III

The Effects of Psychotropic Drugs on Biological Systems of Low Complexity

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Psychopharmacology is a new discipline and its methodology is not yet complete. The need for trying out and developing fresh experimental tools is still acutely felt. A great variety of empirical screening devices has been used and considered as analogues of behavioral phenomena observed in the human. In the main such analogues have been the spontaneous, responsive and learning behavior of mammals at various levels of the developmental scale. However the behavior of pigeons,[1] fish,[2] snails,[3] and spiders[4] has also served for the study of psychoactive drugs.

When employing such analogues of behavior as observed in species very far removed from the human, a number of assumptions are being made. One of these assumptions is the hopeful expectation that similar behavioral manifestations observed in different species are correlated with similar physiological processes. While such an assumption appears to be plausible, there is in most cases no experimental evidence available for it as yet. The special temptation to make such an assumption lies in the possibility it provides for the study of the biological substrate of human behavioral manifestations in biological systems of much greater availability and much smaller complexity than the human central nervous system. Another assumption is implicitly made for most experimental work in this field, namely, that all *behavior* must be studied on neuronal systems although the only requirement of which we can be certain is that *neuronal characteristics* have to be studied on neurons.

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The one common denominator of all models and analogues used in the study of psychopharmacological effects is the fact that they all possess at least parts of a central nervous system. However, in this connection, one might remember that while it is true that there is no psyche without a central nervous system, the converse does not hold—not every central nervous system is capable of manifesting behavior on the psychological level.

This report will concern itself with the effects of psychotropic drugs on seven different systems, five biological and two enzymological in nature. None of the biological systems used for these experiments is endowed with even a rudimentary central nervous system.

In our investigation, we have focused on three characteristics of living systems, namely, metabolism, growth, and reactivity and our experimental results will be presented in this order.

The questions we asked ourselves in studying the effects of psychotropic drugs on biological systems of low complexity were: (a) Is there any consistent corresponding pattern discernible between the reactions of psychotropic drugs on such simple systems and other more complex ones which possess a central nervous system? (b) Of what order is such a corresponding pattern if it appears?

Two orders of meaningfulness might be differentiated here on methodological grounds:

(1) a quantitative or statistical correlation between certain resulting reaction patterns and known psychopharmacological characteristics of the drugs

and

(2) a qualitative correspondence between the behavior of the experimental system and the behavior of animals or humans in reaction to a drug. Such a qualitative correspondence could be referred to as isomorphism of behavior in the two systems. It would be characterized by a close correspondence of the rank orders and time sequences of the phenomena observed in addition to a mere quantitative correlation.

The following six drugs were used in our experiments: (1) secobarbital, as a standard sedative; (2) dextroamphetamine, as a typical stimulant; (3) chlorpromazine and (4) proclorperazine, as representatives of the major tranquilizers; (5) imipramine, representing the group of antidepressants; (6) lysergic acid diethylamide—LSD-25, a typical psychotomimetic.

I. Enzymological Systems

Two simple enzyme catalyzed reactions were studied: the hydrolysis of urea by urease, and the light emission by firefly tail extracts in the presence of adenosine triphosphate (ATP) and oxygen. The two systems were chosen because they represent two distinct types of reactions: single-step hydrolysis by urease and multi-step oxidation by firefly tail extract.[5]

1. *Urease*

Methods: Urease solution was prepared by dispersing 0.75 mg. dry powder (Nutritional Biochemicals Corp.) in 15 ml. H 20, neutralizing with NaOH and extracting the suspension at 0 C. for 10 minutes in an all-glass hand homogenizer. The extract was centrifuged at 3000 rpm for 10 minutes, and the clear supernatant used for the reactions.

The drugs were dissolved in the appropriate amount of water, neutralized, when necessary, with 0.05 HCl or $NaHCO_3$ to as near neutrality as was possible without precipitation. LSD-25 was supplied in intravenous solutions, and thus did not require further manipulation.

Procedure: The procedure used was a modification of Harman and Niemann.[6] Into clean boiling tubes containing 1 ml. 9.1 *M* phosphate buffer at pH 6.7 were placed 0.1 ml. urease and the appropriate amounts of drug solution, and the volume was made up to 3 ml. with H_2O. The tubes were placed in a 30 C. bath and after 15 minutes of equilibration each received 3 mg. urea in 0.1 ml. water. After 30 minutes the reaction was stopped by the addition of 0.5 ml. 2 N HCl. The ammonia formed was estimated by making the reaction mixture alkaline using 3 Gm. K 2 CO_3, and trapping the liberated NH3, in 10 ml. 0.01 *M* HC 1 by aerating for 1 hour and finally back-titrating the HCl with 0.01 *M* NaOH to bromothymol blue end-point.

Results: The activity of urease in presence of the drug is illustrated in FIG. 1, expressed as per cent of activity in the absence of drugs. As can be seen, only LSD-25 shows significant inhibition. The inhibition of urease activity by LSD-25 is very marked even at very low concentrations, amounting to some 50 per cent at 10^{-5} *M*.

