tion A), m.p. 202–245°. There was no absorption which could be attributed to an olefinic double bond. A series of fractions were combined as fraction A since they had similar melting points and identical infrared spectra.

Repeated crystallizations from absolute ethanol raised the melting point to 263–269° without changing the infrared spectrum. A mixed melting point with an authentic sample of 3-aza-A-homo-5α-cholestan-4-one, m.p. 270–272°, gave a m.p. of 264–269°, establishing that these samples are identical.

Further elution of the column with ethanol yielded 800 mg. of starting material, II.

17β-Acetylamino-4-androsten-3-one (VII).—*Method A, from Progesterone.*—Progesterone (VI), 2.0 Gm., was allowed to react with 0.4 Gm. of sodium azide in polyphosphoric acid in a procedure similar to that used for cholestenone. After repeated crystallizations from acetone the crude product yielded 220 mg. of an analytical sample of 17β-acetylamino-4-androsten-3-one (VII), m.p. 275–278°; $[\alpha]_D^{27}$ + 58.0° (c, 1.0 $CHCl_3$); $\lambda_{max.}^{EtOH}$ 239 mμ, log ε 4.11; $\lambda_{max.}^{CHCl_3}$ 2.92 μ (free NH), 3.00 μ (associated NH), 6.01 μ (C=O), and 6.20 μ (C=C). A second crop (500 mg.) was obtained giving a total yield of 720 mg. (35%).

Anal.—Calcd. for $C_{21}H_{31}NO_2$: C, 76.55; H, 9.48; N, 4.25. Found: C, 76.42; H, 9.39; N, 4.69.

Method B, from 17β-Acetylamino-5-androsten-3β-ol (IX).—To a refluxing suspension of 681 mg. of 17β-acetylamino-5-androsten-3β-ol (IX) (14) in a mixture of 100 ml. of dry benzene and 40 ml. of acetone, was added a solution of 4.1 Gm. of aluminum isopropoxide in 20 ml. of benzene. The mixture was refluxed 44 hours. After cooling, 10 ml. of water was added and the mixture was shaken vigorously. Then 40 ml. of dilute sulfuric acid was added and the mixture was again shaken. The benzene layer was separated, washed with water, and dried over sodium sulfate. The residue obtained by evaporating the solvent was crystallized from acetone to yield 345 mg. of 17β-acetylamino-4-androsten-3-one, m.p. 272–277° [reported 270° (12) and 284–286° (13)]. This product was shown to be identical to that prepared by method A by a comparison of infrared spectra and a mixed melting point.

17β-Acetylamino-3-aza-A-homo-4a-androsten-4-one (VIII).—The Schmidt reaction was carried out by the procedure described for cholestenone using 1.6 Gm. of progesterone, 60 Gm. of polyphosphoric acid, and 800 mg. of sodium azide. The residue obtained by the usual workup was triturated with cold acetone and filtered to yield 500 mg. (29%) of a white powder, m.p. 270–280°. A mixed m.p. with VII showed a marked depression, m.p. 230–250°. An analytical sample was prepared by crystallization from methanol-acetone; m.p. 289–291°; $[\alpha]_D^{27}$ − 2.0; $\lambda_{max.}^{EtOH}$ 220 mμ, log ε 4.14; $\lambda_{max.}^{CHCl_3}$ 3.07 μ, 3.20 μ, 5.98 μ, 6.05 μ, 6.30 μ.

Anal.—Calcd. for $C_{21}H_{32}N_2O_2$: N, 8.13. Found: N, 7.43.

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Studies on Sciatic Stimulated Rat Brain Sulfhydryl Levels

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Rat brain nonprotein sulfhydryl levels were increased by electrical stimulation of the sciatic nerve. Pretreatment with morphine sulfate, meperidine hydrochloride, chlorpromazine hydrochloride, or meprobamate blocked the rise in cerebral levels of nonprotein sulfhydryl. The compounds had no effect on nonstimulated cerebral nonprotein sulfhydryl.

UNGAR AND ROMANO (1) demonstrated that electrical stimulation of the sciatic nerve of unanesthetized rats induces increased brain levels of free and bound sulfhydryl groups. Earlier work in our laboratories (2) showed that stimulation of the sciatic nerve of pentobarbitalized rats increased brain levels of nonprotein sulfhydryl (NPSH). Only the NPSH levels of the cerebrum were increased by sciatic stimulation.

