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LYSERGIC ACID DIETHYLAMIDE (LSD) AND RELATED COMPOUNDS

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THERE IS quite frequently some confusion about LSD and lysergic acid itself. Even in the literature examples can be found. I would, therefore, like to show first what LSD is and to show how it differs from several other substances which are more or less similar to it in chemical composition. In 1943, Dr. A. Hofmann from our laboratory detected the strange effects of LSD on himself, and in the first clinical paper on this drug, published by W. Stoll (1), some of Hofmann's experiences are mentioned. It is probably less known that LSD was synthetized by A. Stoll and A. Hofmann (2) as far back as 1938, together with the first semisynthetic ergonovine. Actually, LSD was described at this time as an oxytocic agent and it has, in fact, about the same uterotonic activity as ergonovine.

Figure 1 gives the formula of the compound called LSD-25 which represents the diethylamide of *d*-lysergic acid. It is important to note that there is a double bond between the carbon atoms 9 and 10, and not, as was assumed by Jacobs (3, 4), between C_5 and C_{10}; the LSD formula is sometimes published with the double bond in the wrong place. At first, this may not seem very important, but there are several isomeric forms of lysergic acid and because of this it exists in several different steric configurations. The asymmetric center in position 5 is the reason for a *d* and *l* form of lysergic acid. Besides that, there are two other isomers as a consequence of the asymmetry at C_8, namely lysergic and isolysergic acid. Therefore, LSD and iso-LSD are different only concerning the spatial arrangement at C_8. The difference between lysergic acid and isolysergic acid was once attributed to a shifting of the double bond already mentioned, but, as shown by Stoll and his coworker (5) this is not true. We had the opportunity to test, pharmacologically, the diethylamide derivatives of all four isomers of lysergic acid and now I can state that *l*-LSD as well as *d*-iso- and *l*-iso-LSD are very different from *d*-LSD or LSD-25, in that they are practically inac-

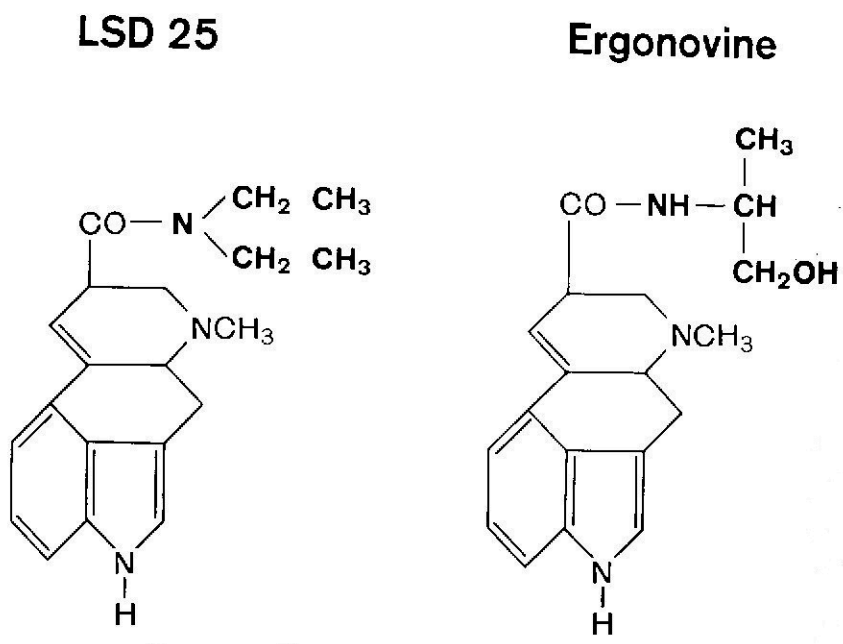

FIGURE 1. Chemistry of LSD-25 and ergonovine.

tive. And the situation is even more complicated since all lysergic acid derivatives, both the natural alkaloids as well as LSD and other similar compounds, can be hydrogenated by saturation of the double bond C_9-C_{10}. In this way, also a dihydro-LSD is obtained. This compound is devoid of specific effects on psychic functions. I may also mention that this hydrogenation creates a new center of asymmetry in C_{10}, thus increasing the possibility of further isomeric forms. Since I am not a chemist, I will not continue with these details. I think it is important to keep some of them in mind, however.

Another compound which will probably also be discussed in this group is 2-brom-*d*-lysergic acid diethylamide, coded BOL-148. This is a derivative of LSD-25 with a substitution of brom in position 2. Also LSD derivatives with different substitutions at the N of the indole part of the molecule are presently being studied, such as *l*-acetyl-LSD, *l*-methyl-LSD, etc. Even if these and some of the other compounds are not of great interest at the present time, one of them might be of importance in the further development of the whole LSD problem.

Rinkel: I would like to commend Dr. Cerletti for pointing out the importance of proper designation. I have repeatedly seen in the literature such expressions as "lysergized individuals" or, "They received

lysergic acid." Such wording is misleading. Great care should be taken to designate the compound properly.

Cerletti: I have forgotten to tell you that a whole series of compounds homologous to LSD, such as the diethyl-, the dipropyl-, the di-isopropyl- and the di-butylamide of lysergic acid, has been prepared by Stoll and Hofmann (6). Besides these, the corresponding monosubstituted amides, for example, lysergic acid methylamide or lysergic acid ethylamide have been studied. The last named substance, known as LAE-32, is of special interest. It is the monothylamide of lysergic acid and is a compound which, when administered in doses about ten times higher than LSD, like LSD, produces very remarkable psychic changes but, in contrast to LSD, seems to be less stimulating and more depressing.

It would be an interesting project to make larger comparative studies with these mono- and di-substituted series of lysergic acid amides in man, to see whether or not only the two ethyl derivatives (LSD-25 and LAE-32) are active in producing psychotic-like disturbances. For example, we have tried the dimethylamide on ourselves and found no psychic effects until doses several times higher than an average LSD dose were administered, but we obtained all kinds of vegetative symptoms which are also observed after administration of LSD. In this respect the substance was nearly as effective as LSD, but there was no interference at all with any psychic function.

Fremont-Smith: What about the propyl compound?

Cerletti: Up to now the dipropyl compound has not been tested in human beings, but it has been tested in dogs. It is less active than the compounds with the shorter chain.

Fremont-Smith: Is there a monopropyl?

Cerletti: Yes; there is a monopropyl compound too, but it has not yet been thoroughly examined. It would be too early for a general statement at this moment, but, after presentation of some further data, it will be more obvious that we must clearly distinguish between the influence of LSD and compounds similar to LSD on the vegetative regulations and on the psychic effects.

Hoagland: These effects do not necessarily parallel at all, there is no relationship, really.

Cerletti: No.

Sherwood: Would you say, from what you just now said—that the one compound has mainly a vegetative effect—that there is any evidence of a difference between the two compounds with respect to permeability of the so-called blood-brain barrier, that is, that while one compound brings about mainly "psychic" effects, the other brings about "peripheral" effects?

Cerletti: I am quite convinced that these vegetative symptoms are of a central origin. The question of differences between these compounds in regard to their passage across the blood-brain barrier has not been studied.

I might continue with some basic pharmacological data on LSD and

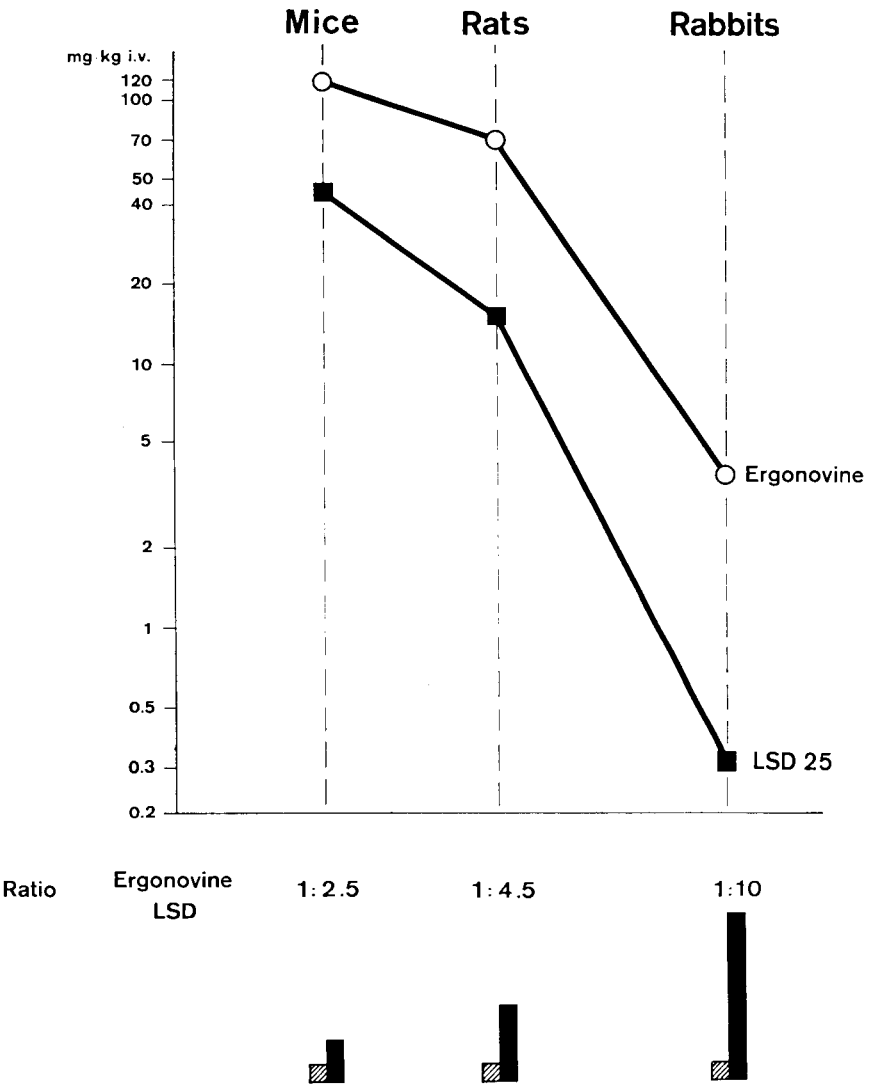

FIGURE 2. Toxicity ($L.D._{50}$ I.V.) of LSD-25 and ergonovine in mice, rats, and rabbits.

show the result of studies of its over-all toxicity (Figure 2). If LSD is compared with ergonovine in a toxicity test, it is quite clear that LSD is in all species thus far studied a much stronger agent. Smaller doses of LSD than of ergonovine are needed to kill animals. This difference between ergonovine and LSD is not of the same order in all animals, but it increases from the mouse to the rat and is most pronounced in rabbits. Rabbits are killed by intravenous doses of LSD of only from 0.2 to 0.3 mg./kg., whereas mice tolerate up to 50 or 60 mg./kg. given intravenously. Rats occupy only an intermediate position, nor are cats and dogs as susceptible to the toxic effects of LSD as rabbits. This is very interesting, because of all laboratory animals the rabbit is the most sensitive to other than toxic or lethal LSD effects. Only ½ μg./kg. of LSD injected intravenously in the rabbit produces a significant increase in rectal temperature. That is a dose at the same level as the minimal effective dose for human beings. It is even a little below the average dose for human beings which is estimated to be about 1 μg./kg. of body weight.

Rinkel: I might say that ½ μg. or even less is effective on so-called normal people.

Osmond: Am I right in assuming that there is another peculiarity in rabbits, in that they have high tolerance for atropine and for mescaline?

Cerletti: Yes; rabbits do have such a tolerance, but for the moment I do not remember the exact values.

Leake: The rabbit is outstanding in resistance to atropine.

Osmond: And mescaline?

Leake: I don't know about mescaline.

Osmond: I understand that is so, which appears to be very peculiar indeed.

Cantoni: The rabbit's high resistance to atropine is probably because of the fact that its plasma contains a very active atropine esterase.

Cerletti: We have not finished toxicity studies in cats and dogs, but it is quite certain that they are less sensitive to LSD than the rabbit. For pharmacological studies, it is very convenient to have both a simple and a sensitive method for testing LSD in the intact animal. The rectal temperature of the rabbit offers at least one criterion to study LSD in a dosage range similar to that of human beings.