None of the other drugs exhibit any inhibitory activity, although

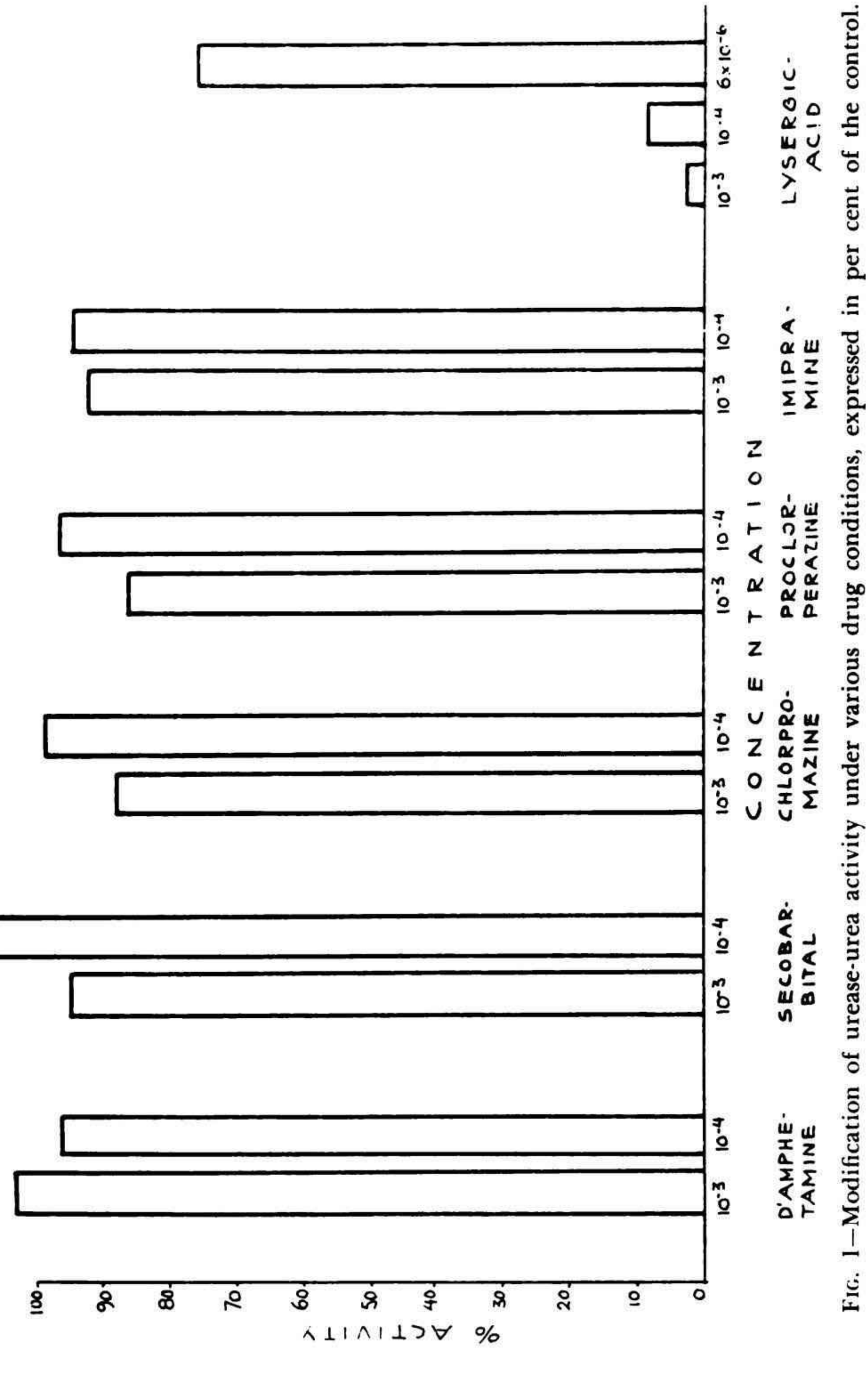

FIG. 1—Modification of urease-urea activity under various drug conditions, expressed in per cent of the control.

certain trends are exhibited by the tranquilizers, chlorpromazine and proclorperazine. Both drugs, at higher concentrations, slightly inhibit the hydrolysis of urea; however, at lower concentration (10^{-4} *M*) the drugs have no effect. Similarly, apparent "stimulations" by dextroamphetamine and secobarbital cannot be considered significant without further experiments.

2. *Firefly Lantern Extract*

Methods: Fireflies were collected near Montreal and while still alive, placed in a deep freeze at —10 C. The frozen animals were freeze-dried and their tails removed and kept in deep freeze until extracted. The method of extraction was similar to that of McElroy (circular supplied by Schwarz BioResearch, Inc.) by suspending 70 mg. tails in 5 ml. 0.04 *M* glycyl glycine buffer at pH 7.7 and homogenizing at 0 C. in a tight-fitting all-glass hand homogenizer. The suspension was centrifuged for 10 minutes at 0 C. (2400 rpm) and the precipitate washed with 2 ml. buffer. The combined supernatants were kept in the deep freeze until use, then thawed and clarified again by centrifugation.

The reaction was carried out in 0.8 ml. capacity cuvettes (1 cm. path) using the following procedure: 0.2 ml. glycyl glycine buffer (0.062 *M*, pH 7.7) containing $MgSO_4$ (0.0125 *M*) was mixed in a test tube with 0.1 ml. drug (to give 10^{-3} or 10^{-4} *M* final concentration) and 0.1 ml. of the enzyme extract. They were mixed by a fine stream of O_2 for 20 seconds, and then quantitatively transferred with a capillary pipette into the cuvette containing 30μg. ATP in 0.1 ml. water. Reading was taken exactly 45 seconds after injecting the liquid into the cuvette, on a Beckman Duspectrophotometer without light source, using a photomultiplier attachment. Both photomultiplier and photometer were adjusted to full sensitivity.

Results: FIG. 2 illustrates the results of these experiments. The light emission values are expressed as per cent of the light emitted by the same system containing no drug. Again, as with urease, only LSD-25 showed significant inhibitory effect, even at concentrations as low as 6×10^{-6} *M*. The two tranquilizers, chlorpromazine and proclorperazine, show a tendency toward inhibition, but this tendency is far from significant. At low concentrations the effect is too small to be significant, at high concentration (10^{-3} *M*) pH changes and precipitation obscure the real effect. Since the enzyme is not very active

