phocytes of C58 mice inoculated with Line U leukemia has occurred in the course of 350 transfers. This increase in the number of mitochondria has occurred concomitantly with a decrease in the killing time, following a standard number of cells, from about 15 days to 4 days. The number of mitochondria in lymphocytes of Line I has not shown a similar increase in the course of 500 transfers made during this same period of time, and the interval between inoculation and death has remained unchanged at approximately 4 days. In making these new counts it was not possible to follow in every respect the technic for staining of mitochondria used by Potter and Ward.

Certain other differences between Line U and Line I have not changed since the time of the previous study of these leukemias. In spite of the increase in mitochondria, the lymphocytes of Line U remain smaller than those of Line I. In morphology, the mitochondria have retained the characteristics which distinguished them previously. Since the range in size of lymphocytes is the same in the two leukemias, although the means are different, if size is inversely correlated with the age of the lymphocyte as has been suggested,[3,4] then the 2 groups of lymphocytes share the same ages and stages of differentiation. The persisting differences between Line U and Line I, therefore, may be characteristics of the lines of leukemia and not differences due to the age and developmental stage of the lymphocytes.

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Distribution and Rate of Disposition of Myanesin in Dogs.

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One of the most interesting characteristics of the action of myanesin* (3-(orthotoloxyl)-1,2-propanediol) is its shortness of action.[1-3] By the use of 3 different analytical methods the duration of pharmacological effect has been correlated with concentration of free myanesin in plasma.[3,4] It has also been demonstrated that less than 1% of myanesin administered intravenously can be recovered in urine as free myanesin. After hydrolysis with acid, material has been obtained from the urine which may be nitrated or coupled with diazotized dinitro-aniline to an extent which accounts for at least 30 to 40% of the total myanesin administered. It would appear, therefore, that myanesin is rapidly degraded in the body and that its short action is due almost entirely to rapid metabolic change. If myanesin is to be used intelligently, more information is needed as to the character of its absorption, excretion, distribution, and disposition. This report summarizes certain aspects of this problem.

Methods. Of the 3 methods proposed for the determination of myanesin in body fluids and tissues,[3,4] the coupling reaction with diazotized dinitro-aniline described by Titus, Ulick and Richardson is most suitable since

* We wish to thank Squibb Institute for Medical Research for the sample of myanesin used in these studies.

[1] Berger, F. M., and Bradley, W., *Brit. J. Pharmacol.*, 1946, **1**, 265.

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[3] Titus, E. O., Ulick, S., and Richardson, A. P., *J. Pharm. and Exp. Ther.*, to be published.

[4] Wyngaarden, J. B., Woods, L. A., and Seevers, M. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 256.

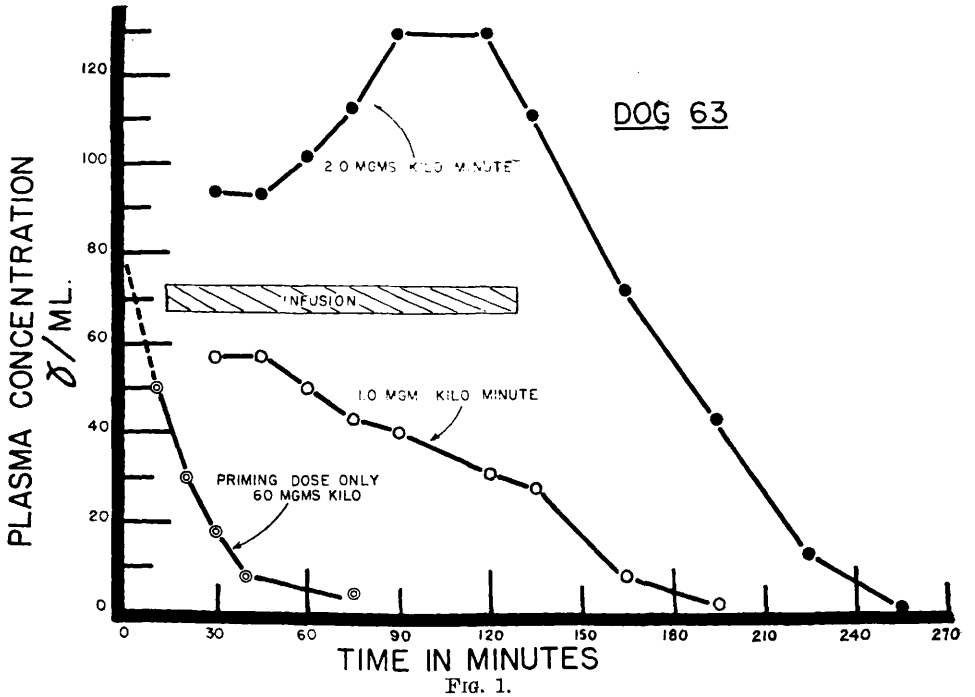

FIG. 1.