Leake: One peculiarity of the rabbit with respect to any type of drug response is the extreme mobility of the water in its blood. The whole blood volume of the rabbit can shift enormously with very slight stimulation.

Seevers: The rabbit also has a high content of esterase in the blood

which splits atropine into its constituents, tropine and tropic acid, which renders it relatively innocuous.

Cerletti: Figure 3 illustrates the temperature effect of LSD on the rabbit. For many years the ergot alkaloids have been known to produce temperature increase, mainly if used in a toxic dosage range. Ergotoxine, for example, is regularly active in doses from 0.5 to 1.0 mg./kg. administered intravenously, but for ergotamine higher amounts are needed. Ergotoxine and ergotamine can be distinguished very easily by comparing their pyrogenic activity. Also ergonovine can produce a temperature

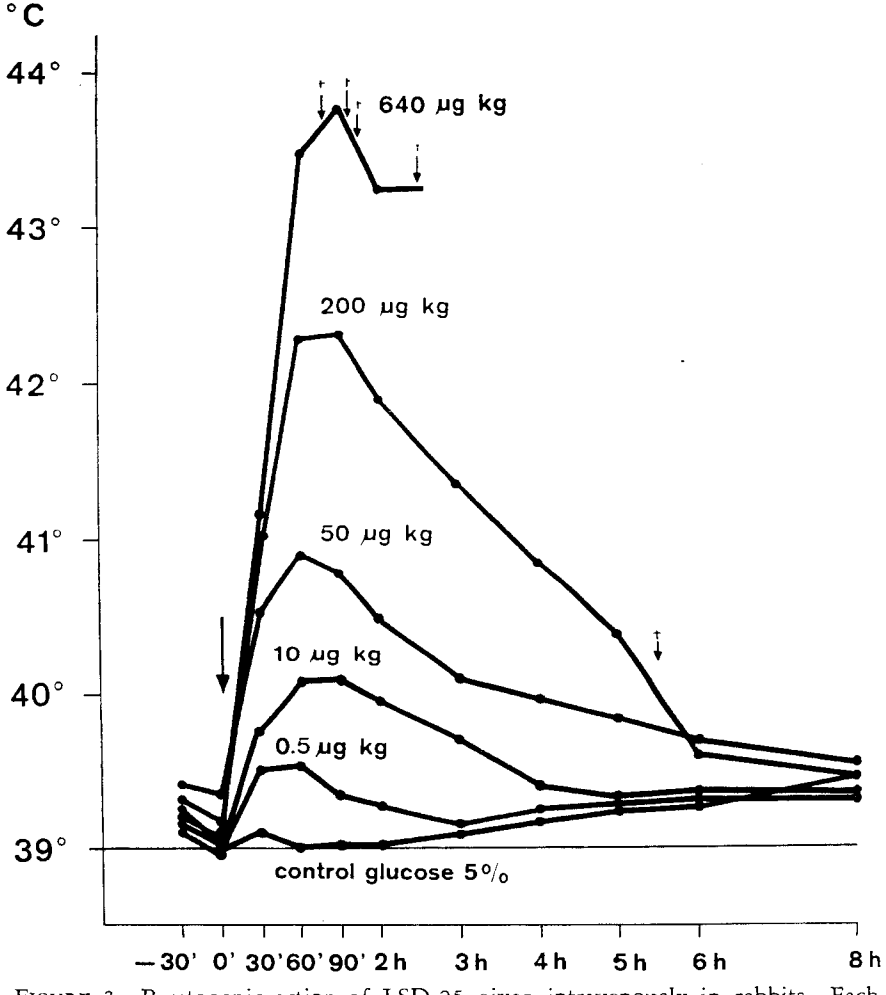

FIGURE 3. Pyretogenic action of LSD-25 given intravenously in rabbits. Each curve represents average values of from 4 to 10 animals.

increase. What distinguishes LSD from the other ergot compounds is the much higher pyrogenic activity. Starting with ½ μg./kg., an increasing response is obtained by augmenting the dose, and when from 150 to 200 μg. on upward are given some animals die in a state of hyperpyrexia. With 500 to 600 μg./kg., all animals are killed at the height of the temperature response. When this is compared with the similar effect of other ergot derivatives, as shown in Figure 4, it is evident that even 1 mg./kg. of ergotamine is ineffective; the minimal dose of this drug that will produce increase in body temperature is about 1.5 mg./kg. Ergosine and the high molecular alkaloids of the ergotoxine group, however, give a stronger pyrogenic response. Compared with a similar temperature reaction after natural ergot alkaloids, the effect of LSD is usually more rapid and it disappears in a shorter time.

Mirsky: Isn't that a dose effect? In Figure 3 you showed where a smaller dose also produced a similar rise in temperature, but at a delayed period.

Cerletti: Figure 3 shows that the maximal rise is obtained practically within 1 hour.

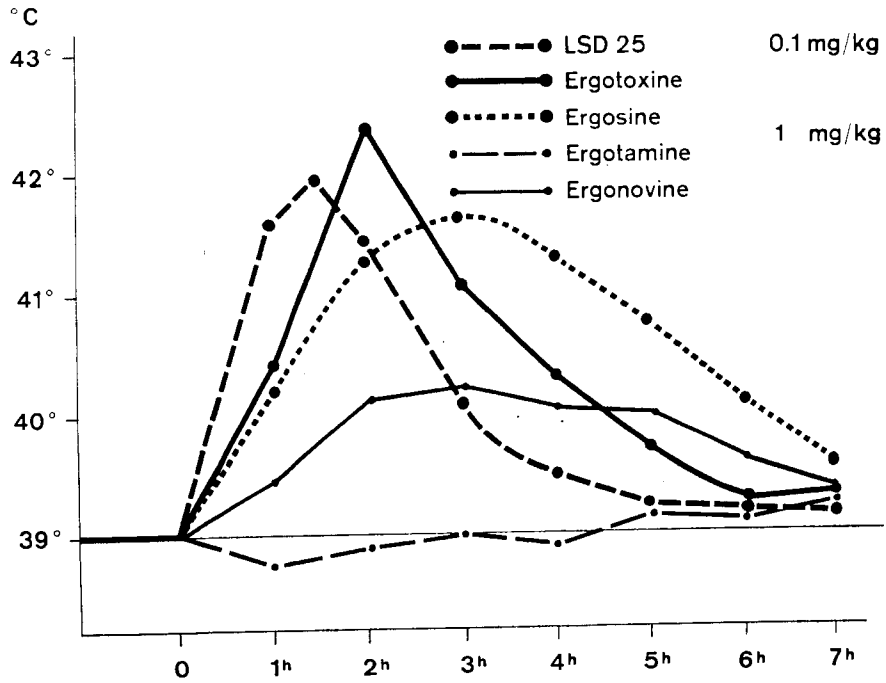

FIGURE 4. Pyretogenic action of different ergot alkaloids in rabbits.

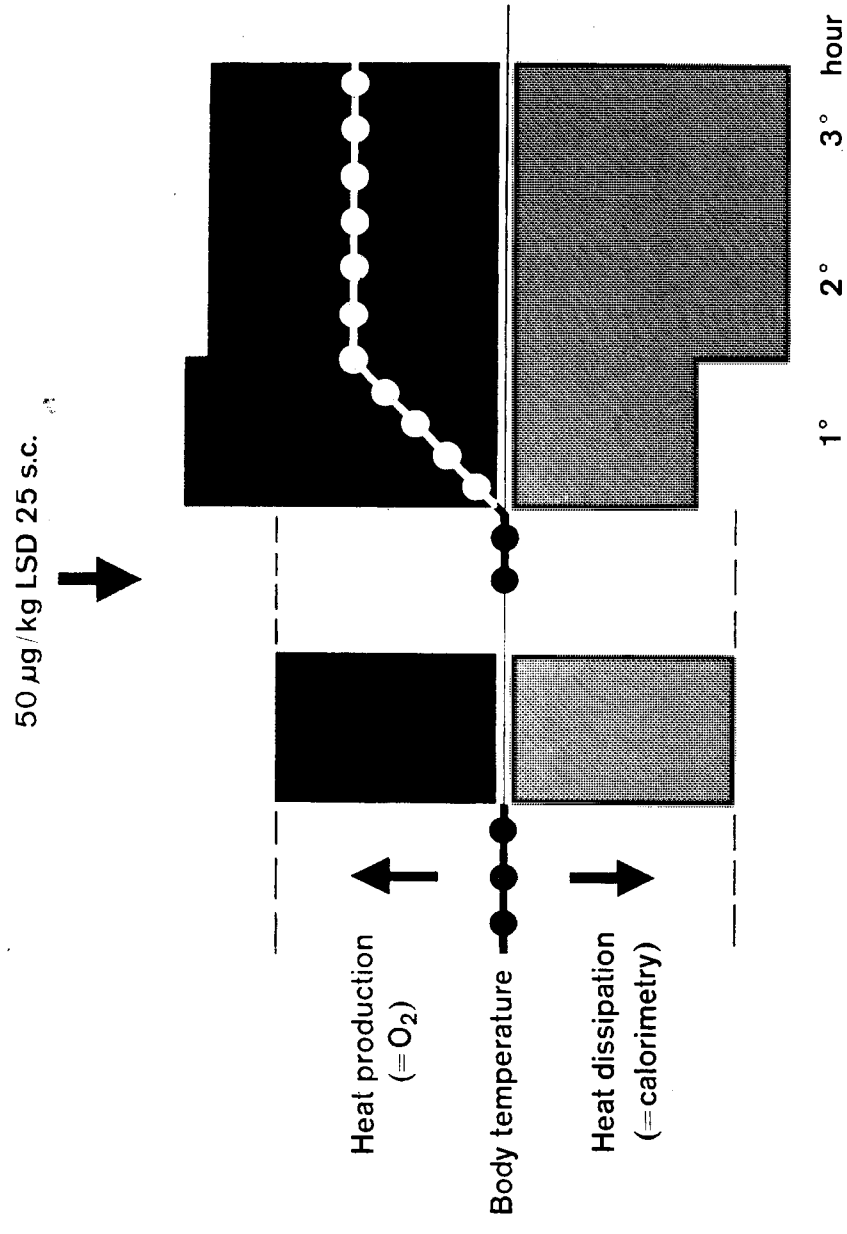

FIGURE 5. Type of metabolic action produced by LSD-25 in the rabbit.

Mirsky: But in all instances, a rise is obtained. There is still a rise with the minimal dosage shown there, but the delay is greater; that is all.

Cerletti: With a minimal dose the maximum temperature is obtained after 30 minutes.

Mirsky: What is the dose below that?

Cerletti: These are the controls.

Mirsky: They rise, too?

Cerletti: No. That is the normal temperature of the rabbit during an 8-hour observation period. All these experiments were carried out during the same hours of the day, and the physiological change of body temperature during the 24-hour period must be taken into consideration.

Sherwood: What did the two animals that had 640 μg./kg. die of?

Cerletti: From respiratory paralysis.

Leake: With the heart still beating?

Cerletti: Yes, with the heart still beating. Respiration stops first.

Leake: Do you know whether or not there is any hemoconcentration with the rise in temperature?

Cerletti: I cannot answer that. That has not yet been studied. We have studied the mechanism of this temperature rise by measuring the O_2 consumption and by measuring the total heat output of the animals in a calorimeter. We obtained the following result: In the first hour O_2 consumption increased (Figure 5).

Cleghorn: It is very unlikely that there would be any major water shift in the rabbit in 30 minutes.

Leake: No; that can occur with startling rapidity. That was the point of my question.

Cerletti: After the injection of LSD, O_2 consumption increases, but if the total output of heat is measured in the calorimeter, it will be found that O_2 consumption first decreases and increases only later on. At least in the beginning of the LSD effect, there is what we call in German "Wärmestauung" (heat congestion). This decreased dissipation of heat surely contributes more to the rapid increase of temperature than the increased heat production. The O_2 consumption rises insufficiently so that the observed temperature increase would be explained only by decreased heat dissipation.