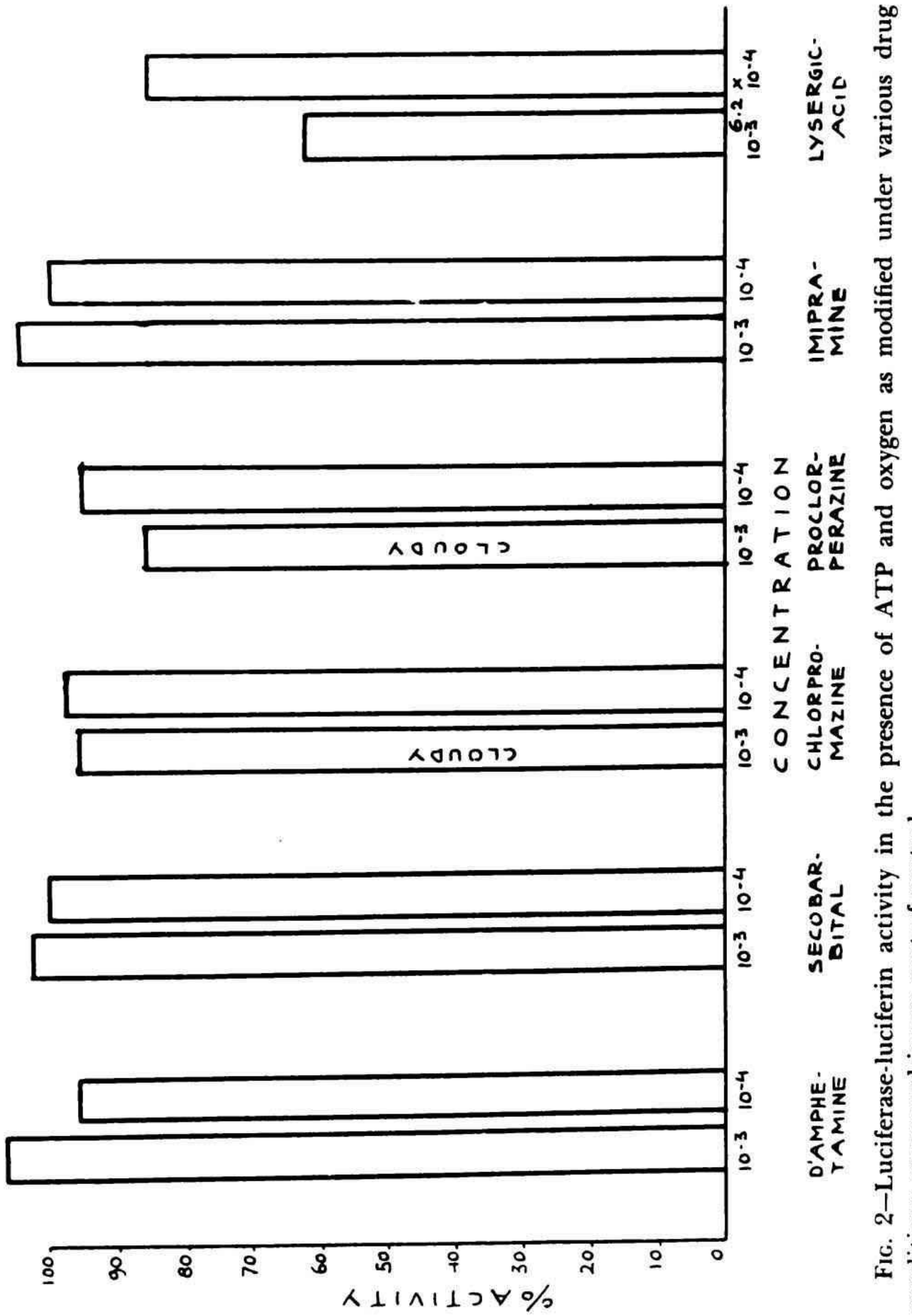

FIG. 2—Luciferase-luciferin activity in the presence of ATP and oxygen as modified under various drug conditions, expressed in per cent of control.

at a pH less than 7.5, the system had to be buffered to this pH. However, chlorpromazine and proclorperazine, at 10^{-3} *M* concentration, start to precipitate in this slightly alkaline solution. As the liquid gets cloudy, part of the light emitted by the system may get absorbed by the cloudiness, thus showing an apparent inhibition of light emission.

Dextroamphetamine and imipramine show a slight tendency to stimulate the reaction at higher concentration; but again, the degree of stimulation cannot be considered significant unless supported by more experiments.

Discussion: The results presented indicate that the two enzyme systems studied are quite refractory to the presence of all drugs, with the exception of LSD-25. It is important to note, however, that the lowest concentration of LSD-25 tried was about 100 times greater than a single human dose when dissolved in 5000 ml. blood. When in one experiment this factor was reduced to 30, LSD-25 had no effect on the activity of urease.

The same factor for the other drugs ranged from 0.6 to 50, the only exception being proclorperazine, which gave 13 per cent inhibition of urease at 10^{-3} *M*, or 200 times the concentration of a normal single dose per 5000 ml. Proclorperazine at this high concentration also inhibited light emission of firefly tail extracts, but this result is unreliable due to the turbidity of the system caused by the drug precipitating out of solution.

It is difficult, without further work, to generalize about the action of the drugs, but it seems possible that with increasing complexity of the enzyme system, there is an increasing tendency in the substances to differentiate as to their effects in vitro according to their effects in vivo.

Summary: The effects of six psychotropic drugs on two enzymes have been studied. Five of the six showed no effect on the rate of hydrolysis of urea by urease, and showed a very slight effect on the light emission by firefly tail extract. One drug, LSD-25, strongly inhibited both enzymes at concentrations as low as 6×10^{-6} *M*.

II. Growth Systems

1. *Proteus Bacteria*

The behavior of proteus colonies over a wide range of concentrations was studied in reaction to five psychotropic drugs. The drugs

were: secobarbital, dextroamphetamine, chlorpromazine, imipramine, and LSD-25. The inhibition of growth and of the swarming phenomenon of proteus served as criteria of the drug effects.

Methods, procedure and results of this experiment are not reported in detail since no significant data were obtained.

It was found that various concentrations of the drugs used were capable of inhibiting the growth of proteus. Around the diffusion zone of some of the drugs, an inhibition of growth as well as growth without swarming were seen together with further growth and swarming. These zones were not sharply defined because of the continuous reduction of drug concentration with the diffusion in the culture medium.

Similar phenomena can be produced with hundreds of different chemical substances and no consistent or specific reaction pattern was observed in this series of experiments with proteus bacteria. As an incidental observation, it may be reported that a late growth of proteus was seen on the zone on which, previously, inhibition of growth had occurred due to the influence of chlorpromazine. It appears that proteus bacteria are capable of adapting themselves to chlorpromazine within a certain period of time.

2. *HeLa Cell Tissue Cultures*

Methods and procedure: HeLa S-3 tissue cultures—that is, cells from a specific type of cervical carcinoma representing pathological epithelial cells of ectodermal origin—were used for this experiment. The same five drugs as in the previous experiment were employed; namely, secobarbital, dextroamphetamine, chlorpromazine, imipramine, and LSD-25. Two concentrations were used, the basic and one hundred times the basic. The basic solution was chosen empirically in rough correspondence with the blood level of the drug if all of a clinical daily dose would be absorbed assuming 6000 ml. as the normal blood level. The relative daily dose of the various drugs corresponding to the solutions employed were dextroamphetamine 30 mg., secobarbital 500 mg., chlorpromazine 400 mg., imipramine 100 mg., LSD-25.1 mg.

Full-grown HeLa S-3 tissue culture bottles were emptied of the supernatant fluid and then replaced by a solution consisting of the drug to be tested (10 per cent), inactivated calf serum (2 per cent) and synthetic medium M-150 (88 per cent).[7] For each drug, both of the ex-

perimental preparations, namely, a basic and the 100-fold solution, were separately induced this way: Control bottles were fed (a) synthetic medium M-150 plus 2 per cent calf serum, and (b) a mixture of 10 per cent sterile water plus 2 per cent calf serum plus 88 per cent M-150. All bottles were re-fed their respective solutions after 48 and 144 hours. To avoid clouding of results by spontaneous new growth of tissue in controls and non-toxic drug preparations, no further feeding was attempted after the sixth day and microscopic readings of all cultures were made until tissue death. Readings were taken after 1 hour and consecutively on 15 occasions over 3 weeks. The results of the microscopic examinations are graphically represented in FIG. 3 in per cent of the control survival time (100 x basic solution).

Results: In high concentration, all the drugs but LSD-25 caused degenerative changes and cell death in a significantly shorter time period than in the two controls. It is interesting to note that chlorpromazine in 1 hour causes significant degenerative changes. The (CPE) cytopathic effect of the basic solutions of chlorpromazine and imipramine was 100 per cent in 24 hours while this effect was equalled in 72 hours by secobarbital, in 144 hours by dextroamphetamine, and in 368 hours by LSD-25. In the lower concentration, on the other hand, only chlorpromazine showed a highly toxic effect, tissue death being completed in 6 days, which is 10 days sooner than in the controls. However, it seems to be significant that the cytopathic (cytostimulating) effects followed the same general pattern in both concentrations and that LSD-25 undoubtedly lengthened the survival of the cell aggregate as compared with the controls.

Discussion: These substances affect the tissue cultures and there are marked differences between the cytopathogenicity of the different drugs.

Because of the unexpectedly high cytopathic effect of chlorpromazine on the HeLa S-3 (carcinoma) cells, cytopathogenicity titrations with this drug were undertaken. The results were $10^{-4.3}$ for HeLa S-3 and $10^{-4.8}$ for simian kidney tissue (end-points calculated by Karber's method) which means that the CP E 50 of commercial chlorpromazine lies in the range of 10^{-4} to 10^{-5} representing 10,000 to 100,000 CP units per 1 ml., possibly independently of the tissue chosen.

