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## ANTICONVULSANT EFFECTS OF STEROIDS

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IT IS known that some steroids are able to influence cellular permeability as demonstrated by the observation of Tipton<sup>1</sup> that cortin depresses the release of potassium from stimulated muscles. Since cellular permeability in the central nervous system is an important factor in determining convulsive reactivity (Spiegel and Spiegel-Adolf<sup>2</sup>), it seemed of interest to study whether some sterols may have anticonvulsant properties. Such an expectation was supported by Selye's<sup>3</sup> observations of hypnotic effects. Preliminary experiments of Spiegel<sup>4</sup> demonstrated that desoxycorticosterone, testosterone, and progesterone are able to raise the threshold for electrically induced convulsions, and experiences of McQuarrie, Anderson, and Ziegler<sup>5</sup> with desoxycorticosterone on two patients with epilepsy seemed to point to a similar direction. In Spiegel's<sup>4</sup> experiments a margin between anticonvulsant and hypnotic dose could hardly be established. It seemed, therefore, desirable to extend these studies to a larger group of steroids, in the hope of finding compounds with a definite margin between these doses.

Similarly, as in Spiegel's<sup>4</sup> previous experiments, the effect upon the threshold for production of convulsions by electric stimulation was determined. This method permits one to ascertain the effect of anticonvulsant agents more unequivocally than the study of antagonistic effects against convulsant drugs such as metrazol (Selye<sup>6</sup>) or cocaine (Aird<sup>7</sup>), since such an antagonism may be due, at least partly, to other factors such as decrease of absorption or of permeability of the cerebral capillaries. The existence of such mechanisms is indicated by the fact that Aird found a protective influence of desoxycorticosterone acetate against cocaine convulsions in doses that are unable to alter the threshold of the brain for production of convulsions by electric stimulation.

We were able to study twenty-nine steroids.\*

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## METHOD

Definite anticonvulsant effects were observed by Spiegel<sup>4</sup> on white female rats. These animals were also employed in Selye's<sup>3</sup> studies of hypnotic effects. In this study, therefore, we used almost exclusively white female rats of from 100 to 150 grams body weight (over 200 animals). Since male rats were resistant even to quantities far beyond the doses effective in female rats and our supply of steroids was limited, we tested these compounds in male rats only occasionally.

The convulsion threshold was determined by using the previously described method of electrical stimulation with the skull intact (Spiegel<sup>8</sup>). The alternating current from the 110-volt, 60-cycle line is reduced by a Variac transformer to the desired voltage (usually 10 volts). After leaving the transformer the alternating current is closed and opened by a relay, the magnet of which is activated by the discharge of condensers (from 1 to 20  $\mu$  F). Since the duration of the activation of the magnet is proportional to the capacity of the condenser, the resistance of the relay circuit being constant, the duration of the stimulation can be varied. The electrodes are placed in the conjunctival sacs. At a certain voltage the duration of the stimulation is increased, step by step, until typical tonic-clonic epileptiform convulsions are elicited. The threshold values are calculated in milliampere seconds since the voltage and the duration of the stimulation are known, and the resistance can be determined on a Wheatstone bridge, the electrodes remaining on the same place as during the stimulation.