Fremont-Smith: So this is the usual mechanism of temperature rise in fever?

Cerletti: That is the same thing. We have compared this effect of LSD with the activity of purified pyrogenic substances from bacteria, and it looks very similar.

Leake: This is compatible with the notion, then, that there is hemoconcentration?

Cerletti: As a secondary phenomenon, yes.

Fremont-Smith: But it is almost more compatible with the idea that it is peripheral vasoconstriction.

Cerletti: Yes, but if the activity of LSD as a peripheral vasoconstrictor agent is measured, it has no such effect in this dose range.

Fremont-Smith: You mean, there is nothing to be shown in the rabbit's ear, no fall in skin temperature?

Cerletti: No. Perhaps some vasoconstriction may be present in the rabbit's ear, but it is quite difficult to tell whether this constriction is caused directly by LSD or by the handling of the animals, etc.

Fremont-Smith: But those two things go together don't they? There is vasoconstriction—a failure to dissipate heat—and a rise in temperature. I am interested that in the rabbit's ear, there is a vasoconstriction, even if it is difficult to explain what causes it.

Cerletti: Yes, but if a block is made at the base of the ear to avoid constriction, the increase of body temperature is not interfered with, so, even if there is a vasoconstriction, this cannot contribute essentially to this increase of temperature.

Fremont-Smith: Unless it is more widespread.

Cerletti: Yes, but it is difficult to measure the vasoconstriction in an unanesthetized rabbit. If LSD is tested in a comparison with other ergot compounds, in very sensitive tests, it will be found to have a very weak vasoconstrictive property as compared with ergotoxine, for example, or with ergotamine. It is quite difficult to show a constrictor action of LSD.

Fremont-Smith: Then there arises the question of how the rabbit fails to dissipate heat if he doesn't do it by vasoconstriction.

Cerletti: Yes.

Leake: Or if he doesn't do it by hemoconcentration, which is what I have been arguing about.

Fremont-Smith: You might be right.

Cleghorn: I don't see why hemoconcentration should be the explanation for the circumstances attending the discussion which Dr. Cerletti has given.

Leake: Hemoconcentration as a factor in fever was very thoroughly examined by Barbour (7) in 1921. He gave all the experimental evidence showing hemoconcentrating effects generally. On the other hand, all the antipyretics in general produce a hemodiluting effect and act as antipyretics, according to this thesis, by producing a reversal of the hemoconcentration.

Cleghorn: Increasing the circulation of the blood?

Leake: That is right, making the water available for loss through the lungs and the skin.

Fremont-Smith: But I think that another factor must be found, because many years ago I made a number of experiments with typhoid vaccine administered intravenously to human beings. And I also made a number of experiments with malaria. In both cases, whether hemoconcentration occurred, which was always very slight, or hemodilution occurred, which could be very striking, depended upon whether or not the patients were allowed to drink water. If they drank water, since there was a very marked antidiuretic effect at the beginning of every fever, there was a marked hemodilution, and this hemodilution in no way interfered with the fever. I think it supports your argument that hemoconcentration might not necessarily have anything to do with fever.

Unna: Hasn't it been agreed, since the times of Dale and Spiro (8), who first investigated the temperature effect of ergotamine and ergotoxine, in 1921, and since presentation of the evidence compiled by Dr. Barger in his splendid monograph (9), that the primary site of action of the various alkaloids of ergot is on the thermoregulatory centers and that hemodilution and vasoconstriction are secondary and may to some extent accompany the effects of these alkaloids?

Fremont-Smith: It is essential to ask in what way it affects the thermoregulatory center, because, after all, when typhoid vaccine or a pyrogen is given, there is an effect on the thermoregulatory center, and this thereby results in vasoconstriction. I would assume that there are only two ways in which a fever can occur: One is by increased heat production, and the other is by decreased heat loss. Either of those can result from a change in the thermoregulatory center. If there is an increased heat production to produce the fever, this does not seem to be enough, from what Dr. Cerletti has said, to explain it.

Unna: But the thermoregulatory centers act like an indicator, and the thermostat can be set higher by drug action.

Fremont-Smith: But by a mechanism also, by a process of influencing heat exchange.

Unna: Yes, of course; if the thermostat is set higher it will decrease heat dissipation and will not decrease heat production.

Fremont-Smith: But it must decrease heat dissipation by a process, and the process must act peripherally, and therefore we come back again to either inhibition of perspiration or vasoconstriction.

Unna: We certainly do, but, Dr. Fremont-Smith, we can still distinguish between ergotamine and ergotoxine, for instance, which, to the best of my knowledge, both have the same effects on isolated tissue as far as vasoconstrictor effects are concerned. Nobody can differentiate between their peripheral vasoconstrictor action, but, as Dale and Spiro

(8) and Rigler (10) have shown, many years ago, they can be distinguished by their effect on the thermoregulatory center. Animals die of hyperthermia after ergotoxine but they do not die of hyperthermia after ergotamine. Both are vasoconstrictors if they are used on the rabbit's ear.

Kety: When some of our pharmacologists speak of vasoconstrictor drugs, they mean drugs which constrict isolated blood vessels. All Dr. Fremont-Smith is saying, and I agree with him wholeheartedly, is that this phenomenon described by Dr. Cerletti can best be explained as a rise in temperature achieved by vasoconstriction and decreased heat loss. This does not necessarily mean that the same dosage would constrict an isolated blood vessel, but, through the thermoregulatory center, it raises body temperature by means of a centrally effected vasoconstriction.

Cerletti: That is the decisive argument. We must distinguish between a vasoconstriction produced by the LSD injected directly in the periphery and a vasoconstriction which is caused primarily by the central stimulus. If the spinal cord is cut, the same amount of LSD can be injected without any effect on rectal temperature, although the vessels are, under these conditions, even more responsive to vasoconstrictor stimuli.

Sherwood: Surely there must be one further mechanism. We know the antidiuretic hormone of the pituitary may well be activated, as suggested by Harris (11), through the sinusoids of the pituitary stalk, and that might, on the one hand, act separately for hemoconcentration or hemodilution, and on the other hand, on the temperature mechanism; it may be an entirely different mechanism, also central. There are two lines leading outward to the periphery which may be entirely different.

There is also the work of Duke and Pickford (12), who have made intracarotid injections and have observed renal excretion following acetylcholine. They were able to stop the effect of carotid injection of acetylcholine by a preceding injection of epinephrine but it had to be timed approximately between 8 and 10 seconds; otherwise, it did not work and they got a reversal of the effect.

Cerletti: My opinion is that the mechanism of the production of this rise in temperature is for the moment less important than the fact that by simply measuring the rectal temperature a very sensitive criterion is given for the central action of LSD. I am absolutely convinced that in the mechanism of decreased heat dissipation vasoconstriction is also involved, but it is not a vasoconstriction produced by a peripheral vascular effect of LSD.

Seevers: Would you add that this is a species-specific characteristic? I would like to add that thought.

Cerletti: It is species-specific. I will show presently that LSD de-

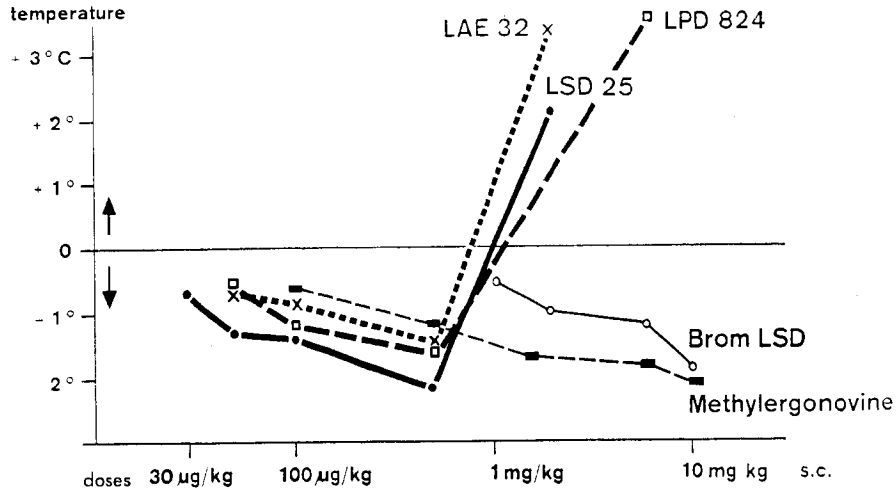

FIGURE 6. Action of different lysergic acid derivatives on body temperature of the rat.

creases the temperature of the rat. The temperature of the dog and the cat are again increased, but when using these species instead of the rabbit higher doses are needed.

Seevers: Not with comparable doses?

Cerletti: No, not with comparable doses. As Figure 6 shows, LSD produces in rats a small but quite a significant fall of temperature in doses up to nearly 1 mg./kg. Only by large sublethal doses, the temperature is increased. That is a behavior similar to that of the hydrogenerated ergot alkaloids, which also decrease the temperature in therapeutic doses, but produce a hyperthermic effect in sublethal doses.

Abramson: What is LPD?

Cerletti: LPD-824 is chemically related to LSD. It is the pyrrolidid of lysergic acid, having the diethylamino group closed to a pyrrolidine nucleus (Figure 7). When this substance was tested in man, doses up to ½ µg./kg. were given intravenously without producing any LSD-like symptoms. Pharmacologically LPD is a very strong hypotensive agent. In animals 10 µg./kg. produces hypotension, both in cats and in dogs. As already said, in human subjects it had no psychic effect, but produced nausea in such small doses that a hypotensive effect could not be achieved.

Abramson: Does it block the LSD reaction?

Cerletti: No. I shall speak later about the different attempts we have made to block LSD reactions, but LPD was not active.

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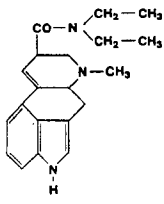
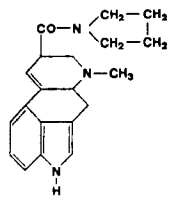
			ratio of activity LSD:LPD
Psychic activity (man)	1 μg/kg effective	10 μg/kg ineffective	> 10:1
Serotonin antagonism (relative activity rat uterus)	100	5	20:1
Hypotensive and bradycardic effect (cat i.v.)	40 – 50 μg/kg	10 μg/kg	1:5

FIGURE 7. Comparison between LSD-25 and LPD-824.

Sherwood: What is a therapeutic dose of LSD?

Cerletti: A therapeutic dose in human beings?

Sherwood: Yes.

Cerletti: I would say doses between ½ and 2 μg./kg., perhaps even 3 μg./kg.

Sherwood: But therapeutic in what sense?

Cerletti: A dose used to produce a model psychosis in man is an experimental tool.

Sherwood: But that is not therapy.

Cerletti: Or used as an aid to psychotherapy. I think the psychiatrists here can tell you better whether or not LSD has a therapeutic value. What I mean by a therapeutic dose is a dose which, from all we can observe, is not toxic in the animal.

Fremont-Smith: A human clinical dose.

Hoagland: It would be about the same order of magnitude as we have been talking about.

Rinkel: If I may answer this question, doses have been given from 100 to 600 μg./kg. They were not differentiated for kilogram of body weight. Even a smaller dose has been used. Dr. Abramson, I understand, gives very small doses therapeutically. But the highest dose we have heard about is 600 μg./kg. (13).

Abramson: For approximately three and one-half years I have been administering LSD-25 to about ten ambulatory patients, in brief psychotherapy, as well as in intensive psychotherapy. This work has been carried on with patients whom I have seen both in my office and in my home. I have used LSD-25 as an adjuvant to psychotherapy in doses between 25 and 40 μg./kg. (14).

Marrazzi: I think the route of administration should be given.

Abramson: It is given by mouth.

Sherwood: My point was, since LSD appears to produce psychosis, it surely cannot be called a therapy.

Hoagland: This question of the use of LSD as an adjunct to therapy may be discussed later.

Cerletti: First of all, I regret having used this term, "therapeutic."

Fremont-Smith: No; I don't regret it.

Osmond: Dr. Cerletti, what color were the animals used in your work?

Cerletti: The rats were all albinos.

Osmond: What color were the dogs?

