quate for bedside studies. It requires much more care and time for its use than the apparatus described above.

We have also used the Fyrite Oxygen Indicator, which is similar in design and use to the CO_2 Indicator. In general, we have not found it as satisfactory when checked against the micro Scholander. This is of little importance since alveolar O_2 concentrations are of much less value to the clinician than the CO_2 concentration.

Summary. The Fyrite CO_2 Indicator Model CND is a satisfactory instrument for bedside measurement of carbon dioxide content of expired air.

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Correlations Between Active Eyelid Closure and Depletion of Brain Biogenic Amines by Reserpine.* (26445)

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In mice and rats active eyelid closure (blepharospasm) is used to evaluate the degree of sedation induced by reserpine(1). Neither guanethidene (15 mg/kg i.p.), which depletes norepinephrine stored in peripheral nerve endings(2), nor Bretyllium (20 mg/kg i.p.), which prevents its release(3), induces active eyelid closure in rats. This suggests that catecholamines stored at peripheral nerve endings are not directly involved in active eyelid closure induced by reserpine. The purpose of this paper is to elucidate the role played by depletion of brain biogenic amines in the mechanism of reserpine induced blepharospasm.

Our results suggest a positive correlation between active eyelid closure and depletion of serotonin (5HT) in the brain but fail to demonstrate a similar correlation with regard to brain norepinephrine.

Method. Male Wistar rats of 150 to 200 g were used in these experiments. Increase of motor output induced by drugs was evaluated with revolving cages. The animals were placed in the cage immediately after drug injection and kept there for 2 hours. A crossover design was used; the interval between retesting of each animal was one week. Eyelid closure was evaluated according to the method of Rubin *et al.*(1). Brain stem norepinephrine was determined according to Bertler *et al.*(4) using perchloric acid extracts. Brain stem 5 HT was measured by a bioassay technic using the rat uterus(5). Brain stems of 2 to 3 animals were combined and homogenized at 0°C with 19 volumes of 0.3 M ice cold sucrose containing 2.5×10^{-4}M of tranylcypromine. An aliquot (5cc) of the homogenate was removed for total 5 HT analysis, a second aliquot (10cc) was used for perchloric acid extraction and a third aliquot (5cc) of the homogenate was centrifuged in a Spinco Model L ultracentrifuge with rotor No. 40 for 40 min. at 20,000 rpm, equal to 26,360 g. Serotonin analysis was then performed separately in supernatant and precipitate. The monoamineoxidase inhibitor (MAOI) d-l tranylcypromine (SKF 385), Reserpine (Serpasil), and d-amphetamine sulfate were given alone or in combination to groups of at least 6 rats. Doses, route of injection, and time elapsed between treatments are indicated in Results.

Results. The effect of tranylcypromine on brain amines of rats is illustrated in Fig. 1. Two mg/kg i.p. of the drug caused an increase of brain 5 HT which reached its peak

* A preliminary report of these experiments was presented at Fed. Am. Soc. Exp. Biol., Chicago, April, 1960 (*Fed. Proc.,* 1960, v18, 279.).