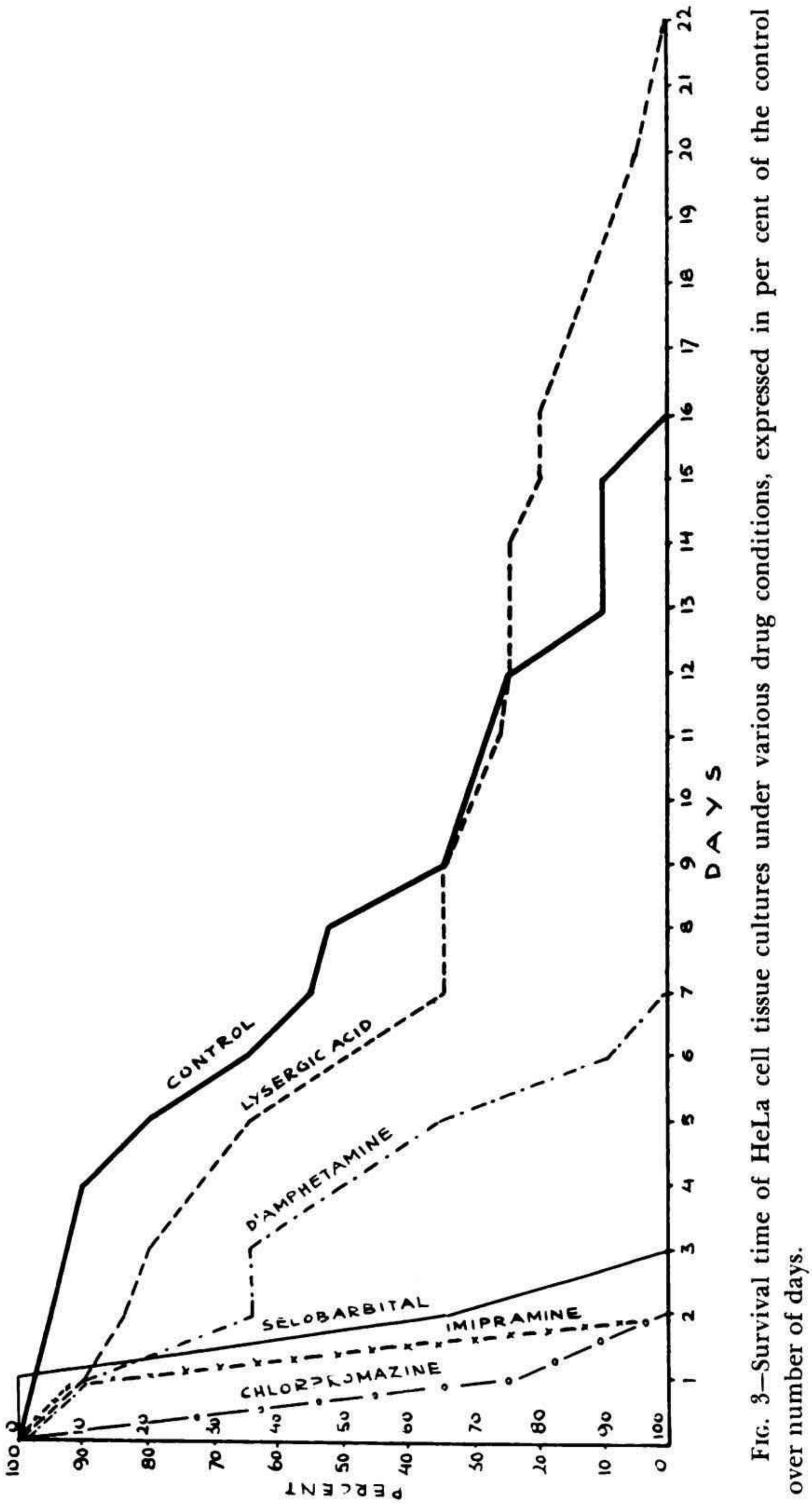

FIG. 3—Survival time of HeLa cell tissue cultures under various drug conditions, expressed in per cent of the control over number of days.

It must be stressed that the drugs used were of commercial quality and the range of concentrations very limited. Therefore, the results obtained are obviously only of a preliminary nature. The rather curious extension of survival time for the tissue culture under LSD-25 might have been due to an inhibition of individual cellular growth which resulted in a longer survival of the cellular aggregates since cellular crowding and choking was postponed. The extremely pronounced cytopathic effects of chlorpromazine on the tissue cultures are noted with interest.

Summary: The effects of five psychotropic drugs were studied on HeLa S-3 tissue cultures in two concentrations. While four of the drugs caused degenerative changes and slow death in a significantly shorter time than in the controls at both concentrations, LSD-25 extended the survival time of the cellular aggregate for several days beyond that of the controls. Chlorpromazine, on the other hand, proved to be extremely toxic for the tissue cultures studied.

3. *Oat Seedlings*

In another series of experiments, the above listed drugs were tested separately on the growth of sections cut from shoots of oat seedlings raised in darkness (etiolated) under standard conditions. Their growth is due solely to extension growth of the preformed cells and for maximum growth, B-indole acetic acid (IAA) or some other auxin is required. The growth of such sections is used as a standard bioassay in the study of plant growth regulators.[8]

Methods and Procedure: Each drug was tested over a five-step range of concentrations from 2×10^{-8} to 2×10^{-4} *M* except for LSD-25 which, due to technical difficulties, was used at 2×10^{-9} to 2×10^{-5} *M*. Controls were established under two conditions: 1) in buffer solution and 2) in buffer solution with 0.1 mg./1000 of B-indole acetic acid added. The growth of sections from the first internode of etiolated oat seedlings was measured. The initial length of the sections was approximately 3 mm.

Results: The results are illustrated in FIG. 4 where each point is the mean of ten replicates. The data have been analyzed statistically and the promotions of growth in the presence of IAA by chlorpromazine and proclorperazine or inhibition of growth by dextro-amphetamine and LSD-25 at various concentrations are significant. Imipramine stands between the two groups promoting