Cerletti: The dogs were just ordinary Swiss mixed dogs of different colors.

Osmond: This may sound absurd, but there is a certain amount of evidence from our work that a difference in color may be very important, and that is why I ask.

Cerletti: But the gray Norwegian rat behaves in exactly the same way. We have this type of animal also in our laboratory.

Osmond: And it dies when given the same amount?

Cerletti: Yes; as far as I remember, there is no difference in toxicity or difference in reaction.

Cantoni: You mentioned before that the dimethylamide and/or the monomethyl can be considered to have the same vegetative effects but not the psychic effects. Would you classify the temperature effect in the rabbit as a vegetative or a psychic effect?

Cerletti: We have not yet finished the comparative study of all compounds concerning temperature reaction in the rabbit. For example, lysergic acid dimethylamide has not yet been investigated, but it has been tested on dogs and on ourselves and it produced vegetative symptoms without psychic disturbances. I do not believe that a temperature increase in the animal determines LSD-like activity. I must, however, agree that several LSD similar compounds, which are inactive in regard to psychic functions, produce much less hyperthermia than LSD. On the other side, ergonovine, the well known uterotonic alkaloid, may increase the rabbit's temperature when given in the dose range of from 30 to 50 μg./kg.

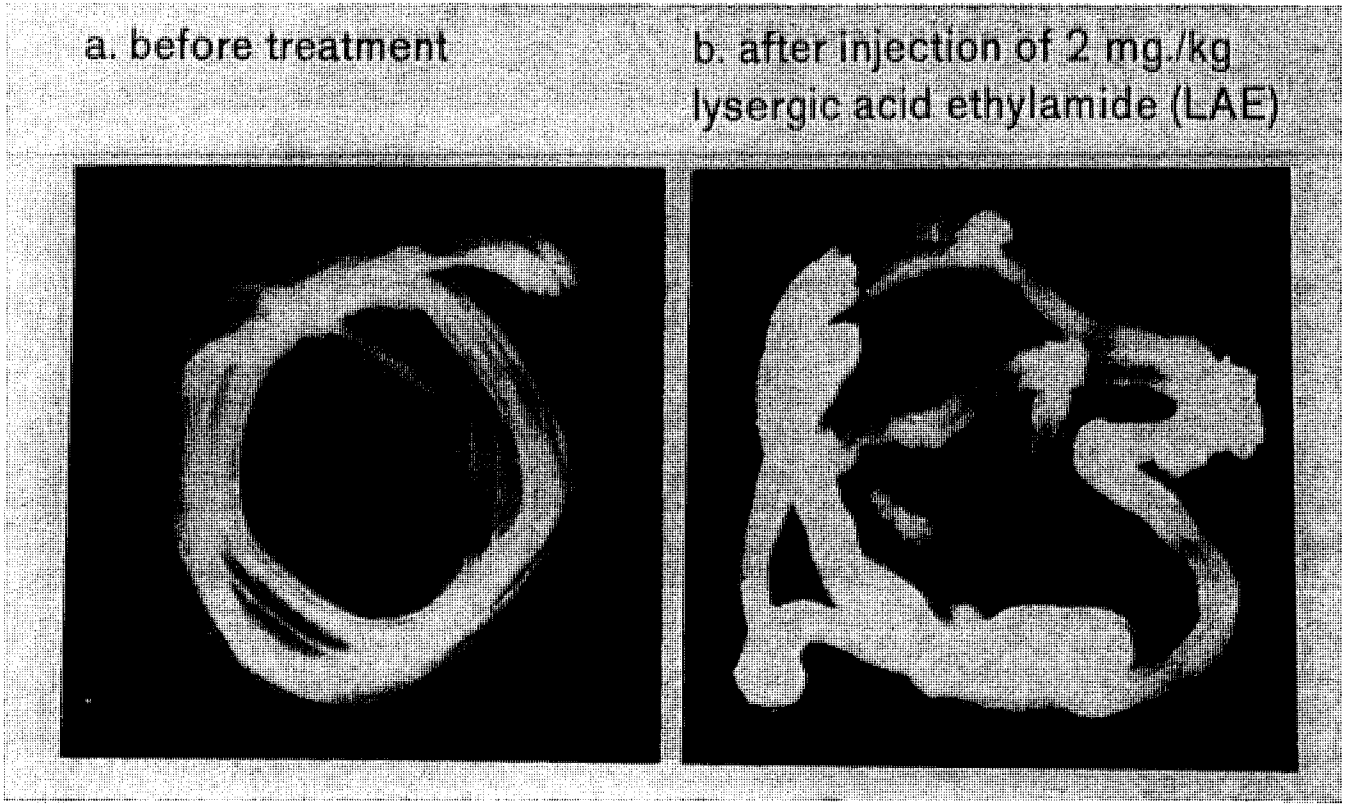


FIGURE 8. Movements of a waltzing mouse. (Time exposure in dark room, the animal being marked with a luminous substance.) Reprinted, by permission, from Rothlin, E., and Cerletti, A.: Über einige pharmakologische Untersuchungen an Mäusen mit congenitaler Drehsucht. *Helvet. physiol. et pharmacol. acta* **10**: 319 (1952).

Cantoni: That is the reason I asked the question, because I saw that, with the other alkaloids, there is a certain correlation between the speed with which the temperature effect comes on and the speed with which the response in the uterus comes on. I was wondering whether that is related to substitution on the amine.

Cerletti: I cannot answer this last point. In any case, all isomeric forms of LSD, which are inactive as psychogenic or as hallucinogenic drugs, are all inactive also with respect to temperature increase and many other LSD effects which can easily be controlled in animal experiments.

Mirsky: But you are not saying that the two are concomitant?

Cerletti: No; there is not enough evidence to say that. But, for example, the question arises whether LSD effects can be blocked with this or that compound. We tried, for instance, to block the hyperthermic reaction by pretreatment with substances which possibly could be competitive LSD inhibitors. In such a moment, it is good to have a clear-cut experimental setup to work with. And it is even better if one can use at the same time doses in a similar order of magnitude as the doses used in human beings. In animal experiments it is surely quite different whether from 10 to 20 μg. or from 1 to 2 μg. of LSD are used. Such large amounts of LSD may create a situation which probably is far removed from the conditions in man. We are always looking, therefore, in our animal work for at least some correspondence to the LSD question in human beings. And one point which, in this respect, should be considered more, is the dosage. Some years ago, we observed an interesting effect of LSD and of LAE in waltzing mice (Figure 8). These animals, having an inherited behavior anomaly, show a typical pattern of circular movements which is disturbed by LSD and LAE. But doses of at least from 1 to 2 μg./kg. must be used, and I would hesitate to say that this effect has anything in common with the effects of LSD in man. Amphetamine influences our waltzing mice in the same way as LSD.

Fremont-Smith: It reduces the dancing?

Cerletti: It does not actually reduce the dancing as, for example, some typical sedative compounds do; some of these compounds very easily stop it. The hydrogenated, high molecular ergot alkaloids also reduce the dancing and eventually stop it completely. But with LSD, the effect is different: The animals are excited but the dancing is continued; however, it is no longer a regular circular moving.

Fremont-Smith: It is disorganized.

Rinkel: If I remember correctly, Dr. Rothlin (15) told me that it was a special strain of mice that had this ability to dance.

Cerletti: Yes; it is an inherited behavior in one special strain.

Rinkel: It is quite an unusual strain, and you discovered that that strain provided a test object for testing a number of compounds.

Cerletti: Yes; you cannot do this on normal mice, and you cannot do it on the so-called Japanese waltzing mice, or in animals with waltzing movements caused by a deficiency in the statoacoustic organ. Our animals can also move quite normally, and the manifestation of the anomaly depends very much upon the prevailing external conditions. Therefore, we were inclined to speak of a psychoneurogenic anomaly.

Mirsky: As I understand it, Dr. Cerletti, you are talking about the effects on body temperature because you are trying to compare dosage levels as used in man, for one reason or another, with a similar dosage level in animals.

Cerletti: Yes.

Mirsky: And that is the only reason you are talking about temperature?

Cerletti: That is the main reason.

Mirsky: It has nothing to do with psychologic or any other phenomena?

Cerletti: No. You will see that, for other pharmacological effects, much higher doses must be used merely to see a result. As to what can be observed in unanesthetized animals, I have a moving picture which demonstrates the effect of LSD in the cat and in the dog; there are some very clear effects. Perhaps a psychologist would try to make an interpretation of the behavioral changes in these animals. What we can observe, as pharmacologists, are mainly vegetative symptoms which are easier to measure and to follow up. These symptoms are quite typical both for LSD and for several other chemically related compounds.

In brief, they can be summarized as follows (Figure 9): LSD, in a dose of about 50 μg./kg. produces regularly a strong dilation of the pupils and a pilomotor reaction. It increases the body temperature. The respiratory pattern is frequently changed; there are attacks of hyperpnea, and then something like panting. Wouldn't you call it panting, Dr. Unna?

Unna: Yes; that is right.

Cerletti: The increased respiration is probably caused, at least in part, by the increased temperature. With higher doses, very strong salivation and even lacrimation are observed. The movements of the animal become atactic, and there is frequently a short paresis, mainly of the hindlimbs. All these symptoms occur early and are more pronounced in the dog than in the cat. The recovery is remarkably rapid. As early as one hour after the injection of a dose, which produces an extremely marked vegetative and motor syndrome, the animals again look quite

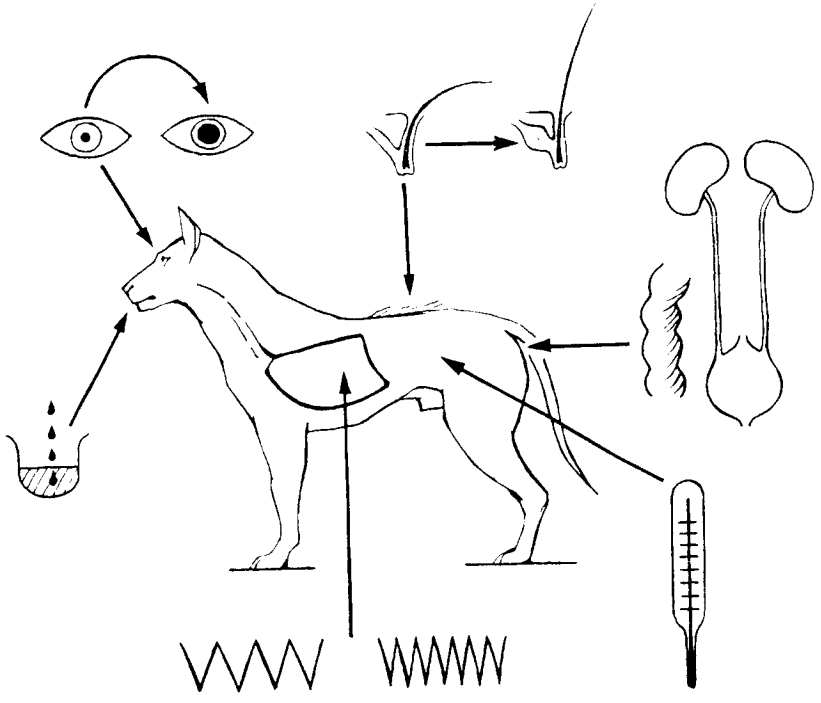


FIGURE 9. Vegetative symptomatology of LSD-25.

normal. In the cat, there is an aftereffect: When the initial vegetative symptoms have more or less disappeared from 50 to 60 minutes after the LSD injection, the otherwise normal looking animal shows a completely altered behavior toward the mouse. I could imagine that there are some possibilities for animal psychologists to work on with LSD.

Rinkel: I might add that we have observed with polygraphic methods, that small amounts, 1 μg./kg. of body weight, increased respiration and sighing, which, perhaps, would be similar to your observations.

Cerletti: Yes, but it will be important to distinguish in man whether such effects, let us say on the pulse rate or blood pressure, or on respiration are primarily caused by LSD, or whether they are only secondary reactions of LSD. When anxiety is produced with LSD, an increased pulse rate can occur as a consequence of this anxiety.

