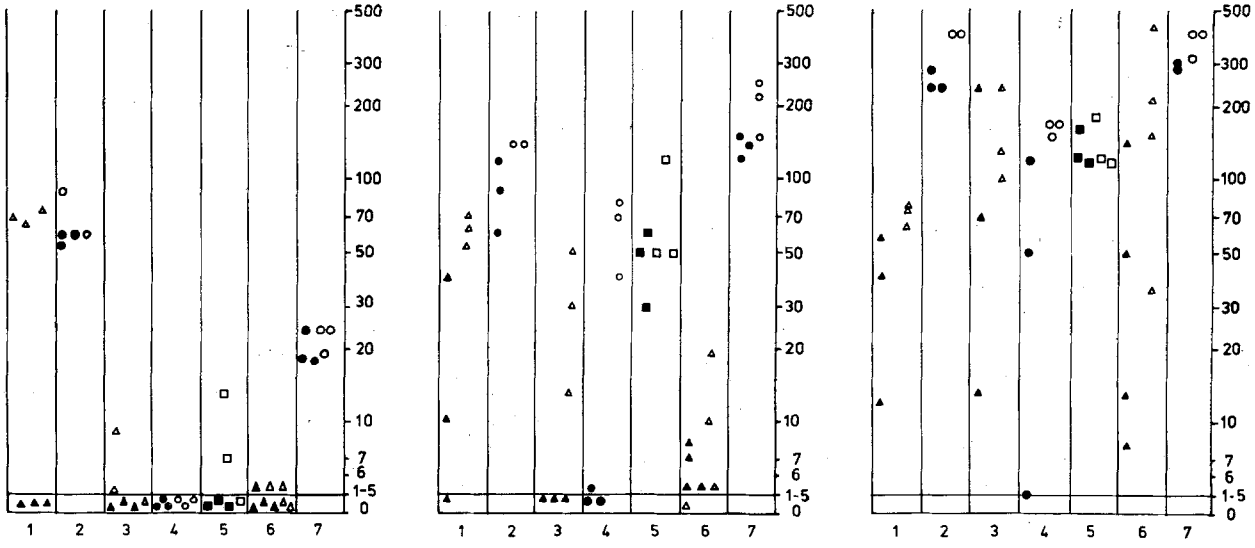


Fig. 1, 2 and 3. FDP in the medium of organ cultures treated and not treated with oxyphenbutazone in various concentrations 24, 48, 72 h respectively after the beginning of the culture. Ordinate (logarithmic): FDP in μg/ml. Abscissa: Number of the culture. Cultures 1, 2, 3, 4, 5 were made of newborn rat kidney; 6 of ovarian cancer. In culture 7 the substrate was a standard fibrin clot of 1 ml. ▲, ●, ■, denote cultures containing oxyphenbutazone in the medium in concentrations of 100, 50, 10 μg/ml, respectively. △, ○, □, denote corresponding controls. The cultures treated with oxyphenbutazone showed a fibrinolytic activity lower than that of the untreated cultures.

culture medium to reach a final concentration ranging from 10 to 100 μg/ml medium. Controls without oxyphenbutazone were run at the same time.

*Results and comment.* Besides a few pilot studies, 7 culture experiments were run based on a altogether 43 separate cultures. The lysis produced by the explants 24, 48, and 78 h after the beginning of the culture expressed in μg of FDP/ml medium is shown in Figures 1, 2 and 3. There is a progressive increase of FDP in the culture medium due to the continuous fibrinolysis.

The fibrinolytic activity of the cultures containing oxyphenbutazone in the medium was generally lower than that of the control cultures. Statistical analysis (Student's *t*-test) of the cultures with 100 μg oxyphenbutazone/ml (Nos. 1 and 3) and with 50 μg/ml (Nos. 2 and 4) showed a significant ($p < 0.02$) difference for the 100 μg/ml cultures after 24 h and 48 h. A lower fibrinolytic activity was also shown by the cultures of ovary cancer (No. 6) and in one where a preformed 1 ml fibrin clot was used (No. 7).

The results showed that oxyphenbutazone in a concentration of 10 μg–100 μg decreases the release of fibrinolytic agents from the cultures of embryonic and tumoral tissues resulting in a delay in the appearance of the dissolution of fibrin[11].

Hypothetically, the effect of oxyphenbutazone on the fibrinolytic activity of the cultures can be attributed to a stabilizing action of the drug on intracellular membranes, which would be followed by a decreased diffusion of fibrinolytic agents from the cells into the culture medium. This means that in this respect the action of oxyphenbutazone would resemble that found by HENRICSON[12] for cortisone.

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## Studies on the Role of Phenethylamine in Methylamphetamine Action Mechanisms

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*Summary.* According to our results, we think that the tolerance developed to central effects of N-methylamphetamine are caused by the liberation and posterior depletion of phenethylamine from its storage places, which is in agreement with our hypothesis about the action mechanisms of amphetamines in the central nervous system.