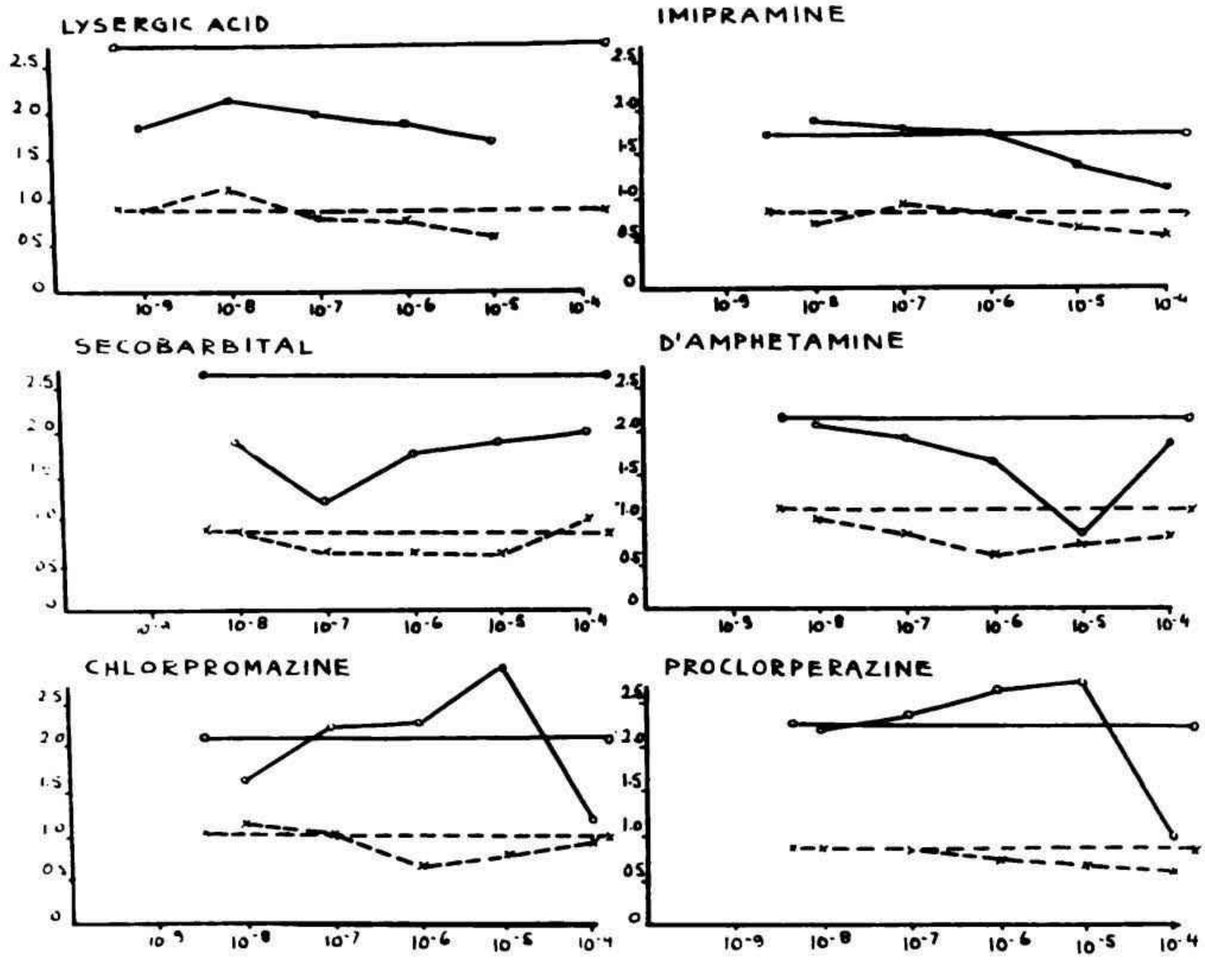

FIG. 4—Growth of etiolated oat seedlings under various drug conditions, expressed in mm. of growth in solutions of different concentration. The interrupted line represents growth under drug condition without addition of the growth hormone, indole acetic acid (IAA). The uninterrupted line represents growth when a standard amount of IAA was added to the experimental solution. The horizontal lines indicate the mean growth of the controls with or without IAA respectively.

growth in the low and inhibiting it at the two highest concentrations. The results obtained by the administration of sodium seconal could not be evaluated with certainty because of the possible interference of the sodium fraction in the complex. It becomes apparent that: 1) the drugs affect the growth of the sections and modify the response to IAA; 2) there are marked differences between the forms of dose response curves for different drugs; 3) drugs with similar chemical and pharmacological properties show similarities in the dose response curves. Thus, for example, the similarity between the phytopharmacological responses to chlorpromazine and proclorperazine—two phenothiazine derivatives—is very striking.

Discussion: The plant physiologist is particularly intrigued by the

growth response of the sections to the different concentrations of dextro-amphetamine. It is quite unusual to encounter a similar substance whose growth inhibiting property diminishes with increasing concentration. Further experiments would be indicated to establish whether similar characteristics are exhibited by other excitants of different chemical composition. It may or may not be significant in view of the importance claimed for serotonin in the normal and pathological functioning of the central nervous system that psychotropic drugs profoundly affect the growth of plant cells which require indole acetic acid for optimal growth conditions. 5-hydroxy-indole acetic acid is excreted in urine as a terminal metabolite of 5-hydroxy-tryptamine (serotonin). On the other hand, it is to be remembered that tryptamine is a frequently encountered substance in the metabolism of a great variety of biological systems.

III. Reactivity Systems

1. *The Hydra Feeding Reflex*

The studies of Loomis[9] demonstrated that reduced glutathione (GSH) is the substance liberated by the wound of the prey, punctured by the harpoon action of a hydra's nematocysts. This substance induces the hydra to open its mouth in an attempt to swallow that prey. Loomis described GSH as an "environmental hormone" which acted on surface chemoreceptors of the hydra and elicited the specific feeding reflex response. Later, Lenhoff and Bovaird[10] demonstrated that the presence of Ca^{++} ion was indispensable if this feeding reflex was to take place. The same researchers demonstrated that the mouth of the hydra would remain open over 20 minutes if the hydra were placed for one minute in a 10^{-3} *M* Ca Cl_2 10^{-5} *M* GSH solution.

Methods and procedure: Asexual hydra littoralis (from Boreal Laboratories, Port Credit, Ont.) were used 24 hours after last contact with food. Reduced glutathione (GSH) was prepared in 10^{-3} *M* stock solution and kept frozen until use.

The drugs were dissolved in the appropriate amount of water, neutralized when necessary, with 0.05 HCL or $NaHCO_3$ to as near neutrality as possible without precipitation. LSD-25 was supplied in intravenous solutions, and thus did not require further manipulation. The basic solution used throughout the experiments was 10^{-3} *M* Ca Cl_2 in distilled water.

Our procedure was essentially the same as that described by Lenhoff and Bovaird[10] except that only three animals were used for each concentration. The hydra were removed from the original container using a wide-tipped dropper, rinsed by sucking up and down a few times in 5 ml. of test solution (10^{-3} *M* Ca Cl_2 in distilled water, containing the drugs, as indicated) and then placed in a porcelain depression plate (soft plate) containing 2 ml. of fresh test solution. After 1 minute, the solution was removed with a fine-tipped dropper and replaced with 2 ml. of a solution containing 10^{-3} *M* Ca Cl_2, 10^{-5} *M* GSH and drug, as indicated. The time of addition of the GSH solution was taken as 0.

The animals were observed through a binocular dissecting microscope at a magnification of 20 x and the time during which its mouth stayed open was recorded for each animal.

Three series of these experiments were done. For the first series, except for LSD-25 which was at concentrations 10^{-5} *M*, the drugs were at concentration 10^{-3} *M*. For the second and third series, except for LSD which was at concentration 10^{-6} *M*, the drugs were at concentration 10^{-4} *M*. However, the effect of chlorpromazine at concentration 10^{-5} *M* was also explored. Before each series, the time during which the mouth of the hydra remained open in the presence of 10^{-3} *M* Ca Cl_2 and 10^{-5} *M* GSH was recorded as control.