Sherwood: I do not see how you can ever distinguish between those two, any more than you can know whether the schizophrenic tells you what he tells you on account of suffering from some kind of internal tension, which he interprets in his own way, or whether the hallucinations he alleges he has are "real."

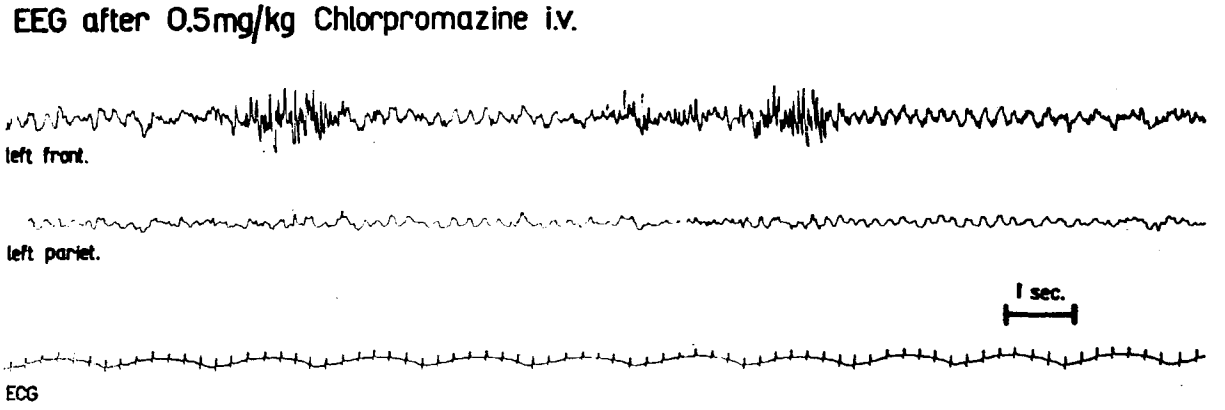


FIGURE 10. Action of LSD 25 on rabbit EEG

Cerletti: But at least you can distinguish that a reaction, which does not correspond to the pharmacological properties of LSD, is probably not a direct effect of the drug. It is, for example, impossible to provoke an increased pulse rate by LSD in any kind of pharmacological preparation suitable for such a test. LSD behaves like a typical ergot alkaloid: If it influences the heart rate, this is always a bradycardic effect. On the isolated heart, there is no effect. But if the heart is innervated, there always is a decrease of heart rate of vagal origin. However, even for this, rather large doses are needed. For example, if there is, in the clinical literature, the indication that 1 μg./kg. of LSD produced some increase in heart rate, I am convinced that that is not the effect of LSD, but rather it is a secondary reaction, i.e., a consequence of the modified psychic situation of the patient.

Hoagland: Dr. Cerletti, as I understand it, what you are concerned with is an attempt to find various empirical criteria of the effects of LSD on the autonomic nervous system or any other part of the organism that can be observed and measured.

Cerletti: Yes; that is right.

Hoagland: One of the major problems has been to produce something like a psychotic response in an animal, and this has been rather unsuccessful. Another approach which may be fruitful is to implant electrodes in the various parts of the brain of an animal to see whether responses can be evoked that are characteristic of some of the substances when they are injected. Dr. Marrazzi has done some of that work and later we may hear from him. We, in our laboratory, have also obtained some rather interesting data on electrical effects of the substances. I wonder if you have done anything of that sort in the Sandoz laboratory, that is, if you have done any electrical recordings from brains directly?

Cerletti: We have done electroencephalograms on rabbits, and these show that LSD has some effects. For example, we have observed a marked synchronization in the rabbit EEG after LSD. Or, if the rabbit is pretreated with chlorpromazine, the EEG changes produced by chlorpromazine can be completely blocked by LSD for a longer period of time (Figure 10).

Mirsky: I think the point brought up by Dr. Cerletti is very important, that is, the distinction he is trying to make between the effects which are secondary to the psychic responses—if that word is used here that way—and the effects which are primary to the drug itself. For the past year or so, we have been trying to find a method whereby the hypothalamus can be activated to secrete the antidiuretic hormone into the circulation. We find that a variety of stimuli can result in an increase in antidiuretic activity of the plasma. But nearly all these stimuli first produce

psychic effects which in turn may be responsible for the increase in the antidiuretic activity of the plasma. On the other hand, the injection of a dose of a bacterial pyrogen, pyromen, which produces a rise in the body temperature in about an hour, results in a marked increase in the antidiuretic activity of the plasma immediately after the injection and before any other discernible physiological or psychological changes occur. As the plasma antidiuretic activity diminishes, the serum 17-hydroxycorticosterones rise and, subsequently, the temperature also rises. Thus, it is possible to develop a technique which permits us to distinguish an effect which is primary, probably on the hypothalamus, and an effect which is secondary in that it is dependent upon the meaning of the stimulus which, in turn, can also induce physiological changes.

Quastel: Do you show that there was polyuria?

Cerletti: Urine incontinentia, and also polyuria.

Quastel: Was the urine measured?

Cerletti: No; it was not measured. Symptoms from the urinary tract are observed only after the large doses of LSD which, at the same time, provoke marked ataxia and even motor paralysis during the first 10 to 20 minutes after the injection.

Leake: Is there increased intestinal motility?

Cerletti: That is the symptom which we have seen only in unanesthetized animals treated with 100 or more μg./kg. If intestinal motility in the anesthetized animal is measured, LSD does not seem to interfere with it, but the anesthetized animal may not respond as well as the unanesthetized animal.

Abramson: Dr. Cerletti, I have noticed that in one out of five non-psychotic subjects that I have regularly studied this year, 25 μg./kg. of LSD will produce paroxysms of hyperpnea irregularly, but always in the same subject. It seems to be specific for the person. Would you interpret that for me, please?

Cerletti: I can only say that paroxysms of hyperpnea are a very typical symptom to be observed in animals. This hyperpnea does not occur in a fixed relation to the time of injection. Sometimes this symptom appears very early, but stops after a few minutes and may reappear several times. In between, the respiration may be normal, but often a very deep respiration accompanied by sighing is observed. It is difficult to predict what kind of respiratory change will be obtained, because this is very irregular, even with the same dose in the same animal.

Abramson: This coincides with the height of the psychic reaction in this individual.

Leake: Isn't that type of reaction a characteristic respiratory accompaniment of anxiety states?

Abramson: Yes; I think so.

Rinkel: I think so, too. We have also observed in our experiments the increase of anxiety.

Abramson: I would think that the explanation is psychological.

Leake: But then one has this to consider: There is part of a feedback —a sort of program where the primary effects, for example, on the autonomic system, leading to visceral reflexes (sensory reflexes) feed back and give the sensation of discomfort or anxiety, which sensation then leads to the hyperpnea.

Elmadjian: Is it known that these substances produced by the autonomic system will produce anxiety when injected? We know what, apparently, is produced by the autonomic system.

Leake: I was simply talking of the sensation which, in humans, we refer to as anxiety which results from a general sense of visceral discomfort and which can result from direct primary effect through the autonomic nervous system.

Elmadjian: Do those symptoms result when any of the compounds known are infused?

Leake: Yes.

Hoagland: They may also be evoked with placebos.

Elmadjian: I would like to say that our experience has been that that is not the case. We have infused epinephrine into a patient in doses ranging from 0.05 μg./kg./min. to 0.20 μg./kg./min. for 30 minutes in addition to a control saline infusion. The subject showed no differences in response in terms of anxiety to these procedures. With the largest dose, the individual fell asleep.

Fremont-Smith: Is this one individual or are you generalizing?

Elmadjian: This is one individual.

Fremont-Smith: But I don't think the fact that one individual did not respond is any reason for saying that twenty other individuals may not.

Elmadjian: That is what I said. I described experiments on one individual.

Fremont-Smith: But you also started off by saying this isn't so, which is a generalization.

Elmadjian: I was giving you an example which questioned the generalization that epinephrine is implicated directly in the symptom of anxiety. Another example which we can submit from our experience is the case of the adrenalectomized patient who was infused with 0.10 and 0.20 μg./kg./min. and who showed no symptoms of anxiety.

Leake: But let me point out here that the feeling of anxiety usually comes after a feeling of nausea, which is associated with increased intestinal activity. Epinephrine of course, quiets that.

Kety: I must hasten to add our own generalizations to this subject. The experience of some of my associates with epinephrine is quite different from what we have just heard. In duplicate series of about 10 subjects each, some of whom were given epinephrine and some of whom were given norepinephrine intravenously over a period of about 20 minutes in sufficient dosage to raise and maintain the blood pressure about 20 per cent above normal, there was a striking difference in the subjective symptoms of the two groups. In practically every instance those who were given epinephrine spontaneously reported anxiety or apprehensiveness, while those who were given norepinephrine showed none of these symptoms. On that basis, it was inferred that there was a difference between these drugs, and that epinephrine showed a rather consistent tendency to produce anxiety (16).

Gerard: In our work, subjects infused with from 2 to 4 μg./kg./min. of epinephrine showed no vasomotor changes that could be measured, and they did show very vigorous changes in the psychological state which can be accurately described as anxiety. Doses of norepinephrine of some ten times the magnitude which produced very violent vascular changes, even to the extent of producing pains in the abdomen, did not produce these anxiety states.

Rinkel: May I call attention to the work that was done at the Psychopathic Hospital in Boston: Funkenstein and his group (17, 18, 19) linked norepinephrine to extrapunitive and epinephrine to intrapunitive types of people. The observation had been made that those who act out their anger are characterized by an abundant release of norepinephrine-like substances and those who do not act out their anger but become depressed are characterized by an abundant release of epinephrine-like substances. There seems to be some similarity to what Dr. Gerard observed.

Fremont-Smith: You mean if it is internalized, the subject might also have a greater sense of anxiety than if he acted it out?

Rinkel: I believe that is true. In fact we use the Funkenstein tests (20, 21) to determine the choice of specific treatments such as electric shock treatment or insulin treatment.

Elmadjian: If these comments are consistent with each other, then it would be suggested that severe anxiety symptoms be treated with double adrenalectomy, since the adrenal medulla appears to be the principal source of excreted epinephrine.

Kety: That would require one other generalization; namely, that clinical anxiety is caused by the liberation of epinephrine from the adrenal medulla.

Cerletti: When we first observed the effects of LSD on dogs, about 7

or 8 years ago, we were quite satisfied with the term "anxiety" which seemed to us very suitable for characterizing the behavior of the animal under the influence of LSD. Pet animals clearly show symptoms of insecurity and of increased anxiety after administration of LSD. They seek protection from the investigator. Toward a stranger they may be more aggressive than normally. Later on, in our work we were disappointed to find that several other lysergic acid derivatives, which have nothing of an LSD-like effect in human subjects, produced the same behavior in dogs, because they also produced similar vegetative disturbances. We have, therefore, the impression that this "anxiety" behavior is only a secondary reaction of the dog to his bad feeling. He suffers from nausea, sometimes even vomiting, and he really looks sick so, because of such effects, the animal probably behaves in this anxiety-like manner. I don't think that we can say that LSD produces anxiety, but rather that the strong vegetative effects of LSD are mainly responsible for it. In the same way also other compounds, such as the *dimethylamide* of lysergic acid, provoke the same reaction in the animal.

Hoch: I might add that if varying dosages are given, vegetative symptoms can be produced without psychic manifestations. If psychic manifestations produced by *d*-lysergic acid diethylamide are blocked with chlorpromazine, these manifestations remain for a while. Therefore, the relationship between psychic manifestations and vegetative symptoms is not so simple. The two are related to each other and the connection is probably an intimate one, but no absolute relationship exists between vegetative manifestations and psychic manifestations. At least we have found it so in our experiments. This is somewhat disturbing because it would be much better if a complete correlation would exist between psychic manifestations and vegetative symptoms at the same time.

Fremont-Smith: We shouldn't be disturbed by human facts.

Abramson: Dr. Hoch, did you say that chlorpromazine eliminates the psychic responses under LSD?

Hoch: Yes, if a high enough dosage is used.