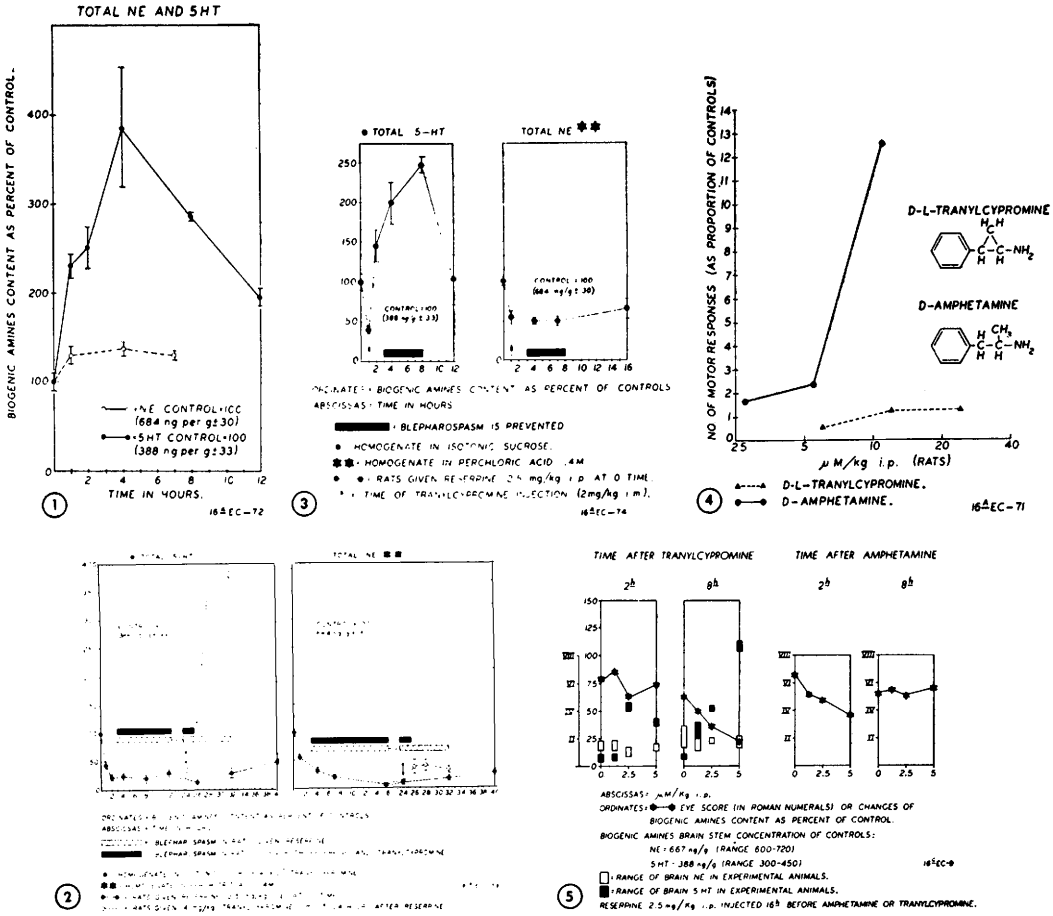

FIG. 1. Effect of d-l-tranylcypromine (2 mg/kg) on rat brain stem serotonin and norepinephrine concentration.

FIG. 2. Effect of reserpine alone and d-l-tranylcypromine 24 hr after reserpine on concentrations of rat brain stem amines.

FIG. 3. Effect of d-l-tranylcypromine given 1 hr after reserpine on rat brain stem amine concentrations.

FIG. 4. Effect of d-l-tranylcypromine and d-amphetamine on motor output of rats.

FIG. 5. Effect of various doses of d-l-tranylcypromine and amphetamine on reserpine-induced blepharospasm in rats.

within 4 to 6 hours and slowly declined toward control values during the next 6 hours. This dose of MAOI caused a small rise of brain norepinephrine. Higher doses of tranylcypromine induced a greater increase of catechol amines. The supernatant and precipitate of brain homogenates from rats given 2 mg/kg i.p. of tranylcypromine were analyzed separately for 5 HT. The supernatant accumulated 5 HT faster than the precipitate. The maximal increase of 5 HT was 3.7 and 2.5 fold respectively; however, the 2 fractions reached equilibrium within 8 hrs.

Reserpine (2.5 mg/kg) decreased the concentration of both 5 HT and norepinephrine in the brain stem of rats (Fig. 2). Depletion was greatest 12-26 hrs after injection, thereafter both amines increased towards control values. In these rats blepharospasm occurred 3 hrs after injection and endured for 29 hrs thereafter. Forty hrs after reserpine 5 HT levels were less than control but were equal to those present 3 hrs after reserpine when eyelid closure had not been established. In contrast, norepinephrine levels at this time equalled those found during blepharospasm.

Tranylcypromine (4 mg/kg) given 24 hrs after reserpine (Fig. 2) abolished the blepharospasm and concomitantly elevated the 5 HT concentration over control values. On the other hand, norepinephrine levels were only slightly elevated. Tranylcypromine (2 mg/kg) given 1 hr after reserpine reversed the active eyelid closure for a period of 5 hrs and the accompanying rise and fall of 5 HT in the brain paralleled the degree of reversal (Fig. 3). However, norepinephrine concentrations remained unchanged during period of reversal.

An attempt was made to determine whether the 5 HT which accumulated after administration of tranylcypromine to rats pretreated with reserpine was preferentially concentrated in either supernatant or precipitate. No such preferential distribution was found. Chemical changes in each fraction paralleled the changes in the whole homogenate.

Tranylcypromine and amphetamine are similar in chemical structure and both antagonized blepharospasm induced by reserpine. They differed considerably however in their action on motor activity in normal rats (Fig. 4) and in their time course of action in reserpinized rats (Fig. 5). Doses of tranylcypromine which produced extensive accumulation of 5 HT in the brain did not increase motor activity in rats when compared with equimolar doses of amphetamine. At all dose levels studied, tranylcypromine was more effective 8 hrs after injection in antagonizing reserpine-induced blepharospasm than at 2 hrs. The reverse was true for amphetamine.