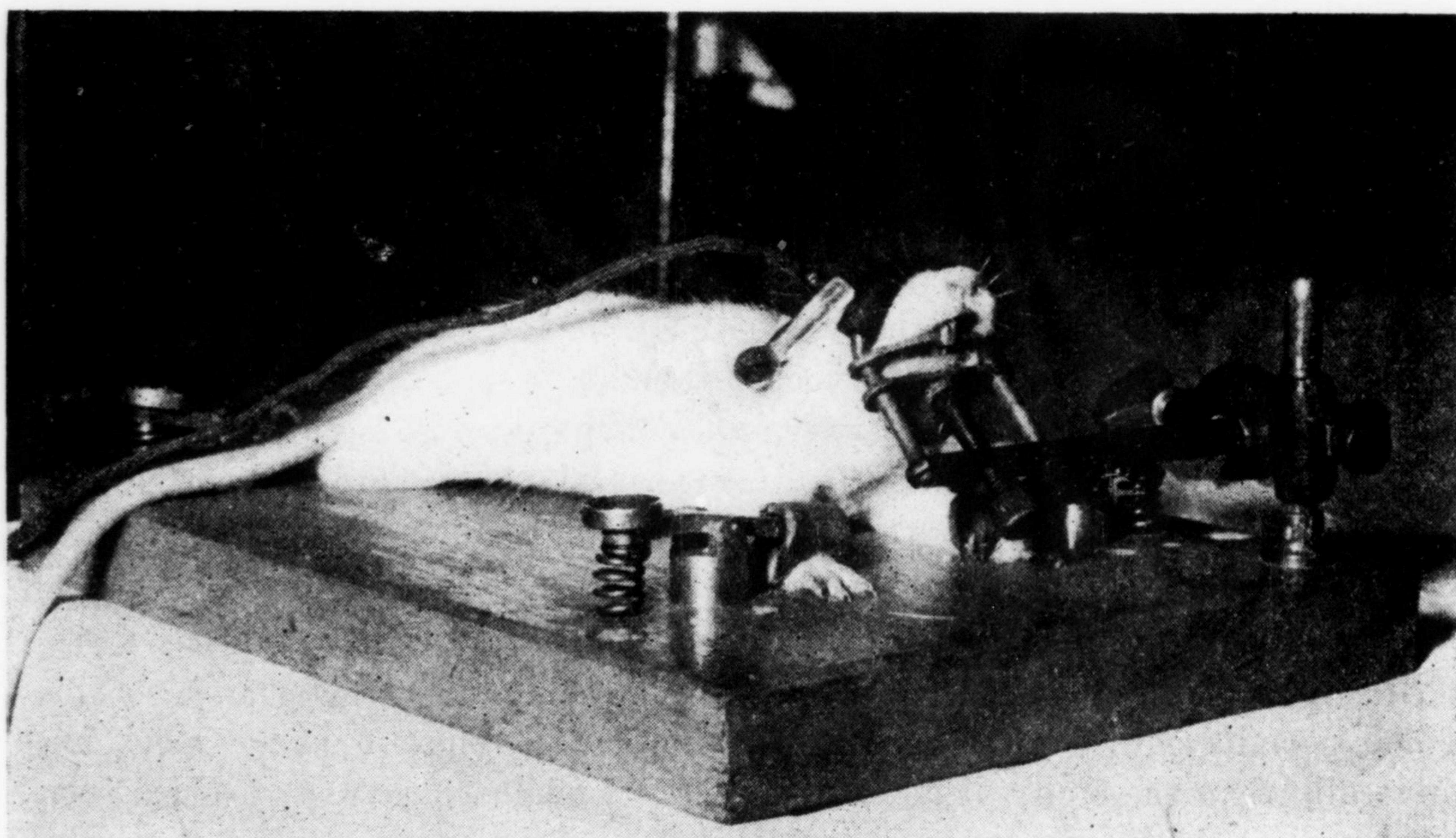


Fig. 1.—Rat board and head holder. Note electrode in right conjunctival sac.

The fixation of the head, application of the stimulating electrodes to the eyeballs, and observation of the convulsions were facilitated by the use of an especially designed head holder (Fig. 1) that fastens the snout, leaving the rest of the head and the eyeballs free. In rats it was of course necessary to apply much smaller electrodes than in rabbits or cats. The electrodes used were slightly concave silver disks with a diameter of 5 mm., which were fitted over the eyeballs. They were held in the conjunctival sacs by small clamps that kept the lids tightly together. With these electrodes the resistance in the circuit usually was between 700 and 1200 ohms. As a rule, at 10 volts a flow of the current from 0.1 to 0.7 seconds sufficed in untreated animals for production of convulsions.

In all experiments the threshold was determined in milliampercere seconds, and the type (extent, localization) and duration of the convulsions were recorded. In order to ascertain the variability of the threshold, at least three tests were made preceding the injection at intervals of from two to three days, and only such animals were used for the injection in which the threshold varied by no more than 30 per cent. The substance under study was applied, as a rule in oily solution, by intraperitoneal injection; occasionally, intramuscular injection of solutions in propylene glycol was used. The convulsion threshold was then determined from one-half to one hour following the injection. The ratio A/B of the convulsion threshold after injection (A) to the average before injection (B) served as a measure of the anticonvulsant effect.

## RESULTS

A substance was considered ineffective, if the ratio A/B was from 1.0 to 1.3 and only slightly effective if this ratio was smaller than 1.5; it was considered anticonvulsant, if this ratio was above 1.5. Applying these criteria, the substances were divided into two groups. In Table I are summarized those which were ineffective or only slightly effective even in relatively large doses.