Abramson: In my experiments I used only 50 mg. of chlorpromazine by mouth, before, at the same time with, and after LSD-25. The effect depended upon the time of administration. When given at the same time, potentiation of the LSD reaction apparently occurred in at least two of five subjects.

Table I shows the number of positive responses and these are summed up from the questionnaire on page 224. The effects on the other variables are given in detail. It is believed that although chlorpromazine, in the doses administered, affects the LSD reaction, the effect is best

described by the statement that the subjects are "LSD inside and chlorpromazine outside."

Hoch: If a rather high dosage of *d*-lysergic acid diethylamide is used and 50 mg. of chlorpromazine are given orally to counteract its action, there will be no success.

Abramson: That would account for the difference, then.

Hoch: Yes. We studied this at different dosage levels, and I think that the dosages are very important. You can probably arrive at many conclusions if you use only one dosage.

Fremont-Smith: What dosage did you find necessary?

Hoch: We used a minimum of 50 mg. of chlorpromazine given intravenously, and when 50 mg. are injected intravenously, the psychic manifestations of LSD can be abolished. Sometimes nausea appeared or the patient was uncomfortable. However, anxiety manifestations and the more involved psychic symptoms did not appear, or if they did, only in a mitigated form.

Beecher: The blood pressure is very low, though, isn't it, when 50 mg. of chlorpromazine are given intravenously?

Hoch: It is also interesting that that is not the case. In some individuals when chlorpromazine is given the blood pressure goes down; in others the blood pressure is hardly affected.

Beecher: With a dose of 50 mg. given intravenously?

Hoch: Yes, with a dose of 50 mg. given intravenously.

Fremont-Smith: In what volume, and over how long a period of time?

Hoch: It was injected probably in about 2 or 3 minutes. In some patients there is a reduction of blood pressure of 10 or 20 mm. Hg while in other patients, there is none.

Beecher: Is blood pressure related to whether or not the chlorpromazine blocks the LSD effect?

Hoch: I do not know that. We could not work out any correlation between alteration of the blood pressure or alteration of the pulse or the changes in the pupils, and the psychic manifestations.

Sherwood: I still find it difficult to distinguish between the primary, "psychic" "mental" effects and the "peripheral" ones. I remember one case of a very disturbed paranoid schizophrenic, who was given 25 mg. of chlorpromazine by mouth. I am sure, although I cannot prove it, that the patient was still disturbed. The only thing that happened was, every time she tried to rise from her bed, she fainted.

Fremont-Smith: I am not quite sure what the implications are.

Sherwood: The implications are that the distinction is so difficult that I would very much hesitate to allege that one of them is the primary effect and the other is the secondary one.

Fremont-Smith: Isn't that partly because you have used the most difficult patient from which to obtain a good report? It seems to me that it is a very difficult distinction, but the suggestions that were made a little while ago with respect to differential doses do justify the attempt and, probably to some extent, promise success in making a distinction between one which is predominantly a direct effect on the psychic aspects of the central nervous system and primary and another which is predominantly on a peripheral organ and secondary, or vice versa.

Sherwood: Yes, but isn't the whole a problem of circulation?

Fremont-Smith: Of course it is, but I do not think this should make us deny in any way the capacity for making some differentiation, or trying to.

Sherwood: The question of differential dosages again is a very difficult one, since we have heard from all speakers that there is such a tremendous individual variability in the response to the drug.

Mirsky: I should like to support what Dr. Hoch said, that a dissociation can be made between those responses which we can regard as neurologic and those responses which we can regard as psychologic. In the psychophysiological preparation, one can demonstrate very well what I would like, for the sake of convenience, to call the suspension of anxiety induced by chlorpromazine.

Sherwood: But isn't any one function of any one organ, even a single muscle fiber, continuously controlled?

Mirsky: We are talking about different levels of organization.

Sherwood: But how can they be dissociated?

Mirsky: The dissociation can be demonstrated, as Dr. Hoch does in the patient and we do with monkeys, where one effect of a drug is still apparent, and another effect is not.

Sherwood: As to that, I might well reply that one is compensated for and the other is not.

Marrazzi: I want to express, with Dr. Cerletti, the difficulty of eliciting any effect in the nature of anxiety or mood changes in animals. We have given up to enormous doses of LSD and mescaline to six laboratory-trained and acclimated monkeys, and we have, with the aid of psychologists and the whole laboratory staff, been unable to detect any anxiety, aggressiveness, or mood changes, although there were autonomic manifestations.

Seevers: I would like to ask Dr. Hoch whether he would accept the idea that if vegetative manifestations become very pronounced they may actually interfere with the psychological manifestations of the drug, in other words, might it be said that they mutually antagonize each other? That is the case, for instance, with morphine. In studying analgesia

with morphine, it will be found that in a group of individuals who become nauseated and vomit, the analgesic responses are very poor. In the same group, if the dose is kept low enough to maintain only an analgesic response, it can be obtained; so, in the final analysis, we are talking about a dose-response effect.

We have done some work with monkeys which have developed great anxiety in response to 4-hour injections of the potent analgesics. The doses are given without interruption for several months, so that the animals become conditioned to a needle response. This group of animals responds to LSD in the doses we have used, which are admittedly large, 25 μg./kg., by a *suppression* of anxiety rather than by an increase. However, a relatively small vegetative response is shown. This suppression of anxiety is in contrast to what is generally seen with the ordinary clinical doses of LSD-25. With still larger doses, where vegetative phenomena occur, such as nausea, vomiting, and the like, this anxiety carries on in the original pattern.

Hoch: We found that there is no reliable relationship, between the two. I do not want to deny, of course, that somewhere there is a connection. I am quite sure that if something which produces a great deal of vegetative stimulation is used, then, of course, it will spill over and will most likely express itself also in some psychic manifestation. But we found that the vegetative stimulation is possible without producing the psychic manifestations. On the other hand, we never found the reverse; in other words, we never found that the patient had marked psychic manifestations without any vegetative alteration.

Abramson: I think that we should be a little more strict about our use of the word, "anxiety," under the influence of LSD-25. It has been our experience that the anxiety of certain subjects will be decreased, for example, in discussing interpersonal relationships, but will be increased if the individuals have to stand alone on the cafeteria line. The anxiety is therefore increased and decreased almost simultaneously, depending on the specific situation to which the subject or patient is exposed.

Hoch: I think what Dr. Abramson has said is very important and I would add that this should, of course, be broken down into its components. One of the most interesting things is that LSD does not produce, uniformly, an alteration of all psychic functions; it alters certain psychic functions but does not alter others. I may go even further and say that in the same individual, with repeated experiments, some basic pattern which is similar can be found. Nevertheless, there could be marked changes, and in a second experiment or in a third experiment, some psychic manifestations might come to the fore which did not come to the fore in the first.

TABLE I

Eight Experiments with Controls on the Effect of Chlorpromazine on the LSD-25 Responses of a Normal Adult Male.

Hours After Ingestion of Drug		½	1½	2½	3½	4½	later
Subj. L. W.	No. of positive responses	4	8	9	8		
Date 6/19/54	Motor behavior		poor	poor	poor		
Drug	Control	poor	poor	poor			
LSD-25	Consciousness	poor	poor	poor			
25 μg.	Concentration	poor	poor				
	Mood	euph.	euph.	euph.			
	Attitude						
	Orientation						
	Memory						
	Remarks	Conscious of people pronouncing words.					
Subj. L. W.	No. of positive responses						
Date 9/24/54	Motor behavior						
Drug	Control			N	O		
L-isomer	Consciousness						
100 μg.	Concentration						
	Mood						
	Attitude	R	E	A C	T	I O	N
	Orientation						
	Memory						
	Remarks						
Subj. L. W.	No. of positive responses	2	7	9	6		
Date 10/1/54	Motor behavior		poor	poor	poor		
Drug	Control		poor	poor	poor		
LSD-25	Consciousness						
25 μg.	Concentration		conf.	conf.	conf.		
	Mood	depr.	euph.	euph.	euph.		
L-isomer	Attitude		introsp.	introsp.	introsp.		
100 μg. ¼ hr. before	Orientation		poor	poor	poor		
LSD-25	Memory						
	Remarks						
Subj. L. W.	No. of positive responses	8	11	9			
Date 10/8/54	Motor behavior		poor	poor			
Drug	Control		poor	poor			
LSD-25	Consciousness			poor			
25 μg.	Concentration		conf.	conf.			
	Mood		euph.	euph.			
Chlorpromazine	Attitude			introsp.			
25 mg. ½ hr. before	Orientation		poor	poor			
LSD-25	Memory						
	Remarks	Muscles trembling. At 2 hrs. subject said he would not be willing to drive car in traffic, only on open road.					

Subj. L. W.	No. of positive responses	3	16	12	10		
Date 10/11/54	Motor behavior	poor	poor	poor	poor		
Drug	Control	poor	poor	poor	poor		
LSD-25	Consciousness				poor		
50 μg.	Concentration		poor	poor	poor		
	Mood	euph.	euph.		depr.		
Chlorpromazine	Attitude				poor		
50 mg. ½ hr. before	Orientation	poor	poor				
LSD-25	Memory				poor		
	Remarks	Subject estimated 40 μg. dose of LSD-25 at, from 2 to 3 hrs.					

Subj. L. W.	No. of positive responses	5	15	13	6		
Date 10/15/54	Motor behavior	poor	poor	poor	poor		
Drug	Control		poor	poor	poor		
LSD-25	Consciousness		poor	poor	poor		
25 μg.	Concentration	poor	poor	poor	poor		
	Mood		apath.	depr.	depr.		
Chlorpromazine	Attitude	introsp.	introsp.	introsp.	conf.		
50 mg. ½ hr. before	Orientation						
LSD-25	Memory						
	Remarks						

Subj. L. W.	No. of positive responses	1	14	12	12		
Date 10/22/54	Motor behavior		poor	poor	poor		
Drug	Control		poor	poor	poor		
LSD-25	Consciousness		drowsy	drowsy	drowsy		
Zero dose	Concentration			poor	poor		
	Mood		depr.	depr.	depr.		
Chlorpromazine	Attitude		introsp.	introsp.	introsp.		
50 mg. ½ hr. before	Orientation		poor	poor	poor		
LSD-25	Memory						
	Remarks	Subject described reaction as "Not LSD-25." Aware of heartbeat and very drowsy throughout.					

Subj. L. W.	No. of positive responses	13	14	11	10		
Date 11/12/54	Motor behavior	poor	poor	poor	poor		
Drug	Control	poor	poor				
LSD-25	Consciousness	poor	poor				
50 μg.	Concentration	poor	poor	poor	poor		
	Mood	euph.	euph.				
Chlorpromazine	Attitude		poor	poor			
50 mg. administered	Orientation	poor					
simultaneously with	Memory						
LSD-25	Remarks	At 1 hr. nervousness. tremors, dizziness. At 1½ hrs. subject estimated 50 μg. dose of LSD-25, and at 2½ hrs. 25 μg. dose.					

It is very difficult to say how much of this is purely dynamic constellation. It depends, of course, on psychodynamic factors, which are influenced differently by the drug at different times, because the psychodynamic constellations are also not fully static. But I fully agree that the constellation is very important in setting the question.

Sherwood: Has anyone made studies on conditioning and extinction of responses under the influence of LSD and without it?

Hoch: In animals, yes.

Hoagland: Dr. Mirsky, you have an answer to that.

Mirsky: Yes; Dr. Robert Miller and Dr. J. V. Murphy (22) have been using the avoidance-conditioning paradigm in our laboratories. Monkeys are trained to avoid a painful stimulus that follows a buzzer by pressing a bar, which is a very simple type of avoidance procedure. Long after the unconditioned stimulus (the painful stimulus) has been discontinued, the animals continue to press the bar when exposed to the conditioned stimulus (the buzzer). That brings up the question raised by both Dr. Abramson and Dr. Hoch. The response of the monkeys does seem to depend on the monkeys and their previous experience. We are planning to test monkeys that were born in the laboratory and who have had different types of experience. We hope to study the effect of previous experiences on extinction of the avoidance response.

Elmadjian: What species were those monkeys?

Mirsky: We have used *Macaque mulatta* monkeys, and we have observed also that during the period of conditioning, there is a concomitant increase in spontaneous responses.

