of the double demethylation.

Materials. The N-ethyl, N-*n*-propyl and N,N'-dimethyl derivatives of barbital were synthesized by Dr. Milton T. Bush of Vanderbilt University(1,3). *Administration of drugs.* N-ethyl barbital and N-propyl barbital were given to dogs intravenously as freshly prepared aqueous solutions containing 1.1 equivalents of NaOH. N,N'-dimethyl barbital was injected intraperitoneally as a solution of 8 mg per ml in saline. *Determination of N-methyl barbital and barbital in plasma.* The method previously described was used(2). Unchanged N,N'-dimethyl barbital, not being ionizable, is not extracted from ether into the carbonate buffer. It is consequently not determined and does not interfere. *Determination of N-ethyl barbital and barbital in plasma.* In a glass stoppered centrifuge tube are placed 4 ml of oxalated plasma, 1 ml of buffer, pH 6 (0.86 moles KH_2PO_4 + 0.14 moles K_2HPO_4 per l), and 25 ml of ethyl ether previously purified by distillation and washing with solutions of NaOH and HCl and finally with water. After shaking and centrifugation, 20 ml of the ether phase is transferred to another tube and shaken with 4 ml of 0.2 *N* NaOH saturated with ether. After centrifugation the lower phase is withdrawn and shaken with 2 ml of ether. The tube is centrifuged and the ether discarded. To 3 ml of the aqueous phase is added 3 ml of 0.2 *M* H_3BO_3. The resulting pH is about 10, and both compounds to be determined are present essentially completely in the form of monopolar anions. The optical density is measured at 241, 250, and 260 mμ with a Beckman ultraviolet spectrophotometer. A mixture of equal parts of the NaOH and H_3BO_3 solutions is used as the blank. At 241 and at 250 mμ the absorption of N-ethyl barbital is equal, and it is from the difference between the densities at these 2 wavelengths that the concentration of barbital is calculated. The concentration of N-ethyl barbital is derived from the difference between the density at 250 mμ and that at 260 mμ. From the observed densities at these 2 wavelengths are subtracted the corresponding densities that would be contributed by the concentration of barbital found by the first calculation. The difference between the corrected density at 250 mμ and the corrected density at 260 mμ is the value from which the concentration of N-ethyl barbital is calculated. Errors due to interference from normal constituents of plasma have not exceeded values corresponding to concentrations of 2 mg per l of either drug. *Determination of N-propyl barbital and barbital in plasma.* The method is identical to that described for the ethyl compound except that a measurement at 242 mμ is substituted for that at 241 mμ.

Results and discussion. Following intravenous injection of N-ethyl barbital and N-propyl barbital in dogs, the resulting concentrations of unchanged drugs and dealkylated products are shown in Fig. 1 and 2, respectively. In both experiments there is an initial rapid drop in the concentration of unchanged drug, this being doubtless due to passage of the drug from blood into tissues rather than to actual loss from the body by chemical destruction or excretion. After this phase the decline in concentration is slow. The pattern of falling plasma concentration of both of these drugs resembles closely that previously found for N-methyl barbital(2).

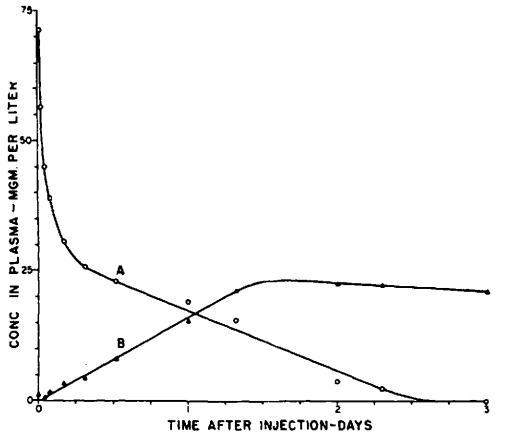

FIG. 1. Curve A (points designated by circles) shows the concentrations of unchanged drug and Curve B (points designated by triangles) the concentrations of barbital found in plasma following the intravenous inj. of 3×10^{-4} moles (64 mg)/kg of N-ethyl barbital in a 12 kg female dog.

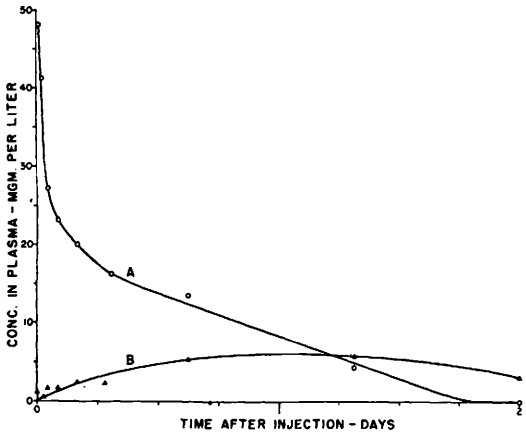

FIG. 2. Curve A (points designated by circles) shows the concentrations of unchanged drug and Curve B (points designated by triangles) the concentrations of barbital found in plasma following the intravenous inj. of 2.5×10^{-4} moles (57 mg) per kg of N-propyl barbital in a 12 kg female dog.