TABLE I. COMPOUNDS WITH NO, OR ONLY SLIGHT, ANTICONVULSANT EFFECT\*

COMPOUND	DOSE IN MG. PER 100 GM. BODY WEIGHT	CONVULSION THRESHOLD AFTER (A): BEFORE (B) INJECTION
Cholesterol (30 mg. per cubic centimeter olive oil injected in rabbits)	18 to 21 (3 to 5 daily injections)	0.5 to 1.3
Allocholesterol (St)	20 and 50	1
Cholesteryl bromide (St)	20 and 50	1
Epicholestanol (St)	50	1
Stigmasterol (St)	20 and 50	1
Stigmasteryl acetate (St)	20 and 50	1
$\alpha$ -Spinasteryl acetate (St)	20 and 50	1
Ergosterol (St)	20 and 40	1
Ergosteryl acetate (St)	20 and 50	1
$\alpha$ -Ergostenyl acetate (St)	50	1
Dehydrocholic acid (St)	50	1
Desoxycholic acid (St)	20	1
	50	1
$\Delta^5$ -3-Acetoxycholenic acid (St)	50	0.7 to 1.2
Sarsasapogenin acetate (K)	20 and 50	1
Pseudo-sarsasapogenin acetate (K)	20	1
	50	1.2
Diosgenin acetate (K)	20 and 50	1
Pseudo-diosgenin acetate (K)	20 and 50	1
$\alpha$ -Estradiol benzoate (Progynon B; HS)	{10,000 rat units	1.3
1.66 mg. per cubic centimeter sesame oil (10,000 rat units)	{20,000 rat units	1.1
5 mg. per cubic centimeter (30,000 rat units)	4.8 mg.	1
Theelin in oil (K) 1 c.c. = 10,000 I.U.	10,000 and 20,000 I.U.	1.3
6 ( $\alpha$ ) Acetoxy-progesterone (E) 10 mg. per cubic centimeter; amorphous modification	5 and 10	1
	20	1 to 1.1
Etio-cholan-3 $\beta$ -ol-17-one acetate (K)	20 and 40	1
	50	1.0 to 1.6
5-Pregnen-3 $\beta$ -ol-20-one acetate (K)	20	1
	30	1.3
	50	1.4
5, 16-Pregnadien-3 $\beta$ -ol-20-one acetate (K)	15	1
	20	1.0 to 1.3
	30	1
Stilbesteron 13 mg. per cubic centimeter olive oil	10	0.9 to 1.1
	20	0.7 to 1.0
20 mg. per cubic centimeter olive oil	30	1

\*If not otherwise indicated, all substances were dissolved in olive oil, 20 mg. per cubic centimeter and injected intraperitoneally in white female rats. The source of the material is marked as follows: St, Dr. H. E. Stavely; K, Dr. O. Kamm; HS, Dr. E. Henderson and Dr. E. Schwenk; E, Dr. M. Ehrenstein.

In Table II are shown the steroids with a definite anticonvulsant effect manifested by an increase of A/B above 1.5 and/or by a decrease of the duration of the convulsions, the reaction being reduced in some instances (for example, after injections of progesterone) to single twitches. In Table I cholesterol also is included. We have already found in previous studies<sup>9</sup> that injection or feeding of this compound fails to raise the convulsion threshold. Likewise in Table I are included compounds similar to cholesterol such as allocholesterol, cholestryl bromide, epicholestanol, phytosterols (stigmasterol, stigmasteryl acetate,  $\alpha$ -spinasteryl-acetate) and mycosterols (ergosterol, ergosteryl acetate,  $\alpha$ -ergostenyl acetate), further bile acids, and derivatives (dehydrocholic acid, desoxycholic acid,  $\Delta^5$ -3-acetoxyl-cholenic acid), and digitalis saponins (sarsasapogenin acetate, pseudo-sarsasapogenin acetate, diosgenin acetate, pseudo-diosgenin acetate). We included in Table I some steroids with hormonal properties and