Sherwood: What is a spontaneous response?

Mirsky: The animals press the bar without exposure to the conditioned stimulus.

Sherwood: So their response is spontaneous behavior, not a response?

Mirsky: By "response," we have reference to the pressing of the bar at the time.

Fremont-Smith: This is spontaneous pressing of the bar?

Mirsky: Yes, spontaneous pressing of the bar, without the buzzer coming on.

Hoagland: Is this a sort of Skinner technique, where they get food?

Mirsky: No; this is avoidance of pain.

Elmadjian: The Miller technique.

Mirsky: Yes; it is similar in principle to the Miller technique. Studies thus far reveal that if, after the spontaneous responses have become constant, one kidney is removed, a marked decrease in the number of spontaneous responses occurs immediately after the operation. This decrease occurs also in animals exposed to any severely noxious stimulus.

Thereafter there is a return to the previous incidence in the spontaneous responses. The spontaneous responses in the controls, from which some perirenal fat was removed, return to the preoperative rate while the spontaneous responses of the animals from which one kidney was removed show a progressive increase above the preoperative level. This increase lasts about 20 days.

Sherwood: Is that response or spontaneous depressing of the bar?

Mirsky: This is depressing of the bar, not in response to the conditioning stimulus. The conditioned response occurs only on exposure to the buzzer.

Fremont-Smith: What happens to that?

Mirsky: That doesn't change.

Sherwood: But that is the point.

Mirsky: The conditioned response is a response to a conditioned stimulus, an external signal. The spontaneous response is a response to an internal stimulus, namely, the removal of the kidney. Although there are no known evidences of biochemical changes after the removal of one kidney, there must be such changes since the remaining kidney does grow. Consequently, it may be inferred that a biochemical change can induce a change in psychological behavior.

Osmond: Dr. Mirsky, I think I am being a bit obtuse here, but this is the kidney with the attached suprarenal gland?

Mirsky: No; we remove one kidney only and do not touch the suprarenals.

Osmond: Can you do this?

Mirsky: Yes; and the animals remain in good condition.

Osmond: This in no way affects the adrenal?

Mirsky: Perhaps temporarily.

Beecher: The anesthesia alone would do this.

Mirsky: Our controls in which laparotomies are performed take care of that. As I have said already, the spontaneous responses drop off in all the animals but the controls return to the preoperative level while the unilateral nephrectomized animals go beyond the preoperative rate.

What I would like to talk about now is the conditioned response. As I have mentioned, the conditioned response persists in most monkeys long after the unconditioned stimulus has been stopped. When such monkeys are given an injection of chlorpromazine, there is an immediate cessation of conditioned responses. When the chlorpromazine injections are stopped, the conditioned avoidance responses come right back.

Using this kind of preparation, one can dissociate between that which is psychological, i.e., the persistence of the conditioned avoidance re-

sponse, and that which is neurological, i.e., the response to the unconditioned stimulus which is not utilized symbolically by the animal.

Leake: With that dosage of chlorpromazine which will suspend the conditioned response, the response to the pain itself will not be suspended?

Mirsky: No. The dosage that we have used in the monkey is 1 mg. of chlorpromazine given subcutaneously.

Marrazzi: Are those monkeys depressed?

Mirsky: What do you mean by "depressed"?

Marrazzi: Do they act as though they had had a sedative?

Mirsky: You mean on the physiological level? No; the effects discussed will occur with no apparent sedation.

Sherwood: Are there any vegetative signs?

Mirsky: With the chlorpromazine?

Sherwood: Yes, when they extinguish.

Mirsky: With chlorpromazine we have not noted any vegetative signs. In rats somewhat similar results are obtained. When rats are conditioned to avoid a painful stimulus, the conditioned avoidance response will be extinguished in from one to several weeks after the cessation of the unconditioned stimulus. If chlorpromazine is administered, the extinction is immediate. When the effect of the drug has worn off, however, the avoidance response occurs just as if no drug had been administered and the animals then go on to extinguish like the normal group. Thus, the drug produces a "suspension" of anxiety or fear.

Fremont-Smith: But at least a suspension of the conditioned reflex.

Mirsky: By definition, the avoidance response is a response to anxiety.

Fremont-Smith: Surely you have suspended the conditioned reflex, and you may have suspended a psychological response.

Mirsky: This is a psychological response by our definition of psychological response.

Fremont-Smith: This could be questioned, because it is quite conceivable that the psychological response is composed of several integrated or interrelated components, some of which are neurological.

Mirsky: Let me put it this way: There cannot be psychological responses that are not neurological.

Fremont-Smith: I agree with you, but there are also interrelations between what you yourself called neurological as distinguished from what you called psychological.

Mirsky: Yes, and I define this as psychic.

Fremont-Smith: I accept your definition, and, it seems to me quite possible that your psychological response may be a complex one and that it may have been cut across, shall we say, by chlorpromazine at a

neurological level. It may be a much more complex thing than just to say you have suppressed a psychological response *in toto.*

Mirsky: When we talk about psychological responses, we are not talking about something that occurs in a vacuum. Of course the drug must act on some neurological process.

Fremont-Smith: But it is not adequately defined, so I think it could still be challenged as being psychological.

Mirsky: I made the definition so that you could change it. In the experiments under discussion, we are talking about a response to the meaning of a situation.

Kety: I don't believe in that. On the basis of your data, why don't you merely say this is a behavioral change?

Hoagland: Dr. Rodnick, as a psychologist, what do you say about this?

Rodnick: It is not necessary for experimenters to be too concerned with concepts which cannot be manipulated by the experiment. The experiment which Dr. Mirsky has described at least attempts to define his variables in terms of certain manipulations. Within the context of this discussion, even before the conditions producing fear can be talked about (that is at least one way of thinking about anxiety), it is legitimate to experiment with avoidance responses. This is what Miller (23, 24) tried to do. In manipulating the conditions making for avoidance behavior, it is necessary to utilize control situations in order to be satisfied that such behavior occurred only in avoidance-producing situations and did not occur in any of the control situations.

Mirsky: The literature (25) will establish that the same animals behave differently when studied in other social situations.

Rodnick: But at least that would be an attempt to define the fear as occurring only in some situations. One could then attempt to determine the conditions under which fear did not occur.

Rinkel: I think that Dr. Mirsky made a very important statement. Human beings have more fear when they have taken LSD individually than when they have taken it with a group.

Marrazzi: Dr. Mirsky, under chlorpromazine the monkeys failed to respond; were they buzzed?

Mirsky: Yes.

Seevers: What was the dose?

Mirsky: One mg. per animal injected subcutaneously.

Leake: The difference in response of individuals alone or in a group to drugs of this type is a characteristic phenomenon learned over many centuries. Because of the previous conditioning of a group to religious ceremonies, during the ritualistic use of alcohol or peyotl or things of

that sort, the responses evoked in the group may be very different from those evoked in an individual alone.

Osmond: There is another factor too; group experiments have not been carried through over a wide range of dosage, have they? We don't know, therefore, what the groups at 300 μg. would do, or what effect that would have. There is still no adequate classification of the individual variation, for example, in the coloring or things of that sort; so there is an enormous number of variables of a different nature to work with in the human experiments.

Hoch: That is quite right.

Cerletti: I will continue with just a few illustrations of circulatory effects of LSD and then go on to a very important topic, the antagonists of LSD. A third topic will be the distribution of LSD and its fate in the body.

A typical circulatory effect of LSD is obtained only with rather large doses. This effect consists mainly of a fall in blood pressure and a decrease in heart rate from about 170 to a range between 130 and 140 beats per minute. In this case (Figure 11) the limb volume was also recorded and it remained practically unchanged in spite of the blood pressure fall. A dose of 40 μg./kg. was given to this cat intravenously, and besides the effects described a depression of respiration was also observed.

Cleghorn: It does not look to me as though that is a resting heart rate in the cat, at 170. Is it?

Cerletti: The heart rate in the cat depends very much on the kind of anesthesia used. This is a cat anesthetized with a mixture of urethane and chloralose, and under these conditions there are usually rather high heart rates (Figure 12). In this example the heart rate is approximately from 170 to 180. Here, with 80 μg./kg. given intravenously, again a drop in blood pressure and a very marked decrease of heart rate occurs. The cardiac rhythm is not disturbed, but the respiratory volume shows a sharp fall. To show that this type of circulatory and respiratory reaction is not a specific effect of LSD, but an effect which LSD shares with many other LSD-similar compounds, a comparison can be made with Figure 13 where LPD, the pyrrolidid of lysergic acid, produces a reaction practically identical with that seen in the preceding figure, a similar decrease of blood pressure and heart rate, and also a decrease of respiration.

Figure 7 shows a better comparison between some results obtained with this last compound, LPD, and with LSD-25. LPD is more effective with respect to the hypotensive and bradycardic effect. A dose of 10 μg./kg. gives this response regularly in cats, whereas with LSD doses

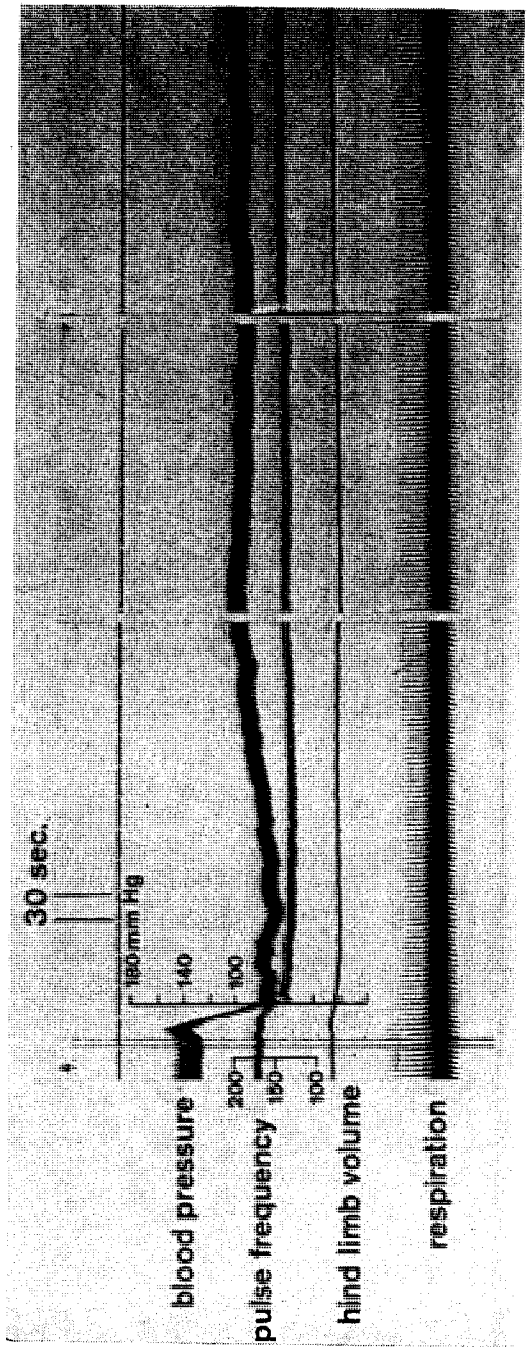


FIGURE 11. Hypotensive and bradycardic effects of LSD in the cat (40 μg./kg. I.V.).