Glutathione, a sulfhydryl-containing tripeptide, has been implicated as playing some role in conditions of mental aberration. The levels of NPSH detected by the amperometric titration

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procedure employed in this investigation consists mainly of reduced glutathione (3). Lowered blood glutathione levels in schizophrenic patients have been reported (4–6) and another report indicated that glutathione was useful in the treatment of schizophrenic states (7). Other workers have been unable to confirm these results. This investigation was undertaken to study further the effects of sciatic nerve stimulation on brain NPSH and to study the effects of chlorpromazine,[1] and meprobamate,[2] morphine, and meperidine on increased cerebral NPSH levels produced by sciatic nerve stimulation.

EXPERIMENTAL

Holtzman albino rats of both sexes weighing 150–250 Gm. were curarized, rather than pentobarbitalized, with 0.02 mg. of dimethyl tubocurarine iodide per rat and artificially respired during the course of the stimulation. The sciatic nerve was stimulated for 20 minutes with a Grass model S4E stimulator with square wave pulses of 40 volts intensity, 3 milliseconds duration at a frequency of 100 cycles per second. After the nerve was stimulated the rat was sacrificed by decapitation. The brain was removed, washed immediately with cold 2% sulfosalicylic acid, the cerebrum removed and frozen with dry ice. The tissue was weighed and homogenized in cold 2% sulfosalicylic acid, centrifuged, and filtered through shark skin filter paper. The filtrate was diluted to a volume in which 2 ml. represented the extract of 100 mg. of tissue. Two-milliliter aliquots were assayed for NPSH by a modification of the amperometric titration procedure of Benesch and Benesch (8). Duplicate titrations of each sample were made, the results averaged and expressed as micromoles per cent of NPSH.

Control rats were curarized and artificially respired for 20 minutes with the electrode placed on the sciatic nerve, but without stimulation.

With the exception of the meprobamate suspension, all drug solutions were prepared daily. Meprobamate was prepared as an 8% suspension with 4% acacia and 0.1% sodium benzoate. All compounds were administered intraperitoneally. Chlorpromazine hydrochloride was administered 4 hours and meprobamate 2 hours prior to stimulation in doses of 10 mg./Kg. and 275 mg./Kg., respectively. Morphine sulfate and meperidine hydrochloride were administered in doses of 15 mg./Kg. and 30 mg./Kg., respectively. Sciatic stimulation was begun 15 minutes later.

The instability of tissue NPSH, even when frozen, necessitated rapid analysis of tissue samples. All frozen samples were assayed for NPSH within 8 hours after removal. Since the experimental procedures were time consuming, a design was employed in which each experimental group was completed as a separate study. An equal number of control animals was used for each experimental group. Nine rats were used for each treatment in the morphine experiment, while 6 rats were used for each treatment in the three remaining experimental groups.

TABLE I.—EFFECTS OF DRUGS ON SCIATIC STIMULATED CEREBRAL NPSH LEVELS

Treatment	Cerebral NPSH μm. % ± S.E.
Control	158.0 ± 1.5
Sciatic stimulation	205.0 ± 5.5
Stimulation plus chlorpromazine 10 mg./Kg. (4 hr. pretreatment)	161.0 ± 4.0
Chlorpromazine control 10 mg./Kg. (4 hr. pretreatment)	160.8 ± 2.3
Control	156.6 ± 1.0
Sciatic stimulation	182.0 ± 4.0
Stimulation plus meprobamate 275 mg./Kg. (2 hr. pretreatment)	152.5 ± 1.5
Meprobamate control 275 mg./Kg. (2 hr. pretreatment)	157.3 ± 2.0
Control	131.0 ± 5.0
Sciatic stimulation	171.5 ± 7.0
Stimulation plus morphine 15 mg./Kg. (15 min. pretreatment)	132.0 ± 7.0
Morphine control 15 mg./Kg. (15 min. pretreatment)	135.0 ± 9.0
Control	162.5 ± 5.5
Sciatic stimulation	210.5 ± 4.0
Stimulation plus meperidine 30 mg./Kg. (15 min. pretreatment)	168.0 ± 5.0
Meperidine control 30 mg./Kg. (15 min. pretreatment)	167.5 ± 2.5

RESULTS

The results of this investigation are summarized in Table I.

Cerebral NPSH levels of sciatic stimulated rats were significantly greater than those of control rats in each of the four experimental groups. The overall mean increase in cerebral NPSH after sciatic stimulation was 22%. When the applied voltage was reduced from 40 to 4 or less volts, cerebral levels of NPSH were still increased. In an experiment in which 4 rats were subjected to sciatic stimulation with 5 volts, an increase of 12.7% over control rats was noted.