Phenethylamine has been found in different animal organisms including man[1–4]. It is a homologue of amphetamine and produces similar pharmacological effects[1,5,6]. In contrast to amphetamine, phenethylamine is a substrate of monoaminooxidase[7]; for this reason it is often necessary to administer it together with a monoaminooxidase inhibitor in order to obtain satisfactory results. Phenethylamine has been postulated as a brain neurohumoral agent[6,8,9]. It can be produced by decarboxilation of its aminoacidic precursor phenylalanine and it is decomposed mainly by aminooxidation. Diminished urinary elimination of phenethylamine was found in certain forms of depressive disease, probably of endogenous origin[3,4]. Treatment of such patients with

aminooxidase inhibitors or with dibenzazepinic antidepressive drugs produced a raise of urinary phenethylamine elimination. The same substances elevated also the phenethylamine level of rat brain, whereas reserpin produced a diminution of it[10,11]. On the other hand, behavioral reserpin effects in the rat were abolished by amphetamine or phenethylamine[12]. It is generally accepted that peripherical amphetamine action has an indirect mechanism mediated by catecholamine release. It was also supposed that amphetamine acts in a similar way on cerebral funcions and behavior. Notwithstanding this, there are some facts that contradict such an interpretation of the central effects of amphetamine. It was observed that only peripherical amphetamine effects are prevented by catecholamine depletion produced by reserpin, while the behavioral amphetamine actions are not antagonized[13]. It was proposed by FISCHER and HELLER[8] that central and behavioral amphetamine effects may be mediated by phenethylamine release and/or by an occupation of active phenethylamine receptors by amphetamine.

Such an interpretation is also supported by the great similarity of amphetamine and phenethylamine structure and effects[5]. It is known that prolonged amphetamine administration produces tolerance phenomena. Such tolerance could be explained by a depletion of phenethylamine in brain in consequence of the constant release during the prolonged amphetamine administration. In fact, we could show[14] that after a treatment of rats with N-methyl amphetamine for 3 weeks, the brain phenethylamine concentration gradually diminished. The original value of approximately 0.5 µg/g was lowered to 0.05 µg/g. It seemed to us important to study whether a prolonged phenethylamine administration would also, produce a tolerance, for this should invalidate such an interpretation. We shall report here the results of the corresponding assays, using as test the observation of lethal effects and the suppression of conditioned responses in the rat.

*Methods.* We did not use a combination of phenethylamine with monoaminooxidase inhibitors in order to avoid complications and difficulties of interpretation, trying to compensate the short duration of phenethylamine effects by an elevation of its doses. Instead of amphetamine we used N-methylamphetamine, for practical reasons. Assays of lethal effects were carried out with groups of 20 Wistar rats (10 male and 10 female), of 120 to 160 g in weight. The lethal dose was determined by i.p. injections, observing the animals subsequently during 96 h and considering the dose that produced death in 50% of the animals as $LD_{50}$. Such assays were carried out with untreated animals and also with rats pretreated for 10 days with methylamphetamine (40 mg/kg) and phenethylamine (100 mg/kg), respectively. Blocking of conditioned responses was assayed according to the method of COOK and WEIDLEY[15]. In each assay 20 rats under these conditions were used. The animals were trained to climb on a wooden pole after receiving an

Table I. Assay of lethal Doses of N-methylamphetamine in rats using the i.p. route

| Dose (mg/kg) | Lethal effects (%) | Probits | $LD_{50} \pm$ SE |
|---|---|---|---|
| Animals without pretreatment | | | |
| 105 | 20 | 4.18 | 115 ± 2.22 |
| 110 | 35 | 4.65 | |
| 120 | 65 | 5.38 | |
| 140 | 100 | 7.00 | |
| Animals pretreated with 1 daily dose of 40 mg/kg of N-methylamphetamine for 10 days | | | |
| 130 | 0 | 0 | 156 ± 2.45 |
| 140 | 15 | 3.95 | |
| 150 | 35 | 4.65 | |
| 160 | 65 | 5.38 | |
| 170 | 80 | 5.85 | |
| 180 | 100 | 7.00 | |

Table II. Assay of lethal doses of phenethylamine in rats i.p.)

| Doses (mg/kg) | Lethal effect (%) | Probits | $LD_{50} \pm$ SE |
|---|---|---|---|
| Animals without pretreatment | | | |
| 200 | 0 | 0 | 245 ± 4.56 |
| 220 | 15 | 3.95 | |
| 250 | 60 | 5.25 | |
| 275 | 90 | 6.25 | |
| Animals pretreated with 1 daily dose of 100 mg/kg of phenethylamine during 10 days | | | |
| 200 | 0 | 0 | 240 ± 5.02 |
| 220 | 20 | 4.1 | |
| 250 | 65 | 5.35 | |
| 275 | 85 | 6.1 | |

Table III. Suppression of conditioned responses by N-methylamphetamine in rats (i.p.)