Results: TABLE 1 gives the duration of the feeding reflex in reaction to the interference of a number of psychotropic substances. Recorded in minutes is the average time the hydra's mouth remained open.

These results are graphically represented in FIG. 5 where the results of the second and third series of experiments (concentrations of 10^{-4} *M*) were averaged.

Discussion: These results suggest that dextroamphetamine shortens the period of sustained feeding reflex and this inhibitory effect of the drug is apparently more pronounced at the lower concentration. Chlorpromazine and proclorperazine also exhibited a very pronounced inhibitory action on the feeding reflex. However, since they are also highly toxic for the animals who did not survive long in the presence of these substances, it is difficult to determine whether the inhibitory action of these drugs is a specific one. The results of the influence of imipramine and LSD-25 on the feeding reflex are so variable that they can hardly be interpreted.

TABLE 1.—Modification of the Feeding Reflex of Hydra Littoralis Elicited by Reduced Glutathione Under Various Drug Conditions

	10^{-3} *M*		10^{-4} *M*		10^{-4} *M*		10^{-5} *M*
	Minutes	*% of Control*	*Minutes*	*% of Control*	*Minutes*	*% of Control*	*Minutes*
No drug	24	100	17	100	16	100	
Dextroamphetamine	13	54	4	24	4	25	
Secobarbital	12	50	15	89	10.2	63	
Chlorpromazine	+*	–	0†	0	0.3‡	2	
Proclorperazine	–§	–	3	20	6	37.5	
Imipramine¶	+*	–	12, 20 Sp‖	95	8	50	
LSD-25	+*	–	4	24	14.6	81	

* The animals died shortly after the experiment began.

† The animals died 38 minutes after addition of chlorpromazine and GSH without appearance of the feeding reflex.

‡ The animals died after 45 minutes.

§ Experiment was not done. The solution precipitated, making the observation impossible.

‖ Imipramine at concentration 10^{-4} *M* caused alternating "spastic" opening and closing of the mouth in the hydra which made observation difficult.

¶ LSD-25 was at concentrations 10^{-5} *M* for the first series and 10^{-6} for the second.

While these experiments seem to offer an interesting experimental approach to the effects of psychotropic drugs on surface chemoreceptors, they must be considered as only preliminary in nature since the three series of experiments were carried out on three different populations of hydra. In future experiments, it would be desirable to test the whole range of concentrations on a larger number of hydra, all taken from the same population.

2. *Dandelion Sleep Movements*

These experiments report some effects of a number of psychotropic substances on the sleep movements of the common dandelion (*Taraxacum officinale Weber*).

The dandelion inflorescence opens for the first time on one day, and closes at night. This cycle is repeated in the following 24 hours. On the third day, the inflorescence remains closed. After some days it opens again, to form the familiar "dandelion clock," containing

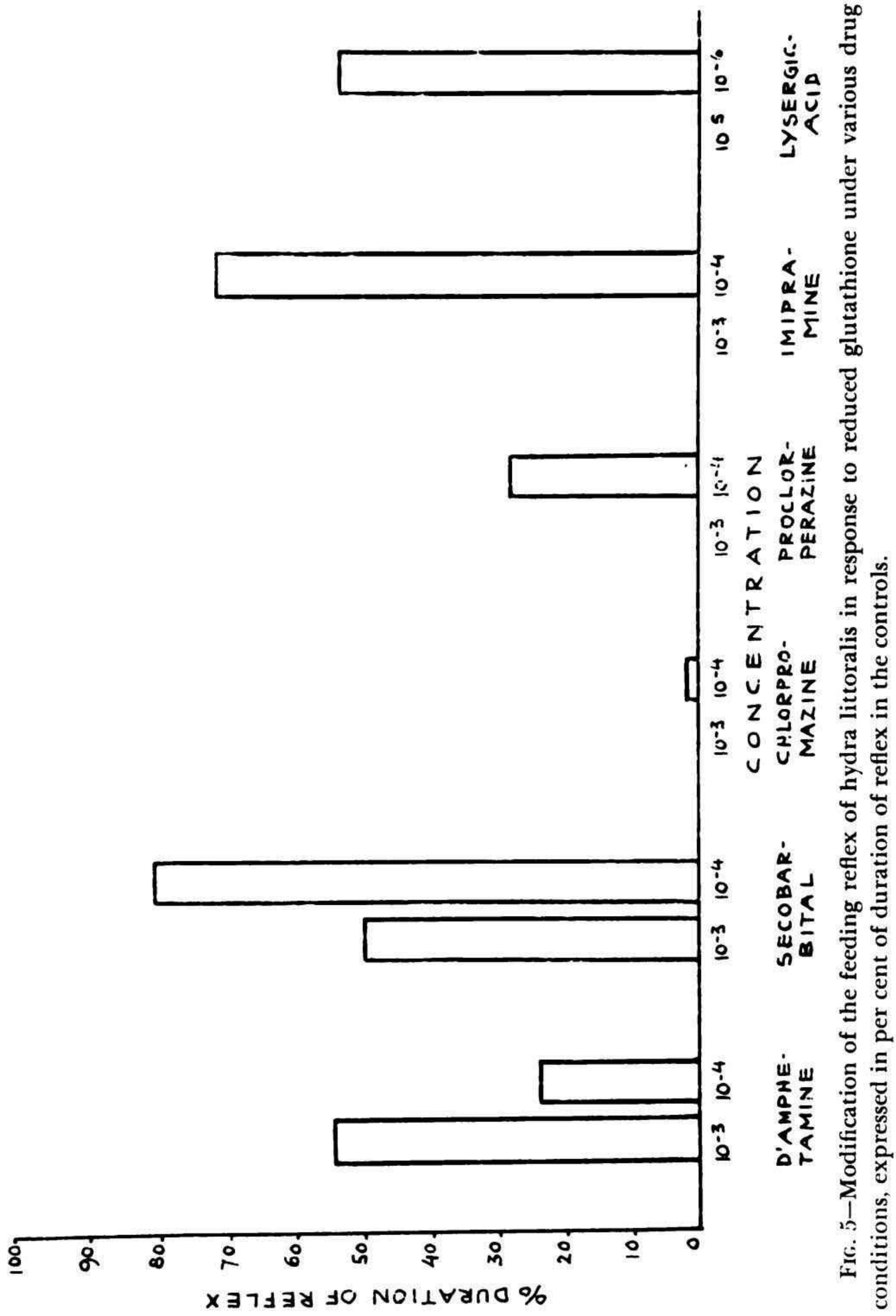

FIG. 5—Modification of the feeding reflex of hydra littoralis in response to reduced glutathione under various drug conditions, expressed in per cent of duration of reflex in the controls.

the seeds with their attached parachutes. The dandelion inflorescence consists of numerous, yellow, individual flowers inserted upon a flat or dome-shaped receptacle. Around the rim of the receptacle are two rings of green bracts. These are referred to as inner and outer bracts. When the inflorescence closes, the flowers move towards the center, and the inner bracts move upwards to enclose the flowers. The outer bracts remain projecting outward. After the inflorescence closes, at the final "sleep movement," the yellow petals die, leaving a small projection, the beak on the top of each seed. At the top of the beak is a ring of hairs. The seed begins to swell, and grows in length, and the beak grows into the stalk of the parachute. The ring of hairs forms the parachute. When the seeds are ripe, the inflorescence opens again. The inner and outer bracts are reflexed against the stalk, and the seeds with the parachutes are exposed.

