The preparation is palatable and taken readily by these patients. A variety of mild and serious common childhood illnesses responded promptly to this medication.

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## Histology of the Brain and Blood in Chronic Experimental Sedation with Barbiturates and Presidon.* (19017)

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The appearance of histologic changes in the central nervous system following indiscriminate use of barbiturates is held as a major argument against the unrestricted and prolonged use of these drugs. However, the evidence for this idea is not strong, especially in human beings. Hassin's(1) report on acute intoxication in man and Neumann's(2) recent report of a case surviving severe intoxication for five months show only non-specific changes in the brain that could well be the result of anoxia due to respiratory depression by the drug. More conclusive evidence as to effects of barbiturates on the human brain is lacking. Seevers and Tatum(3) examined dogs maintained for as long as three years on daily barbital administration. The post mortem examination showed thickened meninges and walls of cerebral vessels with some degeneration of neurons of the brain. However, such damage was poorly localized, varying in degree and location with the individual animals. Krop and Gold(4) could find no changes in the brain of the dog following barbiturate intoxication with lethal dosage. The present study was undertaken to compare the effects of barbiturates with those of 3,3 diethyl-2,4 dioxotetrahydropyridine, Presidon, a non-barbiturate hypnotic, on the histology of the central nervous system of dogs chronically sedated over long periods of time.

*Method.* Twelve mongrel dogs, fed on a stock diet throughout the experiment, were divided into two groups of six. Three animals of each group were given a minimum hypnotic dose of Presidon twice daily, two animals received a similar dose of barbital, and one animal received phenobarbital. The animals in one group were sacrificed after six months, and those in the remaining group after one year. The animals were sacrificed during the sleep following their daily drug intake, the brains perfused with formalin (U.S.P. 1:10), and the viscera examined grossly and samples taken for microscopic examination. The spinal cords and brain stems were frozen by Marshall's technic and sectioned serially. The cerebral and cerebellar cortices were sampled and prepared for examination in a similar manner. Alternate sections were stained with thionin, Weil's and a few with Masson's stains. During the last seven months of the experiment complete blood counts were made biweekly on all the dogs, and a sample of sternal marrow was taken at autopsy in order to observe any possible development of blood dyscrasias especially agranulocytosis which has been reported clinically following the use of Presidon.

*Results.* A single dose of Presidon produced a light sleep of about two hours duration. The dogs could always be aroused from such sleep at any time and upon undisturbed awakening were immediately alert. In none of the animals receiving this drug were overt

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3. Seevers, M. H., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1931, v42, 217.

4. Krop, Stephen and Gold, Harry, *J. Pharm. and Exp. Therap.*, 1946, v88, 260.

TABLE I. Drug Dosages and Blood Counts of Treated Dogs.

| Dog No. | Daily oral dosage* Drug | Daily dose (mg/kg) | Blood counts No. of counts | Initial WBC | Avg WBC | Avg RBC (million/cmm) |
|---|---|---|---|---|---|---|
| 1 | Barbital | 106 | 13 | 16550 | 11104 | 7.43 |
| 2 | | 121.5 | 13 | 15350 | 12893 | 8.25 |
| 3 | | 103 | 2 | 12500 | 10375 | 6.55 |
| 4 | | 152 | 2 | 12400 | 13400 | 6.13 |
| 5 | Phenobarbital | 61.3 | 13 | 10450 | 14400 | 6.25 |
| 13 | | 70.8 | 2 | 12300 | 10800 | 5.25 |
| 7 | Presidon | 342 | 2 | 8150 | 10500 | 4.65 |
| 8 | | 333 | 2 | 18750 | 13675 | 5.86 |
| 9 | | 353 | 2 | 11050 | 11750 | 7.44 |
| 10 | | 312 | 13 | 7600 | 10290 | 8.20 |
| 11 | | 343 | 13 | 8400 | 12704 | 7.53 |
| 12 | | 361 | 13 | 8300 | 9139 | 7.27 |

* Range of body wt—7.3 to 15.8 kg.

neurological disturbances observed even after 12 months of continuous medication. Barbital and phenobarbital, on the other hand, resulted in periodic sleeping which was inconstant both in time of appearance and duration; furthermore, there was a tendency for accumulation of the effect of these drugs so that often the animals would be deeply narcotized for hours necessitating the omitting of the drug for a day. Awakening from sleep in these dogs was accompanied by ataxia and general disorientation for several minutes, and the animals receiving barbital for one year were constantly mildly ataxic during the last two months of the experiment. Withholding the drugs for as long as 4 days never resulted in convulsions in these dogs, although the animals on barbital and phenobarbital became quite restless.