All 3 of these alkyl derivatives of barbital disappear at rates that are of the same order of magnitude.

As unchanged N-ethyl barbital disappears from the plasma, barbital appears and increases slowly in concentration for more than a day. Comparison with the corresponding experiment in which the same molar dose of N-methyl barbital was given to another dog (2) shows that barbital was produced somewhat more rapidly from the methyl than from the ethyl derivative but that the rates at which these 2 alkyl groups are removed from nitrogen are of the same order of magnitude.

Calculation of the yield of barbital by the procedure outlined in the earlier study(2) indicates that approximately 60% of the N-ethyl barbital that has disappeared after 2 days can be accounted for as barbital produced. While this estimate is necessarily rough, it does corroborate the conclusion(1), based on isolation of barbital from urine, that N-ethyl barbital is dealkylated to a large extent. If allowance is made for losses, the amounts isolated from urine are consistent with a yield of the order of that estimated here.

The metabolic fate of the N-propyl derivative of barbital is in striking contrast to that of the 2 lower homologues in that much less barbital is produced. In the experiment of Fig. 2, the highest concentration of barbital found is less than 4 times the normal blank. The measurements are consequently subject to a large proportional error, but do suffice to indicate that not more than about 10% of the N-propyl barbital was dealkylated. This experiment is thus corroborative of the earlier discovery(1) that N-propyl barbital does not give rise to significant amounts of barbital in the urine. It was suggested(1) that failure of N-propyl and some of the other N-substituted barbitals to be converted to any great extent to barbital might be due to their destruction by more rapid reactions rather than to any inherent incapacity of the body to remove the substituent groups from nitrogen. The brief duration of action of a single intravenous dose of N-propyl barbital was at that time taken as evidence of rapid chemical destruction. It is now known that the transient character of the effects seen after intravenous injection of this drug (and also other N-substituted barbituric acids and thiobarbituric acids) can be attributed to physical redistribution of the drug in the body rather than to chemical inactivation. It is evident in the experiment of Fig. 2 that N-propyl barbital persists in the body for a long time and that the low production of barbital cannot be due to rapid removal of the parent compound by other processes. Either the propyl group is less susceptible to enzymatic attack than the

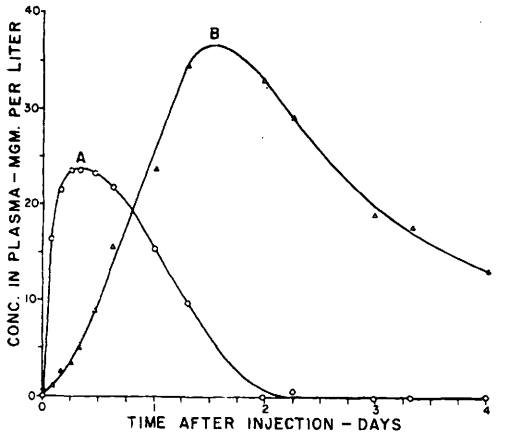

FIG. 3. Curve A (points designated by circles) shows the concentrations of N-methyl barbital and Curve B (points designated by triangles) the concentrations of barbital found in plasma following the intraperitoneal inj. of 3×10^{-4} moles (64 mg) per kg of N,N'-dimethylbarbital in a 12 kg female dog. Unchanged drug was not determined.

methyl and ethyl groups, or the initial product of enzymatic action is one in which there is less tendency toward rupture of the nitrogen-carbon bond.

N,N'-dimethyl barbital was injected intraperitoneally in a dog and the concentrations of the singly and doubly demethylated products in plasma measured. The results are shown in Fig. 3. The monomethyl compound appears at a relatively rapid rate. The final product of demethylation, barbital, appears more slowly and is increasing in concentration long after its immediate precursor has passed its maximum concentration. If it be assumed that the volumes of distribution are the same as found in an earlier experiment(2), it can be calculated that the initial rate at which dimethyl barbital is converted to monomethyl barbital is several times that at which the latter compound is converted to barbital. It might be expected that the probability of a methyl group being lost within a given period of time would be higher for a molecule containing 2 identical methyl groups than for a molecule containing only one. This factor alone would not, however, suffice to explain the high rate at which the first methyl group is removed from N,N'-dimethyl barbital.

Summary. Analytical methods are described for the simultaneous determination in plasma of N-ethyl barbital and barbital or N-propyl barbital and barbital. These methods have been used to study the metabolic fate in the dog of the N-ethyl and N-propyl derivatives of barbital. Both of these compounds disappear slowly from the plasma. N-ethyl barbital is converted in considerable part to barbital, the concentration of that substance in plasma increasing for more than a day. N-propyl barbital is converted to barbital only to a very small extent. By means of methods previously developed for the simultaneous determination of N-methyl barbital and barbital in plasma, the fate of N,N'-dimethyl barbital has been studied. Both methyl groups are removed from this compound, the first much more rapidly than the second.

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