Methods and procedure: The dandelion inflorescences used in this study were taken from a natural population growing on the top of the covered reservoir on MacGregor St. in Montreal. The inflorescences were selected for uniformity and cut on the first day of opening. The cut stalks were each 5.0 cm. in length. The inflorescences were placed immediately in different drug solutions with distilled water as a control, contained in test tubes. Each tube contained 8.0 ml. of solution.

Six psychoactive drugs were used, and each was tested at three concentrations. The drugs with the concentrations in brackets were: Chlorpromazine (25 mg., 50 mg., 75 mg.), proclorperazine (25 mg., 50 mg., 75 mg.), LSD-25 (0.025 mg., 0.050 mg., 0.075 mg.), secobarbital (25 mg., 50 mg., 75 mg.), dextroamphetamine (5 mg., 10 mg., 20 mg.), imipramine (12.5 mg., 25 mg., 50 mg.). The concentrations arbitrarily chosen for each drug were around that average concentration known to be effective in single dose in humans. Each drug treatment and the control contained five replicates. The opening and closing movements were measured over a 2-day period, every 2 hours, with the exception of the period between 10 P.M. and 6 A.M. The inflorescences were then left for a third day and observations were then made on the appearance and position of the morphological parts of the inflorescence.

Results: All the dandelions were closed by sunset and, to a greater or lesser extent, opened during the next day. An estimate of the

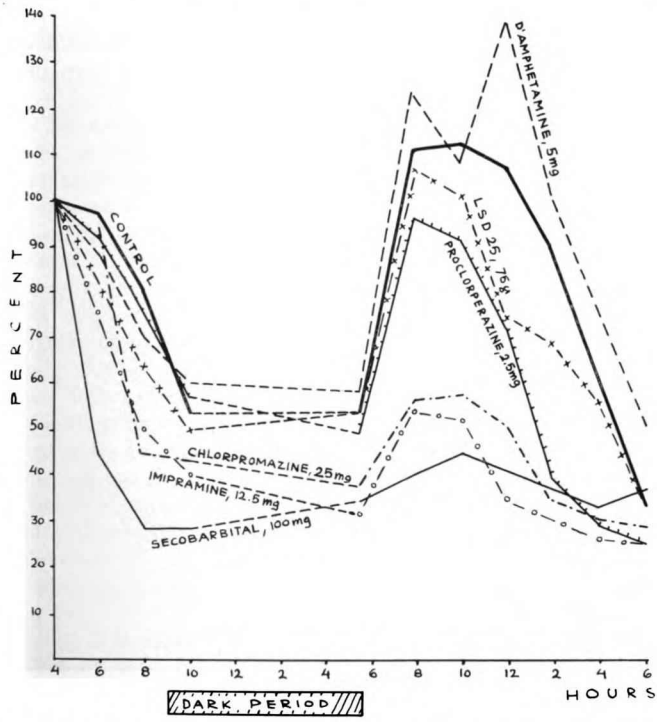

FIG. 6—Sleep movements of excised dandelion inflorescences in different drug solutions. The mean diameter of the inflorescences for each drug condition at each time interval is expressed as a percentage of the mean diameter of the same inflorescence at the start of the experiment.

degree of opening was obtained by measuring the diameter of the inflorescence as seen from above. The changes in opening at the drug concentration which gave the most pronounced opening on the second day are shown in FIG. 6.

The three highest responses, as regards opening movements on the second day, were obtained with dextroamphetamine, LSD-25, and proclorperazine. In the intermediate range we found two drugs,

chlorpromazine and imipramine. Secobarbital increased the rate of closing and produced the smallest movements on the second day or, expressed otherwise, it strongly inhibited the normal sleep movements as compared with the control.

Discussion: It is interesting to note that dextroamphetamine, LSD-25 (lysergic acid diethylamide), and proclorperazine (a phenothiazine derivative) are all characterized by excitatory effects on the human central nervous system. The amphetamines are amongst the most typical stimulants known, and possess an almost exclusively excitatory action. LSD-25 is a psychotomimetic which produces both excitatory and inhibitory effects on the human central nervous system with the excitatory action prevailing. Proclorperazine is employed clinically as a tranquilizer, but, in addition to some marked inhibitory effects, also possesses distinct excitatory ones.[11] The action of chlorpromazine, also a phenothiazine derivative, is mainly characterized by marked inhibitory effects on most psychomotor functions. It seems to possess no excitatory potential when behaviorally observed. Imipramine, an antidepressant in the psychiatric sense, is characterized by an immediate action which is much like that of a sedative, both objectively and subjectively.[12] Secobarbital, one of the classical hypnotics, is characterized by its powerful inhibitory action on the central nervous system when given in sufficiently high doses.

In addition to these dynamic changes during the first 48 hours, there were changes in the morphological appearance of the inflorescences which were observed at the end of the third day. At this time the inflorescences of the controls were tightly closed. In marked contrast to this, imipramine and, to a lesser extent, secobarbital and chlorpromazine produced globose, fluffy heads. Other effects are less obvious, but whereas imipramine and secobarbital both cause the inner bracts to become incurved, dextroamphetamine and chlorpromazine cause the upper half of the inner bracts to project outwards. It was also found that with proclorperazine the length of the seed and of the beak were greater than in the controls. Dissection of the inflorescences indicated that LSD-25, and dextroamphetamine might also stimulate the elongation of the seed and the beak. FIGS. 7a and -b show the striking difference between the appearance of inflorescences treated with dextroamphetamine and imipramine.

These experiments suggest that the behavior of some plant prep-

FIG. 7a—The appearance of inflorescences after three days of treatment with dextroamphetamine. The front row of tubes is the distilled water control. The succeeding three rows are in the order of increasing concentration of the drug. (For explanation, see text.)