| Dose (mg/kg) | Blocking effects (%) | Probits | $ED_{50} \pm$ SE |
|---|---|---|---|
| Animals without pretreatment | | | |
| 3 | 20 | 4.2 | 5.5 ± 0.55 |
| 6 | 60 | 5.25 | |
| 9 | 90 | 6.25 | |
| Animals pretreated with 1 daily dose of 3 mg/kg of N-methylamphetamine during 10 days | | | |
| 3 | 5 | 3.4 | 8.5 ± 0.55 |
| 6 | 20 | 4.2 | |
| 9 | 55 | 5.15 | |
| 12 | 90 | 6.25 | |


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Table IV. Supression of the conditioned responses by phenethylamine in the rat (i.p.)

| Dose (mg/kg) | Blocking effects (%) | Probits | $ED_{50} \pm SE$ |
|---|---|---|---|
| Animals without pretreatment | | | |
| 25 | 0 | 0 | 38 $\pm$ 1.64 |
| 35 | 30 | 4.48 | |
| 40 | 65 | 5.4 | |
| 50 | 90 | 6.25 | |
| Animals pretreated during 10 days with 1 daily dose of 35 mg/kg of phenethylamine | | | |
| 25 | 0 | 0 | 36.5 $\pm$ 2 |
| 35 | 27 | 4.4 | |
| 40 | 69 | 5 | |
| 50 | 87 | 6.1 | |

electrical shock to the feet and conditioned to do the same also after hearing the sound of a buzzer.

According to unpublished observations, phenethylamine and amphetamine are amongst the substances that block the conditioned responses. We did not differentiate between specific and unspecific blocking since we thought this of no importance from our point of view. We considered simply as blocking when the animals did not react to the buzzer's sound. The effective dose 50 ($ED_{50}$) was calculated by taking into account the percentage of positive answers, according to MILLER and TAINTER[16]. The tests were repeated after 10 days of treatment of the rats with N-methylamphetamine (3 mg/kg) and phenethylamine (35 mg/kg), respectively. Control tests were performed giving 25 and 50 mg/kg of phenethylamine to non-tolerance conditioned rats 24 h before test. The injections were always administered by the i.p.

*Results.* The results obtained in assays of lethal effects are shown on Tables I and II. It can be seen on Table I that the $LD_{50}$ of N-methylamphetamine is raised from 115 to 156 mg/kg after a pretreatment with 40 mg/kg of the same substance for 10 days. The difference is significant ($p < 0.005$). Table II shows that the lethal dose of phenethylamine remains practically unaltered after a pretreatment with 100 mg/kg of this substance daily for 10 days. The results obtained in assays on conditioned responses are shown on Tables III and IV. Table III shows that the $ED_{50}$ of N-methylamphetamine is raised from 5.5 mg/kg to 8.5 mg/kg after a daily pretreatment with 3 mg/kg of this substance for 10 days. The difference is significant ($p < 0.005$). Table IV shows the results on control tests according to which 25 and 50 mg/kg of phenethylamine do not produce tolerance to the conditioned responses in rats.

*Discussion.* Our supposition that amphetamine tolerance is produced by phenethylamine depletion would have been invalidated if phenethylamine itself had produced a tolerance. This did not happen. Taking into consideration these circumstances and the fact that chronic administration of N-methylamphetamine produce a depletion of cerebral phenethylamine[14], we think that our observations are at least consistent with the supposition that amphetamine acts in the tests employed by us mainly by liberating phenethylamine.

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## Lithium Effects Upon Components of Activity in Rats

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*Summary.* When lithium chloride was administered to rats several changes occurred in a number of components of activity, some of which were sex-related. There was a reduction in both ambulatory and rearing activity and in behaviour associated particularly with the mouth and nose. The findings are related to a suggestion that lithium effects on behaviour may be more subtle than hitherto thought.

Almost alone amongst psychoactive agents presently finding widespread usage in clinical medicine, lithium has still not been subjected to thorough psychopharmacological analysis leading to any consistent picture of the behavioural effects of its salts in animal subjects. A number of recent studies have produced widely varying results[1]. Some investigators have indeed reported a lack of effect, except upon animals previously treated with combinations of other psychoactive agents[2].

It may be that lithium modifies behaviour subtly, and that the screening tests used to date either impose too rigid a control upon the actions of experimental animals, or direct the experimenter's attention to too narrow a band of activities, effectively masking what may be important lithium effects[3].

To overcome this problem the present study was designed to observe rats under the influence of lithium chloride in a test situation which allowed the animals relatively free movement and in which a number of behavioural components could be simultaneously observed.

*Methods.* The animals were placed singly into a box having a square floor area 30.4 × 30.4 cm, and 32.6 deep. The interior of the box was painted white and the floor was marked into 4 equally sized squares, 15.2 × 15.2 cm. Fluorescent room lighting, directly above the apparatus, was used throughout the experiment. The experimenter noted the occurrence of particular kinds of behaviour by speaking into the microphone of a tape recorder, for later analysis.

The taped records were used to assess the frequency of occurence of a number of behavioural components, namely: ambulation (moving all 4 feet from one floor square to another); rearing (standing on the hind legs, either against the walls of the box or more centrally in the box); freezing (a sudden cessation of all movement); crouching (lowering

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