In preliminary experiments, pretreatment with 10 mg./Kg. of chlorpromazine 10 minutes prior to sciatic stimulation was not effective in preventing the rise of cerebral NPSH. These rats were deeply sedated and had lost their righting reflexes. A longer pretreatment period of 4 hours was used before sciatic stimulation. The rats were beginning to regain their righting reflexes before stimulation was begun. As illustrated in Table I, this latter treatment with chlorpromazine completely blocked the rise in cerebral NPSH after sciatic stimulation. Chlorpromazine alone had no effect on cerebral NPSH levels in nonstimulated rats.

Bradley, *et al.* (9) had reported that pretreatment with 400 mg./Kg. of meprobamate intraperitoneally 20 minutes before stimulation was ineffective in preventing the rise of cerebral NPSH. In this study, a 275 mg./Kg. dose of meprobamate given 2 hours prior to stimulation blocked the NPSH rise, as did chlorpromazine. Meprobamate itself had no effect on the NPSH levels of the nonstimulated brain.

[1] Generously supplied by Smith Kline and French Laboratories, Philadelphia, Pa.

[2] Generously supplied by Wallace Laboratories, New Brunswick, N. J.

Morphine and meperidine-treated rats exhibited analgesia at the time stimulation was begun, and retained their righting reflexes. The results obtained were almost identical as those obtained with the meprobamate and chlorpromazine-treated rats. Both drugs prevented the NPSH rise in stimulated rats and neither affected NPSH levels of nonstimulated rats.

DISCUSSION

Ungar and Romano (1) found an increase of 55% in brain protein-bound sulfhydryl and a nonsignificant rise of 29% in dialyzable sulfhydryl in sciatic stimulated rats. In our studies, the 22% increase in NPSH observed was highly significant ($t_{10} = 6.3$; $P < 0.01$). The coefficients of variation in our studies were only about 4%, whereas in their data they were 12 and 16%. The fact that the dialyzable sulfhydryl was estimated by difference may account for the greater variability. It is possible that dialyzable sulfhydryl is not equivalent to the NPSH remaining after protein precipitation with sulfosalicylic acid. Another factor to be considered is that only cerebral NPSH levels were measured in our studies since NPSH of the cerebellum, medulla, and midbrain did not rise after sciatic stimulation.

Ungar and Romano postulated that the increased levels of protein-bound sulfhydryl after stimulation resulted from structural rearrangement of proteins participating in the mechanism of excitation. If NPSH changes parallel protein-bound sulfhydryl changes, then one possible explanation of the blockade of the NPSH rise by morphine, meperidine, chlorpromazine, and meprobamate is that these drugs may have prevented the excitation of the cerebrum, perhaps by a blockade of the neuronal paths from the sciatic nerve. One would have to assume, then, that pentobarbital, which did not block the NPSH rise, is relatively ineffective in preventing excitation of the cerebrum. Whether blockade of neuronal paths occurs can be confirmed by the use of electrophysiological approaches.

The biological roles of glutathione have not been established with certainty. Krimsky and Racker (10) have found glutathione to be a firmly bound prosthetic group of the glycolytic enzyme phosphoglyceraldehyde dehydrogenase. Glutathione reductase has been identified (11) and found to catalyze the oxidation of reduced triphosphopyridine nucleotide in the presence of oxidized glutathione with the resulting formation of reduced glutathione.

Szent-Gyorgyi (12) has suggested that glutathione and ascorbic acid, with their respective enzymes, might form a respiratory chain. The reduced and oxidized forms of these substances form reversible redox systems, and are found in highest concentrations in tissues with the highest metabolic activity. Mapson and Moustafa (13) constructed a model respiratory system in which oxygen consumption fell when levels of reduced glutathione decreased and oxidized glutathione increased. They proposed that oxidized glutathione is reduced by reduced triphosphopyridine nucleotide in the presence of glutathione reductase. The reduced glutathione is then oxidized by dehydroascorbic acid in the presence of dehydroascorbic acid reductase. This involvement of glutathione in carbohydrate metabolism suggests that the rise in cerebral glutathione may possibly be a consequence of accelerated cerebral metabolism which occurs when the sciatic nerve is stimulated.

The psychotropic agents employed in this investigation had no effect on nonstimulated cerebral NPSH levels and, as has been previously shown in this laboratory (9), behavioral changes induced by psychomimetic agents are not reflected by changes in cerebral NPSH. The tranquilizers as well as the analgetics employed in this study blocked the NPSH rise produced by sciatic stimulation. It is interesting to note that the analgetics used block the conditioned avoidance response. It is apparent that induced rises in cerebral NPSH levels are blocked by only certain types of central nervous system depressants since pentobarbitalized animals respond to the sciatic stimulation. Also, loss of consciousness is not essential to the blocking effect. Further work is contemplated to elucidate the mechanism of this blocking action.

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