Comments and Communications

Use of Nembutal as an Anesthetic for Large Wild Mammals[1]

DURING the study of hibernation in the black bear, it was necessary to attach instruments to the animal and to take periodic samples of blood. This required the complete immobility of the bear. Ether anesthesia took too long a time before complete sedation occurred. Nembutal (the trade name of sodium pentobarbitol), because of its ease of administration, safety, and rapid action, was therefore tried and found satisfactory.

An adult male American black bear (*Ursus americanus*), weighing 211 pounds, was made available through the courtesy of Edward Johnson, superintendent of the Woodland Park Zoological Gardens, Seattle, Wash., and was anesthetized upon three occasions. Several attempts at oral administration of Nembutal, both in a bolus of ground raw meat and mixed with powdered brown sugar, proved unsuccessful, so intraperitoneal injections were used. The bear, without food for 24 hr, was incarcerated in a squeeze cage and held steady while the Nembutal, dissolved in water at a concentration of 25 mg/kg body weight, was injected intraperitoneally. The bear was then allowed to move freely within the cage, while observations and records were made on its heartbeat, respiration rate, and subsequent actions until sedation occurred.

Immediately following the injection a respiration rate of 28/min and pulse rate of approximately 160/min were recorded. Within 17 min surgical sedation was attained. The respiration rate dropped and remained fairly uniform at 12/min, with the pulse rate at approximately 110. Surgical sedation on this occasion remained for about 83 min, during which many blood samples were taken from the scaphenous vein. The bear showed no deleterious aftereffects. The data obtained from these and subsequent blood samples are being described elsewhere.

A second intraperitoneal injection with a smaller dose of 20 mg/kg body weight proved inadequate, so a supplementary injection of sodium pentothal, 7.5 mg/kg, was given intraperitoneally. In approximately 43 min after this second injection, or 2 hr and 5 min after the initial injection of Nembutal, sedation occurred. Food had not been withheld for the previous 24 hr, and this might have prolonged the time required. Although sedation was not as deep as in the previous trial, since both the heartbeat at 120/min and the respiration rate at 22 were higher, nevertheless, it persisted for approximately 1 hr. No ill aftereffects were noticed.

A third trial similar to the first was made after food had been withheld for 24 hr. In 13 min complete surgical sedation, which lasted approximately 1 hr was reached, with the pulse rate remaining at about 100/min and a respiration rate of 12. Blood samples were taken, and electrocardiograms, involving numerous manual operations in the cage with the bear, were recorded. Again there were no ill aftereffects. Repetition of dosage and effects indicated that Nembutal injected intraperitoneally at a concentration of 25 mg/kg body weight can be safely administered to bears.

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[1] Acknowledgment is made to the Air Force under contract AF 33 (038)-18509 for aid and assistance in making this study.

The Peyote Cult

I WISH to express appreciation for, and to concur with, the communication dealing with peyote, signed by Messrs. La Barre, McAllester, Slotkin, Stewart, and Tax, in your November 30 issue. The subject is of importance to American Indians, to civil liberties, and to anthropology.

In late 1922, when federal and state proscriptions against the Native American (Peyote) Church were being pressed, the peyote cult members at Taos Pueblo laid their case before me. They offered (with an understanding of what was involved) to submit themselves individually and as a group to the fullest scientific investigation. They understood that such investigation would be pharmacological, biological, psychological, and social, and that it would involve experimentation, using part of their number as a control group.

In 1924, at the meeting of the Committee of 100 on Indian Problems (a Committee assembled by Secretary of the Interior Hubert Work), I reported this offer; a resolution was enacted, calling upon the National Research Council to plan and execute an investigation into peyote. The council never initiated this requested investigation.

Some years later, for the American Indian Defense Association, Donald Collier (at present a staff member of the Chicago Natural History Museum) canvassed all the then-existing literature on peyote, totaling some 400 published books and papers. His conclusion was identical with that set forth in the communication in SCIENCE.

Subsequently, in 1933, I became U. S. Commissioner of Indian Affairs. I introduced the above-mentioned analysis of the existing literature into the *Congressional Record* (the hearings, as I remember, of the House Sub-Committee on Interior Department Appropriations); and Secretary Harold L. Ickes and I prohibited absolutely any interference by the Indian Bureau with the religious practices of the Native American Church. We were abused in a good many quarters for this action; but the administrative

policy that we established has been adhered to up to the present time, although the Navajo tribe has passed and tried to enforce ordinances against the use of peyote. Administratively, we felt that we had no right to veto the Navajo Tribal Council's action.