Discussion. Tranylcypromine, an effective inhibitor of MAO(6), increased brain stem 5 HT levels markedly in both normal and reserpinized rats (Fig. 1, 2, 3). Norepinephrine levels were not elevated as much. Amphetamine and tranylcypromine both reversed reserpine-induced active eyelid closure. The antagonism displayed by tranylcypromine was related to its antienzymatic action rather than to an amphetamine-like central nervous system stimulation for the following reasons: (a) In normal animals 5 μm/kg of tranylcypromine did not affect motor output when compared with 5 μm/kg of amphetamine (Fig. 4). (b) This dose of tranylcypromine was still effective on eyelid closure 8 hrs after injection whereas amphetamine was maximally effective 2 hrs after injection and was not effective 6 hrs thereafter (Fig. 5). (c) The antagonism produced by MAOI was paralleled by an increase of brain stem 5 HT. (d) It is unlikely that amphetamine reverses eyelid closure by restoring the deficit of brain norepinephrine since the MAOI reversed reserpine effects without markedly changing norepinephrine concentrations.

These results therefore support Brodie's hypothesis(7) that release of brain 5 HT rather than norepinephrine is important for understanding the mechanism of action of reserpine. Other amines such as dopamine may also be important. These findings do not clarify the mode of action of reserpine on 5 HT binding sites. It may be suggested that adverse effects of reserpine on 5 HT transport mechanisms(8) can be overcome only when appropriate concentrations of free 5 HT are accumulated intracellularly as a result of MAO inhibition. For example, when 5 HT concentrations in the brain stem were brought back to control levels (Fig. 2, 3) reserpine-induced blepharospasm was abolished. It is not clear why tranylcypromine had less effect on brain norepinephrine than on brain 5 HT. This finding could result from either slower turnover of norepinephrine or lack of action of tranylcypromine on other enzyme systems involved in the metabolism of norepinephrine in brain tissue (9).

Summary. Reserpine-induced closure of the eyelids is antagonized by amphetamine and tranylcypromine *via* different mechanisms. Antagonism by tranylcypromine is a function of increased 5 HT content in the brain stem but is independent of changes in norepineprine concentration.

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Effect of Drugs on Amino Acid Levels in Brain: Excitants and Depressants. (26446)

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Previous studies on the action of drugs on amino acid levels in the brain have brought to light a variety of effects. Okumura, *et al.* (1), using ion exchange chromatography to analyze brain amino acids, found that repeated injections of chlorpromazine produced a rise in glutamine, aspartic acid and γ-aminobutyric acid (GABA). Repeated electroshock treatment and repeated injections of β-phenylisopropylmethylamine produced no marked changes in free amino acid levels, although the latter produced an increase in N-acetylaspartic acid. That a reduction occurs in the level of GABA in brains of rats treated with semicarbazide was demonstrated by Killam and Bain(2), but this effect was not observed in animals with seizures induced by pentylenetetrazole. Baxter and Roberts (3) found significant increases in GABA levels in brains of rats treated with hydroxylamine.

The effect of 4 hypoglycemic agents on free amino acid levels in the rat brain has been described(4). The present paper describes results obtained by applying the same analytical procedures(5) to a study of a larger group of agents affecting the central nervous system. These were divided into compounds exerting a predominantly stimulating effect (including the so-called hallucinogens, mescaline and LSD) and compounds exerting a predominantly depressing effect (hypnotics, analgesics and "tranquilizing" agents).

Experimental. A. *Design of experiments.* Wistar rats (250 g males) were fasted 24 hours, then injected with the material to be tested dissolved in normal saline. Amounts injected varied and have been recorded under Results. The amount of time allowed for the drug to act also varied. In every experiment 6 groups of 5 rats were used, the experiment being so designed that 2 groups functioned as controls (injected with normal saline), 2 groups received one compound to be tested, and the remaining 2 groups received the second compound to be tested. Thus each experiment consisted of 30 rats distributed as shown below:

Group	Control	Compound A	Compound B	Total
I	5	5	5	15
II	5	5	5	15
	10	10	10	30

Each of these unit experiments was repeated 2 or more times, the brains being extracted in one experiment with picric acid and in the other with alcohol. Both extraction procedures and chromatographic methods of determining amino acid levels have been described(5).

Estimations of the significance of treatment effects were made by the method of paired replicates as described by Snedecor (6). This involved obtaining the difference in density reading between each of the treated groups and its own control in each experiment. From the sum of such differences derived from all the experiments carried out, a mean difference was obtained, the