A typical experiment illustrating the effect of rate of infusion on plasma concentration of myanesin following a priming dose of 60 mg per kilo. Drug administered intravenously at the rate of 1 ml per minute and concentration adjusted to give the appropriate dose of drug.

it is both sensitive and the analysis is not complicated by high tissue or reagent blanks. The results reported here have all been obtained with this method.

For the determination of "total" myanesin, samples were acidified with an equal volume of concentrated hydrochloric acid; heated for 10 minutes; extracted into chloroform; evaporated; residue taken up in 85% phosphoric acid; and coupled as in the method for determination of free myanesin. In the absence of definitive information as to the nature of the degradation product it was not possible to say exactly what our recoveries were for "total" myanesin, nor can one be certain that this is the best way in which the analysis could be carried out. However, since it was possible to account for 30-40% of the compound administered, the method described must be at least an approximation of "total" myanesin.

Rate of disposition of myanesin. The general plan of these experiments is illustrated by results presented in Fig. 1 for a single dog. Plasma concentrations were determined at intervals following the intravenous injection of a single dose of 60 mg per kg, until all drug had disappeared from the plasma. Several days later a glass cannula was inserted into a femoral vein under local anesthesia; a priming dose of 60 mg per kg of myanesin was injected intravenously and 5 minutes later a continuous infusion of drug into the cannulated vein was begun. Rate of infusion was kept constant at 1 ml per minute and concentration of drug adjusted so as to give each animal either 1 or 2 mg per kg per minute of infusion. Infusion was continued for a period of 2 hours. Blood samples for determination of plasma concentration were taken at half hour intervals during the period of infusion, and at hourly intervals thereafter until plasma concentrations had fallen to zero.

Rate of disposition of myanesin following infusion of both 1 and 2 mg per kg per minute was determined in 6 dogs. Ten other dogs were infused only at a rate of 2 mg per kg per minute.

Fig. 2 summarizes all experiments in 16 dogs carried out as described above. For pur-

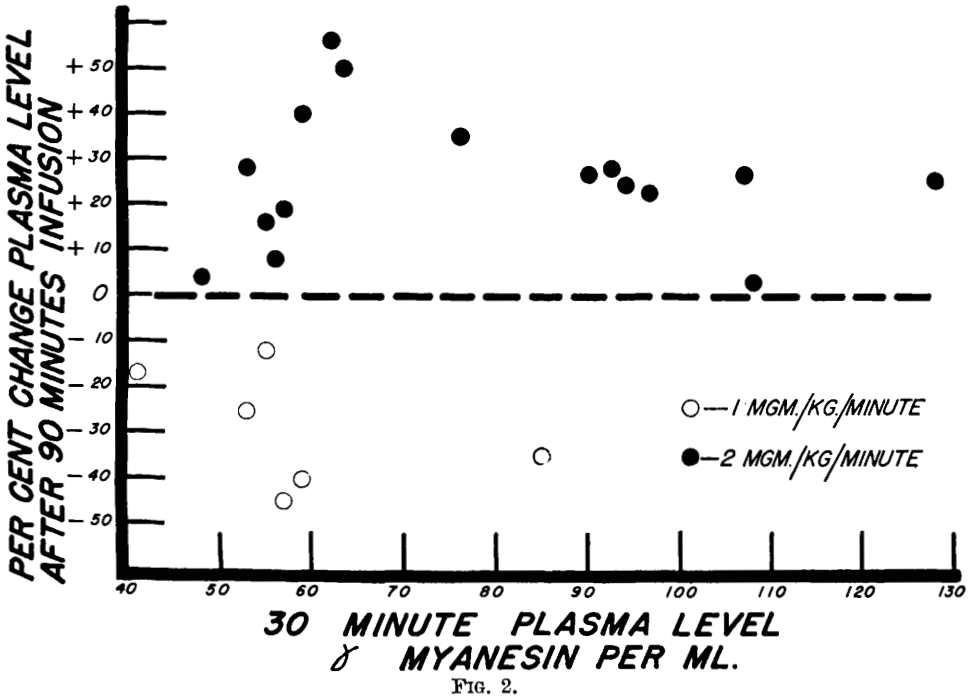

FIG. 2.