There remain on the books of a number of states (procured by the Indian Bureau and by missionaries in earlier times) statutes that declare the ceremonies of the Native American Church (in effect) to be misdemeanors; and there is an occasionally renewed drive at Washington to secure the classification of peyote as a habit-forming and injurious drug. The subject concerns Indians in a dozen or more states; and it is hoped that the communication in SCIENCE will be given attention.

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Measuring Rate of Growth of Leucocytes

RECENTLY, Osgood, Li, Tivey, Duerst, and Seaman (*1*) reported a method for quantitatively measuring the rate of growth of leucocytes in tissue culture, by determining $DNAP^{32}$ (radiophosphorus in desoxyribonucleic acid). In the opinion of the writer, the paper presents no acceptable evidence for the validity of the method. On the contrary, it serves to illustrate a number of errors that must be avoided in this type of work.

Interest in $DNAP^{32}$ has been stimulated by the fact that its rate of formation is so low that it may be dependent upon the rate of formation of new DNA, associated with mitosis. Indeed, advantage has been taken of this fact (*2*) to obtain a qualitative indication of mitotic activity. But, as Hevesy has remarked (*3*), it is of the utmost importance to purify the sample of DNA very carefully, in order to remove phosphorus compounds that may have a much higher specific activity. Many workers (*3–5*) have perfected elaborate methods for isolation of DNA and have presented analyses to characterize their preparations—in other words, to show that DNA was actually isolated and that $DNAP^{32}$ was actually determined.

For isolating DNA, Osgood *et al.* utilized a procedure introduced by Schmidt and Thannhauser (*6*) —alkaline hydrolysis of ribonucleic acid under conditions that do not render DNA acid-soluble. The authors of the Schmidt-Thannhauser method did not specifically study its application to blood leucocytes, nor did they claim that it offered the precision necessary for isolation of $DNAP^{32}$ of any tissue. Furthermore, Osgood *et al.* have omitted the important first part of the method, without any stated reason. Thus their method for determination of $DNAP^{32}$ was essentially a new one, but they presented no analytical data of any sort to justify calling their preparation "DNA." Nevertheless, it is possible to judge the soundness of their procedure, from certain papers that they do not cite. (These studies were concerned with tissues other than blood leucocytes. Although Davidson, Leslie, and White (*7*) applied the Schmidt-Thannhauser method to bone marrow, they apparently did not confirm their results by use of any other method for determining nucleic acids.)

Schneider (*8*) prepared DNA from six different rat organs, according to Schmidt and Thannhauser. To this "DNA" he applied his hot trichloracetic acid extraction procedure, which, as he has shown, separates nucleic acid from non-nucleic acid phosphorus. It was then evident that Schmidt-Thannhauser "DNA phosphorus" was 3–66% too high (compare columns IV-D and V-B of his Table I). Later the Schmidt-Thannhauser method, modified as suggested by Schneider, was applied by the present writer with others (*2*) to $DNAP^{32}$ determination in whole rat liver and hepatomas. It was also applied to nuclei isolated from these tissues. "$DNAP^{32}$" values were greatly reduced, in most cases, if cytoplasm (containing no DNA) was removed. Thus the Schmidt-Thannhauser procedure is not generally applicable to determination of $DNAP^{32}$ in whole tissues. Whether Osgood *et al.* actually isolated $DNAP^{32}$, cannot therefore, be determined from their preliminary report.

That the true rate of $DNAP^{32}$ formation in the cultured leucocytes was actually much lower than the reported rate is suggested by the observations disclosed concerning the degree of vitality possessed by the cells. Generally speaking, the cell counts graphically presented in Figs. 1 and 2 take the form of logarithmic death curves. It must be remembered that it is not possible, with Osgood's techniques, to separate the mitotically active cells, if such are present, from those that are dying. In a paper on marrow cultures (*9*), which Osgood *et al.* cite, Osgood states that his cultures "show degeneration after one to three weeks, although mitoses have been found at thirty-four days. . . ." However, in the tracer experiments reported recently, blood leucocytes, not marrow cells, were cultured, and the culture conditions were stated to be especially unfavorable. Apparently, no mitotic figures were observed.

Finally, Osgood *et al.* claim that by determining $DNAP^{32}$ it is possible "to obtain a quantitative measure of the rate of formation" of new cells. As has been remarked (*5*), the immediate precursor or precursors of DNA phosphorus are unknown. Until they can be identified and the specific activity of phosphorus entering DNA can be determined, there can be no *quantitative* estimate of the rate of formation of DNA, or of new cells, by such an approach.

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