Necropsy findings upon the gross and microscopic examination of the viscera were in general devoid of significant changes. The spleens of the animals receiving barbital and phenobarbital were the exception. Pinkish gray tumors, 2 to 3 mm in diameter, lined the border of the spleen, and microscopic examination showed them to contain splenic tissue and large vessels congested with erythrocytes. There were no histologic changes in the central nervous system of any of these dogs that could be classified as pathologic in the usual sense. Perhaps an exception to this was the presence of small round elements similar to the amyloid bodies often seen in the human brain including presumably normal cases. These were scattered through the brain stem and cortex. Mott, Woodhouse, and Pickworth(5) described similar stuctures in the brains of cats and monkeys chronically intoxicated with barbiturates. However, their chemical analysis showed such bodies to be mucinoid, rather than amyloid in composition, and they presumed them to be of degenerative origin. A chemical analysis was not made in our experiment, and it is perhaps possible that these structures may be indicative of some change in brain chemistry since they appeared only in the animals receiving the drug for one year.

Table I lists the results of the blood counts of these animals, and the values are compatible with those for normal dogs as given by Mayerson(6) and Wells and Sutton(7). The post mortem examination of the sternal marrow showed ample amounts of all myeloid leucocytic elements, and the peripheral blood was free of immature leucocytes.

*Discussion.* The failure to observe histologic changes aside from the amyloid-like bodies in these animals seems somewhat in disagreement with the literature; however, when considering the differences in dosage, condition of the animals and method of preserving the brain, a latitude in results of different experiments is most likely, and as has

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been mentioned no very marked changes in the brain of the dog have been previously observed following chronic barbiturism. Lack of histologic change, however, does not necessarily preclude the possibility of a derangement of physiological processes, and the ataxia of the barbital dogs is a good indication of this fact. Failure to observe further neurological signs or withdrawal symptoms may very well be due to the dosages used which, as can be seen in Table I, were not large when compared to the daily intake in human barbiturate addiction. Table I also shows that large doses of Presidon were needed to produce sleep as compared to small doses of the barbiturates, yet the sleep after the former was much more uniform without cumulative symptoms or after effects such as ataxia or disorientation. The presence of the amyloid-like bodies could not in this experiment be related to any neurological signs for they were present in the dogs receiving Presidon as well. However, their appearance only in those animals treated for one year does suggest a relationship to prolonged use of these drugs.

Whether or not there are species differences in reactions to these drugs is of course not determined. However, in comparing our results with the literature, it would seem such differences, at least with regard to the nervous system, would be a matter of degree. With regard to the blood, on the other hand, species differences may be a factor in the development of dyscrasias following the use of drugs. The dog appears to have a much more active hemapoietic system than man and conceivably could be more resistant to drug effects.

*Summary.* (1) Twelve dogs receiving barbital, phenobarbital or Presidon daily for six months to one year were observed for neurological disturbances and alterations in the histology of the viscera, blood and brain. (2) Dogs receiving barbital for one year developed a mild ataxia. These dogs and one receiving phenobarbital for one year had small splenic tumors at autopsy. All other post mortem findings were negative with respect to histologic changes in the viscera and blood. Small structures similar to amyloid bodies seen in man were found in the brain stem and cortex of all dogs treated one year, but not in dogs treated six months. These structures could not be correlated with neurological changes in behavior, nor is their significance known.

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## Mechanism of Enzymatic Reduction of Triphenyl Tetrazolium Chloride.* (19018)

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Although considerable attention is being paid to tetrazolium salts in various fields of biology(1,2) the mechanism of their reduction by mammalian tissues requires further elucidation. It can be assumed that in an enzymatic reducing system, composed of an enzyme protein, pyridine-nucleotide and flavo-protein, the immediate electron donor of triphenyl tetrazolium chloride (TTC) is the dye-reducing flavo-protein of Straub(3). This mechanism was suggested by Bieling, *et al.*(4) and Kun(1) and clearly demonstrated by Brodie and Gots(5) with yeast triose phos-

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