Summary of experiments showing relationship of rate of infusion to change in plasma concentration. Each point represents per cent change in concentration from thirty minutes after priming dose as compared to plasma concentration 90 minutes later.

poses of comparison it was assumed that 30 minutes after the priming dose (and 25 minutes after starting the infusion) was a sufficiently long period of time to allow for a balance to be established between the processes of distribution, degradation, and excretion. Change in plasma concentration beyond this point compared to rate of infusion should give an estimate of the ability of each animal to dispose of the drug. It is readily seen from Figs. 1 and 2 that infusion of 2 mg per kg per minute resulted in an increasing plasma concentration and therefore is greater than the rate of disposition. On the other hand infusion of 1 mg per kg per minute was insufficient to maintain plasma levels, thus one can conclude that under the conditions described dogs can dispose of between 1 and 2 mg per kg per minute.

Plotting the results as shown in Fig. 2 also illustrates another point, namely, that between plasma levels of 50 and 130 μg per ml the rate of disposition can not be correlated with plasma concentration.

That disposal of myanesin is due to metabolic change rather than excretion was suspected from earlier experiments.[1,3,4] Additional evidence for this point of view was obtained from some of the present continuous infusion experiments in which urine was collected during the infusion period and analyzed for free myanesin. In the several experiments in which this was done less than 1% of the drug infused could be found in the urine. Since a major portion of myanesin does eventually appear in the urine, in combined form, it is obvious the rapid disposal of the drug must be due to metabolic alteration.

Distribution in body fluids and tissues. Preliminary calculations of the apparent volume of distribution of myanesin following a single intravenous injection[3] gave a value of approximately 70% of body weight. It was to be expected therefore that the drug was widely distributed possibly in total body water. In order to study this more exactly 7 unanesthetized dogs were infused intravenously with myanesin at the rate of 2 mg per kg per minute as described above. After 2 hours infusion, blood, saliva, and spinal fluid samples were

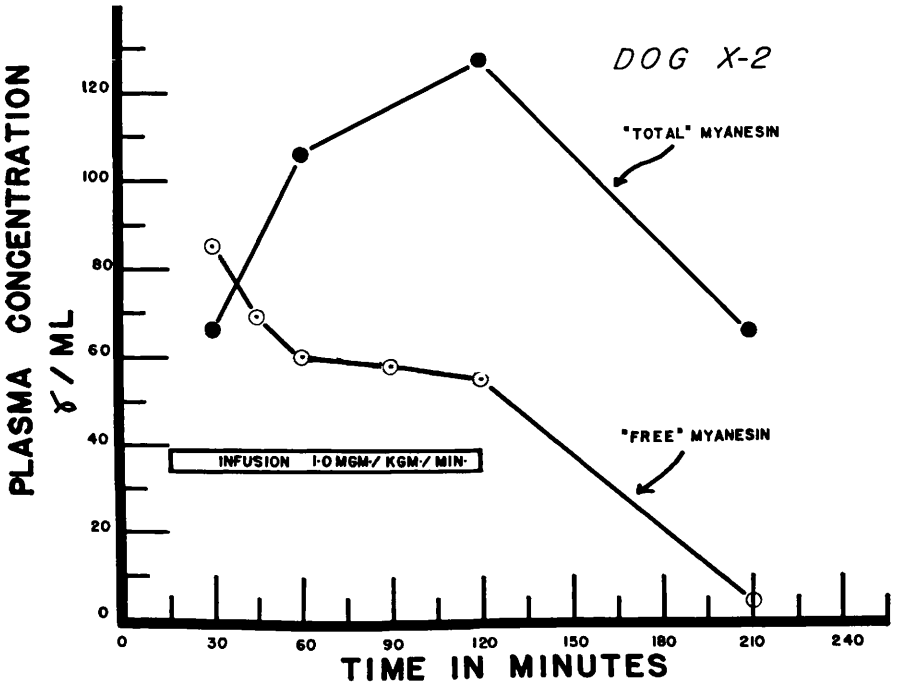

FIG. 3.

Typical experiment illustrating relationship of free and "total" myanesin in plasma following intravenous infusion. "Total" drug determined after hydrolysin with hydrochloric acid.