TABLE II. COMPOUNDS WITH DEFINITE ANTICONVULSANT EFFECT

COMPOUND	DOSE IN MG. PER 100 GM. BODY WEIGHT	CONVULSION THRESHOLD AFTER INJECTION (A): THRESHOLD BEFORE INJECTION (B)
Testosterone (25 mg. per cubic centimeter sesame oil, HS)	17	1 to 1.2
	18	1.5
Thirteen animals tested	19	1 to 2
	21	1.7 only very mild convulsions
	25	2
	26	1.7 in large animal (169 Gm.)
Androstenedione (20 mg. per cubic centimeter olive oil, HS, St)	5	1.2 to 1.5
	7	1.5 to >2.0
Twenty-seven animals tested	8	>2
	10	1.0 to 3.0
	11	>3
	12	3 to >4
	15	2 to >4
	20	3
Dehydroandrosterone (20 mg. per cubic centi- meter olive oil, HS, St)	8	1.1 to 2.5
	10	1.8 to 2.5
Thirteen animals tested	15	2.4
	20	1.6 to 3.0
	30	1.6 to 4.0
	40	2.4
Progesterone (12 mg. per cubic centimeter sesame oil, HS)	5	1.0
Ten animals tested	6.5	Ratio could not be determined exactly because only rudimentary twichings were elicited
	8	Same
Acetoxy pregnenolone (4 and 5 mg. per cubic centimeter sesame oil,* (HS))	2.0 and 2.5	1.0
	3	1.0 to 1.7
Thirty-five animals tested	4	2.1 to 3.7
	8	2.0 to 3.0
Desoxycorticosterone acetate (5 mg. per cubic centimeter cod-liver oil, HS)	20	1
	23	1.2
Eight animals tested	25	1 to 2.0
	27 and 28	>1.5; marked reduction of duration of convulsions
(15 mg. per cubic centimeter sesame oil, HS)	1 to 2	1.0 to 1.2
Eleven animals tested	2.5	1.7
	3.2	1.2 marked reduction of duration
	4.3	Unexcitable
	5	2.0 (duration reduced)
(15 mg. per cubic centimeter in male rats)		
Five animals tested		
(Weight, from 200 to 300 Gm.)	8 to 40	0.6 to 1
(Weight, from 150 to 170 Gm.)	35 to 48	No definite convulsions (current produced only brief single twiches)

\*Four milligrams per cubic centimeter prepared by us and 5 mg. per cubic centimeter prepared by Schering Corporation.

parent substances or derivatives that had no, or only a small, anticonvulsant effect in the doses at our disposal. This group comprises estrogenic hormones ( $\alpha$ -estradiol benzoate, theelin), the amorphous modification of 6( $\alpha$ ) acetoxyprogesterone, etiocholan-3 $\beta$ -ol-17-one acetate, 5-pregn-3 $\beta$ -ol-20-one acetate, and 5, 16-pregnadien-3 $\beta$ -ol-20-one acetate. Finally, in Table I is contained stilbestrol that chemically does not belong in this group, but has folliculoid effects.

The steroids that manifested definite anticonvulsant effects as summarized in Table II belong to the groups of sex hormones and adrenal substances. It should be emphasized that as a rule anticonvulsant effects could be obtained with much smaller amounts, if high concentration rather than low concentrations of a compound were injected; for example, anticonvulsant effects were obtained with desoxycorticosterone acetate in a solution of low concentration (5 mg. per cubic centimeter) only if doses above 23 mg. per 100 Gm. body weight were injected, while from 2.5 to 3.2 mg. per 100 Gm. sufficed if a more concentrated solution (15 mg. per cubic centimeter) was used, apparently because an efficient blood concentration was more easily reached in the latter case.\* A further factor is the size (age) of the animal. In general, in rats weighing 100 Gm. or less, smaller relative doses (per 100 Gm. body weight) were sufficient to obtain an anticonvulsant effect than in rats weighing 150 Gm. or above. The sexual factor has already been mentioned. It is illustrated by the experiments with desoxycorticosterone acetate. In doses from 8 to 40 mg. per 100 Gm., this compound in concentrated solution produced in male rats weighing from 200 to 300 Gm. no change or even a slight increase of the convulsive reactivity; in smaller animals weighing from 150 to 170 Gm., a reduction of the reactivity was obtained with doses of from 35 to 48 mg.; this is about ten times the dose that was sufficient in female rats for such an effect.

In estimating the efficiency of an anticonvulsant substance, it seems desirable not only to determine its effect upon the convulsion threshold, but also upon the margin between anticonvulsant effect on the one hand and hypnotic effect on the other. In Table III these margins are shown for the steroids that we found to be distinctly anticonvulsant. In determining the minimum hypnotic dose, signs of depression of the activity of the central nervous system were looked for, such as reduction of spontaneous movements, ataxia, impairment of the righting reflexes, and reduction of the reactions to painful stimuli.