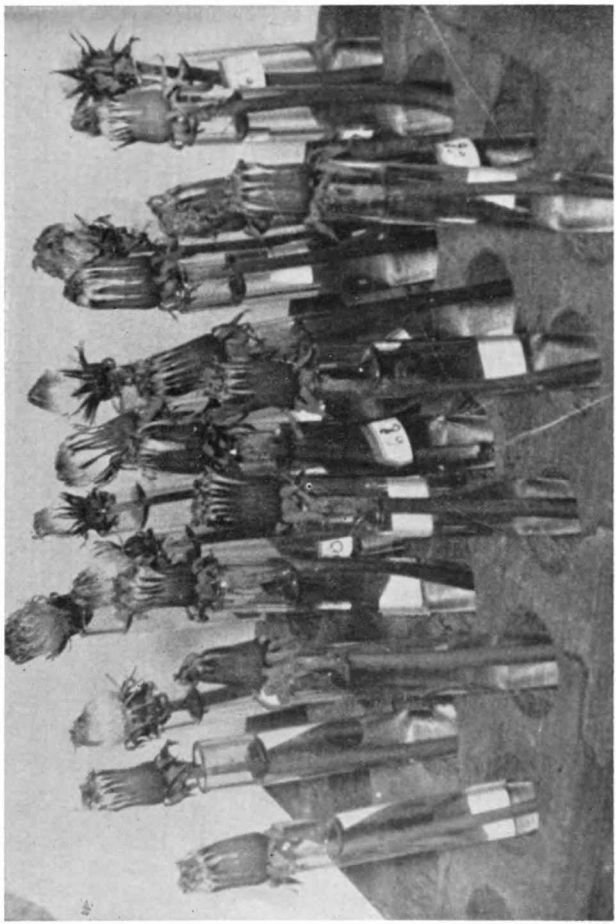

FIG. 7b.—The appearance of inflorescences after three days of treatment with imipramine. The front row of tubes is the distilled water control. The succeeding three rows are in the order of increasing concentration of the drug. (For explanation, see text.)

arations may under certain conditions be isomorphic to the behavior of biological systems possessing a central nervous system. Whether such an occurrence is more than a curious coincidence cannot be determined at this time.

Summary: Experiments were performed on the "sleep movements" of dandelion inflorescences which were excised and placed in tubes containing either water or solutions of various psychoactive drugs. Marked differences were observed between the action of various drugs on the subsequent closing and opening of the inflorescence. Distinct differences appeared between the effects of excitatory and inhibitory drugs on the sleep movement of the excised plants. Furthermore, some of the morphological structures of the inflorescences were differentially altered by different drugs.

Discussion: What has been achieved by performing these rather strange experiments and by assembling this array of data, which at first sight certainly strikes one as somewhat incongruous, if not irrelevant? Is such wildcat exploration justified?

We are not the first to have used models without a central nervous system for the study of psychopharmacological effects. Other investigators, however, did not experiment on more than one or two systems as a rule. One notable exception is Decourt in France who studied the effects of a variety of drugs on many biological systems of high and low complexity and introduced the concept of narcobiotic action. The characteristic feature of this action, he claims, is an inhibition of the fundamental metabolic processes of living matter, essential to the normal activity of all cells.[13] He found that chlorpromazine had the most powerful narcobiotic action of all drugs he had examined. The following four standard tests were proposed by him for the assaying of a drug's narcobiotic action: reduction in the rapidity of absorption of grains of carmine by infusoria; reduction of the rate of development of the embryo of the sea urchin; reduction of activity of an anaerobic microbe; study of mitostatic action on a plant root.

Our own data do not seem to confirm his theory of a general and uniform "narcobiotic" action which characterizes certain drugs and is revealed in the same strength on all living cells. In our experiments with oat seedlings, chlorpromazine exhibited a pronounced growth-stimulating effect in certain concentrations.

It is our impression that even at the cellular level and at rather

low molecular concentrations, the specific pharmacodynamic effects of a drug—in our case, of a psychotropic substance—depend on its concentration. The points of inversion where the gradient of drug activity changes direction, toward or away from the norm, above or below it, might hold particular interest. It is, for instance, rather intriguing to note that in four of our seven different systems, dextroamphetamine produced more inhibition at a lower than at a higher concentration. Does this mean that the difference between inhibition and stimulation at the cellular level, or at the enzymatic, depends on the molecular density of the substance under investigation? At this point we can only ask the question, since the exploratory nature of our experiments does not permit us to answer it, on the basis of statistical analysis or crucial evidence.

Another question offers itself when one inspects our findings: Is lysergic acid diethylamide as "poisonous" to other hydrolytic enzyme systems as it happened to be in our experiments with urease? And if this proved to be the case, is its psychotomimetic effect in part or wholly due to this effect on hydrolytic systems in cellular aggregates in the brain which are responsible for the regulation of synaptic transmission?

In line with our attempt to reduce our hypothetical preconceptions to a minimum, we did not adhere to one particular method of determining "dose ranges" for our experimental systems. In some experiments we used molecular concentrations, in others a rough approximation to probable blood concentrations in the human, and a third extremely arbitrary method consisted in the administration of clinical doses appropriate for adult humans to plant preparations (dandelions). While molecular concentrations should probably be used exclusively if one searches for the mechanism of drug action in these simple biological systems, our findings lend some support to the possibility that the clinical dose method may be productive of results if such simple systems are used as screening devices for psychotropic drugs.

It is conceivable that experiments like those described, covering a very broad spectrum of biological activity, might provide new ways of classifying psychotropic substances. Our observations on oat seedlings, for example, suggested the following ordering of the drugs studied: 1) quantitative modifiers (secobarbital and dextroamphetamine), sedatives and stimulants; 2) organizers (chlorpromazine

and proclorperazine), drugs with anti-psychotic properties; and 3) disorganizers (LSD-25 and imipramine), drugs which produce or enhance certain psychotic manifestations in the perceptual and ideational spheres. On the other hand, psychopharmacology is already plagued with a plethora of proposed classifications, and any new proposal should be solidly grounded in objective criteria.

Researchers working with tissue cultures might wish to pursue the apparent very high toxicity of chlorpromazine for cell aggregates characterized by low levels of organization which stands in distinct contrast to the low toxicity of this drug for the highly organized tissues which are found in the structures of the central nervous system. Is the survival extending property of LSD for HeLa cell cultures of any significance or simply a curiosity in view of its very high toxicity for the human brain? Can we really consider the sleep movements of dandelions a quantitative and qualitative analogue of mammalian sleep?

Barring all speculation, we may conclude that our experiments have yielded highly suggestive evidence that certain meaningful relationships can be demonstrated between the reactions to psychotropic drugs of organisms possessing a central nervous system and biological systems of low complexity. Whether these relationships can serve for the elucidation of still obscure neuropharmacological mechanisms or for the screening of psychopharmacological effects, only future investigation will establish.

Summary: The effects of six psychotropic drugs on seven biological systems of low complexity were studied. The drugs were secobarbital, dextroamphetamine, chlorpromazine, proclorperazine, LSD-25 and imipramine. The biological systems studied were grouped into those primarily representing metabolic processes or growth or reactive phenomena. They consisted in the urease-urea hydrolytic enzyme system, the luciferase-luciferin oxidase system, proteus bacteria cultures, HeLa cell tissue cultures, etiolated oat seedlings, the hydra feeding reflex and dandelion sleep movements. Some of the experimental data suggest that meaningful relationships exist between the reaction patterns of these simple biological systems and those of organisms possessing a central nervous system when subjected to the action of psychotropic substances. This relationship may be quantitative or in some instances possibly qualitative in nature (isomorphism of behavior). The significance of these findings and their

possible application to the study of basic mechanisms or to the screening of psychotropic drugs has been discussed.

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