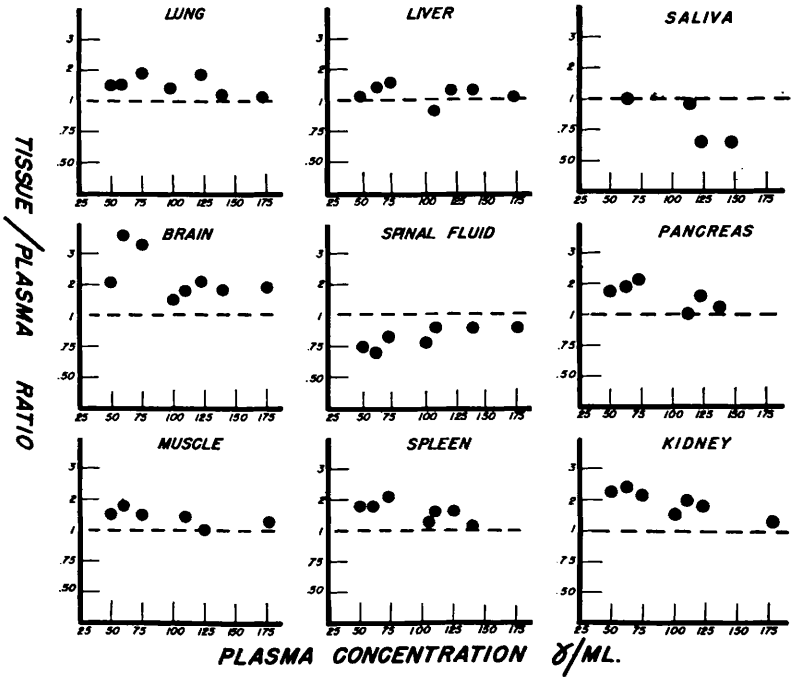

FIG. 4.

Tissue/plasma concentration ratio after continuous infusion of myanesin for 2 hours. All determinations are of free drug.

taken and animal killed by the intravenous injection of air. Tissue samples were removed as rapidly as possible and prepared immediately for analysis. Each sample of tissue was weighed; ground in a Waring blendor to form a coarse suspension and finally ground with a Ten Broeck tissue grinder until each sample could be handled with a tuberculin syringe. In order to facilitate grinding each sample of tissue was mixed with an equal volume of saline, except for muscle with which 2 volumes of saline were used for each volume of tissue. Preliminary experiments indicated that added myanesin could be recovered satisfactorily from such tissue mashes. However, it was not possible to analyze for "total" myanesin since tissue-reagent blanks were excessively high.

The results of all experiments are summarized in Fig. 4 in which ratios of tissue/plasma concentration are plotted against plasma concentration. As predicted it is obvious that myanesin is widely distributed, and approximately according to water content of each tissue. A notable exception to this, however, is seen in the case of brain in which the ratio of tissue/plasma was always more than unity and average approximately 2. The explanation for this finding is at present obscure, but of considerable interest since the outstanding actions of myanesin are on the central nervous system. Since spinal fluid and saliva concentration were consistently lower than plasma, one wonders if perhaps distribution of myanesin may not in part be conditioned by lipoid solubility as well as water solubility.

Discussion. The above experiments furnish concrete evidence that the brief action of myanesin is due primarily to rapid metabolic alteration. Since myanesin has been suggested for use in a number of chronic diseases[5,6] where prolonged action is desirable, it is important that more data concerning the characteristics of the processes involved in the alteration of the free drug should be obtained. Such information may furnish important leads as to means whereby longer acting drugs may be synthesized.

Summary. 1. From a study of plasma concentrations of animals infused continuously, it has been shown that dogs can dispose of between 1 and 2 mg of myanesin per kg per minute, irrespective of plasma concentration between the limits of 50 and 130 μg per ml. 2. The rapid disposition of myanesin is due primarily to metabolic alteration. 3. Myanesin is widely distributed in the body approximately according to water content. A notable exception to this is found in the brain where tissue/plasma ratios of greater than 2 are observed.

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Effect of Cycloheptenylethyl Barbituric Acid, "Medomin," on Intestinal and Uterine Smooth Muscle.*

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The following investigation was undertaken to determine the effect of cycloheptenylethyl barbituric acid "Medomin" on intestinal and uterine smooth muscle and the effects of a number of short and ultra-short barbiturates on the intact intestine of the non-anesthetized dog.

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Methods. Excised Intestine. Excised longi-