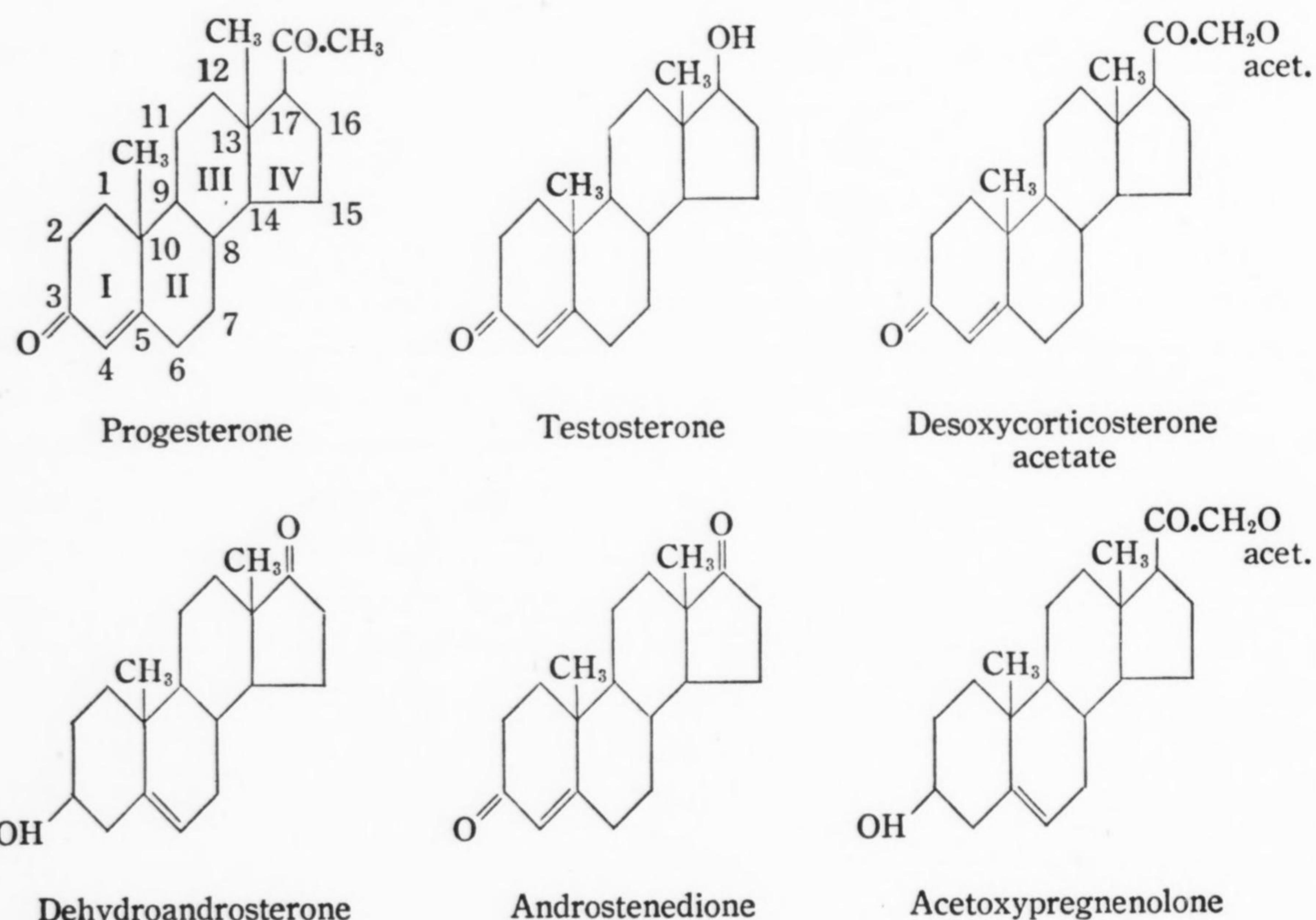
TABLE III. MARGIN BETWEEN MINIMUM ANTICONVULSANT AND HYPNOTIC EFFECT

COMPOUND	MINIMUM ANTICONVULSANT DOSE (MG.)	MINIMUM HYPNOTIC DOSE (MG.)
Testosterone	18 to 19	18 to 19
Androstanedione	7 to 10	10 to 12
Dehydroandrosterone	8 to 10	12 to 15
Progesterone	6.5	6.5
Acetoxypregnenolone	3 to 4	4 to 5
Desoxycorticosterone acetate		
5 mg. per cubic centimeter	25 to 26	25 to 26
15 mg. per cubic centimeter	2.5 to 3	2.5 to 3

\*It is, of course, of paramount importance to overcome the poor solubility of many of these substances. Steroids that were definitely effective in clear solution proved ineffective if only coarser dispersions were injected. Shaking the suspensions for several hours, or even for days, in a shaking machine helped only slightly. Gently heating on a water bath resulted in much better solutions but seemed in some instances, for example, acetoxypregnenolone, to impair the effectiveness of the substance under study. In such a case the steroid was dissolved in small amounts of chloroform; this solution was mixed with the oil and the chloroform evaporated by keeping the solution for several days in a vacuum at room temperature. In control experiments, such chloroform-oil mixtures, without the steroid under study, were kept under the same conditions in the vacuum; intraperitoneal injections of corresponding quantities of these control solutions were ineffective.

It can be seen from Table III that the anticonvulsant dose lies rather close to, or is identical with, the hypnotic dose for testosterone, progesterone, and desoxycorticosterone acetate, while a definite margin between these doses exists for androstenedione, dehydroandrosterone, and acetoxy pregnenolone.

Regarding the chemical structure of those compounds that revealed definite anticonvulsant effects, we deal with tetracyclic derivatives of cyclopentenophenanthere (see Sobotka<sup>10</sup>), having only one double bond in the first ring except dehydroandrosterone and acetoxy pregnenolone, which are unsaturated in the 5, 6 position (see below). At positions 10 and 13 all have angular methyl groups; at C<sub>3</sub> all are oxygenated, except acetoxy pregnenolone and dehydroandrosterone, which are here hydroxylated. The only other side chain is at position 17; here are found most of the differences, testosterone having an OH group, androstenedione and dehydroandrosterone, an O; progesterone, a CO·CH<sub>3</sub>;



acetoxy pregnenolone and desoxycorticosterone acetate, CO·CH<sub>2</sub>O acetate. A comparison between testosterone and androstenedione seems to indicate that ketogenic oxygen at C<sub>17</sub> is favorable to an anticonvulsant effect. However, we do not wish to draw definite conclusions in this respect before testing further compounds with similar structure.

#### SUMMARY

Among twenty-nine steroids tested, desoxycorticosterone acetate, progesterone, testosterone, acetoxy pregnenolone, androstenedione, and dehydroandrosterone were able to increase the threshold of electrically induced convulsions. A definite margin between anticonvulsant and hypnotic dose was found for the latter three substances only.

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### CERTAIN ASPECTS OF THE BRONCHIAL REFLEXES OBTAINED BY STIMULATION OF THE NASOPHARYNX

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IT HAS been known for many years that stimulation of the nasopharynx produces reflex bronchoconstriction.<sup>1-5</sup> The pathway of this reflex has been believed to be afferent via the sensory fibers of the trigeminal nerve and efferent down the vagus. It has been demonstrated, however, that atropine is incapable of entirely blocking the reflex cardiac slowing from nasal stimulation,<sup>6</sup> and a later report indicates that atropine is also incapable of blocking the coronary constriction induced by nasal stimulation.<sup>7</sup> Both of these effects have, therefore, been assumed to be due in great part to a "sympathetic inhibition" of tone. In view of these findings we decided to investigate further the nature of the nasobronchial reflex.

All the experiments were done on decerebrated dogs. It is necessary to obviate the influence of higher centers which inhibit reflexes. The more commonly used anesthetic agents in adequate dosage are prone to depress or abolish reflexes. The animals were rendered decerebrate by exposing the cortex, dissecting down to the region of the red nucleus and severing the brain stem in this region. The dogs thus rendered decerebrate displayed typical spasticity, tremors, clasp-knife rigidity, etc. The method of recording bronchial constriction was by means of a very small inflated rubber balloon inserted into one of the small bronchi and attached to a tambour and writing lever. During the production of these reflexes an open pneumothorax was established on the same side as the balloon. This was done to so equalize intrabronchial and intrathoracic pressure that any change in the force of respiration would not affect the pressure on the balloon in the bronchus. Several experiments were done, however, without the open pneumothorax, which showed essentially the same results as the others. To validate this method we injected several well-known bronchoconstricting agents (acetylcholine, histamine, mecholyl) and stimulated electrically the peripheral end of the cut vagus nerve (Fig. 1). In every instance we noted a