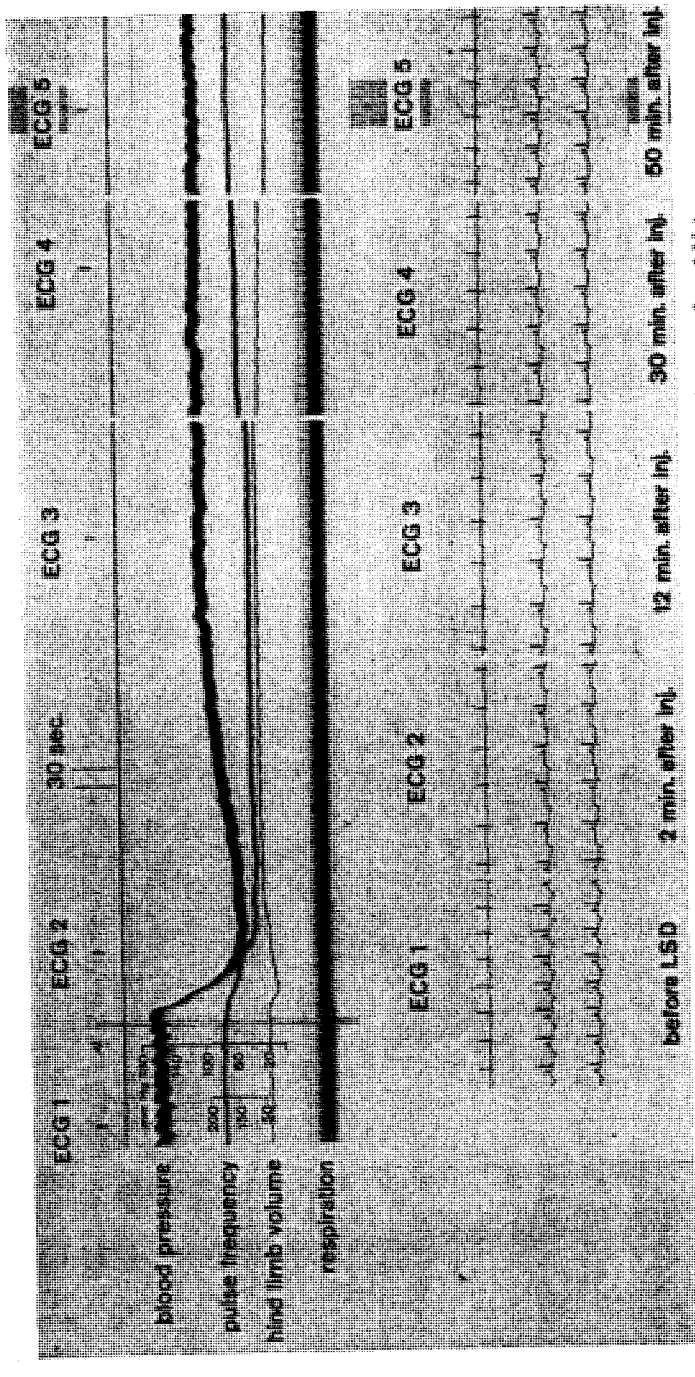


FIGURE 12. Hypotensive and bradycardic effects of LSD in the cat (80 μg./kg. I.V.).

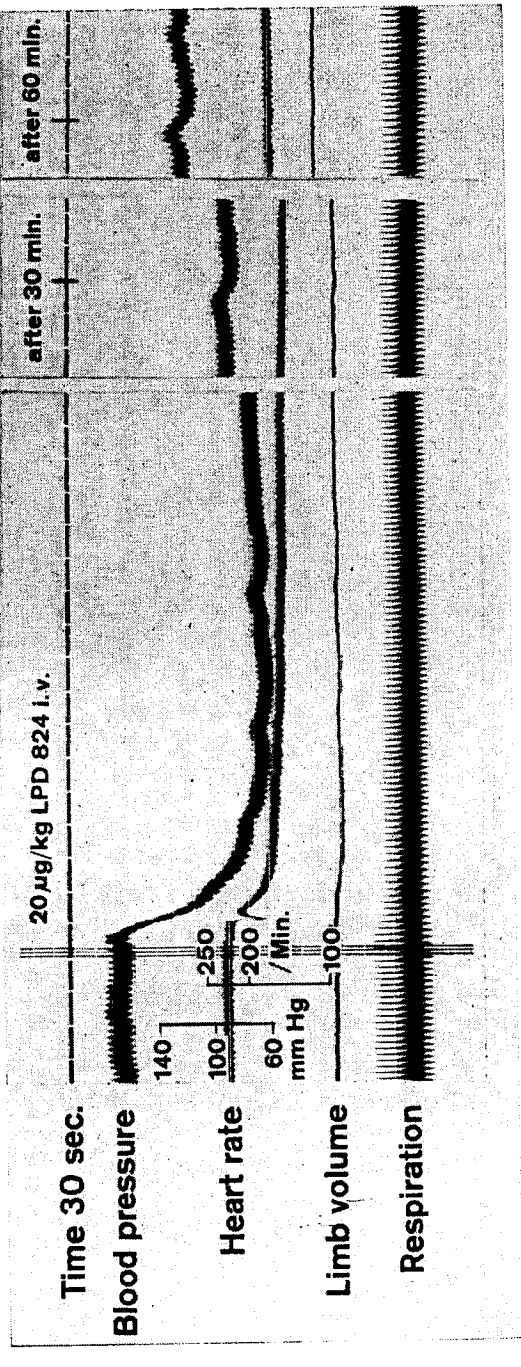


FIGURE 13. Circulatory effect of LPD-824 in the cat (20 µg./kg. I.V.).

about four or five times higher are needed in order to induce bradycardia and a similar fall in blood pressure.

Concerning activity in man, as far as we know from a few limited experiments, there are no psychic alterations at all after a dose of several hundred micrograms of LPD. So there is at least a ratio of more than 10 to 1 compared with LSD. The serotonin antagonistic effect of LPD is even twenty times less than with LSD, if the two drugs are comparatively tested on the isolated rat uterus. I would not say that the circulatory effects of LSD can help us very much in understanding some of the specific action of LSD in man, because these effects are observed to be more pronounced after administration of another compound which, to our knowledge, is ineffective in man as a psychosis-producing compound.

Abramson: Are there any other effects in man besides the absence of a psychosis? Are there any positive effects?

Cerletti: Yes. LPD was used in a preliminary clinical trial mainly because of its hypotensive action which in animals is stronger, for example, than the effect of hydrogenated ergot alkaloids of the polypeptide type. But in man, LPD showed a too strong nauseating and emetic action, and so the effect on the blood pressure could not be studied.

Abramson: Did this nausea and hypotension occur after a dose of 10 μg./kg.?

Cerletti: The nausea in man may be evident with a dose of about 3 to 5 μg./kg.

Abramson: It was all given by mouth, I assume?

Cerletti: No; it was also given by intravenous administration. The highest intravenous dose was 300 μg.

Mirsky: Dr. Cerletti, you spoke of the pyrogenic effects in various species. Did you make any studies of this in man using LSD?

Cerletti: No; we have no experience concerning temperature effects of LSD in man. I wonder whether this has been controlled. I could imagine that there would also be disturbance of thermoregulation in man with LSD.

Hoagland: I don't know of any.

Rinkel: There is a publication (26) on the temperature effect of LSD.

Fremont-Smith: Are there any publications on skin temperature in man?

Rinkel: I don't know. At the Boston Psychopathic Hospital, we have done some skin temperature measurements, but we haven't published the work as yet. Alberto DiMascio and Elsie Suter, of our research division, have taken some temperature measurements by placing a thermocouple on the surface of the index finger of the right hand of a

subject who had received LSD. They recorded on the polygraph, in a few instances, the skin temperature with, of course, appropriate controls. Some preliminary findings indicated that about 2 hours after LSD was administered, the temperature dropped somewhat, as compared to the control base in the same subject, but it returned to the base line by the end of the day. What this means has not yet been analyzed.

Mirsky: The reason I asked the question is that there is an interesting difference between species, as brought out previously in this discussion, and your main approach is to use dosages which are comparable in animals to those employed in man, so I wondered how the temperature changes correlate with changes in other species.

Cerletti: The results of Dille and Horita's (27) work on rabbits are the same as ours, and they also mention that they observed temperature increase in the dog and in the cat after administration of LSD, but experiments on man were not done.

Fremont-Smith: We also must remember that it is not only a question of species but also of anesthesia. There is no reason at all to assume that a cat's response under one anesthesia is in any way a characteristic response of organisms to drugs, because with a different anesthesia, there may be a reversal; for instance, the influence or the effect, of histamine on spinal fluid pressure, which regularly rises. Histamine with barbiturates causes a rise in the spinal fluid pressure of the cat. With ether it causes a fall in the spinal fluid pressure; in man, histamine without anesthesia causes a fall in spinal fluid pressure.

Hoagland: I should like to say in this connection that the electrocorticogram and hypothalamogram in the rat are both modified in the same way by LSD but are suppressed in the animal anesthetized with pentothal and show increased activity in the unanesthetized rat. LSD seems to have little effect on these electrical activities in rats anesthetized with ether.

Mirsky: I would like to go back to my question, which had nothing to do with anesthesia, but which concerned the temperature changes in animals receiving LSD-25, without anesthesia.

Cerletti: With anesthesia, there is no more temperature increase after LSD. All information that I gave previously was the result of experiments in the unanesthetized animal. As soon as the rabbit is anesthetized, there is no more increase; if pentothal is injected into the rabbit at the height of the LSD reaction, a rapid drop of temperature results.

Magoun: Dr. Cerletti, I wonder if you would tell us what you think is the significance of the discussion that we are having. Does our discussion show that these autonomic effects can be eliminated as being

related to the psychic influences of this material, in that the same autonomic effects are induced by other materials that lack a psychic influence?

Cerletti: Yes.

Magoun: Or do you feel that these changes in the autonomic sphere are of value in indicating the general area of the brain where LSD has its action and are, hence, of value in getting us into the areas of central action of LSD? I wonder if we are just beginning again an elaborate analysis of what the peripheral effects of this material are. Could we orient ourselves briefly?

Cerletti: I wanted to provoke a discussion on this point, because we have seen that some absolutely identical effects as obtained with LSD can also be produced by other compounds which, however, are not effective in the sense of a psychotic-active substance. Therefore, the question comes up whether these two groups of LSD effects, the vegetative and psychic symptoms, are interrelated in some way, or whether they are distinct from each other. This problem arises again when we discuss the distribution and fate of LSD in the body. LSD disappears quite rapidly from the blood and tissues, and is eliminated in large quantities by the bile secretion in the gastrointestinal tract.* From these experiments it would seem that most of the LSD is already inactivated at the moment when the psychic effects on man are at the maximum, whereas the vegetative symptoms seem to be more related to the presence of LSD.

Hoagland: The evidence clearly points to the view that the peripheral autonomic nervous system effects have little to do with the central problem, as far as they can be determined. There may be interrelations because of the effect on the hypothalamus, but in this type of experiment things that are central cannot be distinguished from those that are peripheral. It seems to me that is clearly established.

Quastel: Does serotonin act antagonistically to all reactions of LSD?

Cerletti: That is not absolutely clear. LSD is very active against serotonin.

Quastel: But not the other way around?

Cerletti: I will show a simple example of an antagonism of serotonin against LSD, but, to my knowledge, that is the first clear example which proves that this kind of antagonism is possible.

Perhaps, to start with this question of serotonin which, for the pharmacologist, again has many different aspects, it might be useful to point out that we must begin with the periphery and then try to climb up to higher levels of organization. For studying the antagonism of LSD against serotonin, we can rely upon several clear and relatively simple

*In animals. Data in man are not available.

methods. A very sensitive preparation for testing the serotonin inhibition by LSD is the isolated uterus of the rat, pretreated with estrogen (Figure 14). The effect that serotonin has of stimulating smooth muscle in this particular organ can be blocked by extremely small quantities of LSD. Serotonin is added every 10 minutes, and at the point of the arrow (Figure 14) LSD is put in the bath. With a concentration of 3×10^{-9} a partial, and with 6×10^{-9} a practically complete, block of serotonin is obtained after some 20 to 30 minutes. It is typical of LSD that the maximal effect is not obtained instantly, but only after a certain length of time. Later on, the serotonin effect slowly recovers, and the reaction comes back to the original size. This is an easy way to show the antagonism of LSD against serotonin. We know today that several antagonists against serotonin act very well on such isolated preparations, but many of them are practically ineffective as soon as they are used in the intact animal in showing a similar antagonism. There is quite some evidence, however, that LSD may also act on the intact animal as a blocking agent against serotonin.

This test of serotonin was also very helpful in tracing LSD in the body before a labeled LSD was at our disposal. Extracts of blood and organs of animals which had received an injection of LSD are very strongly antagonistic to serotonin, and, as far as we know, this is caused by the presence of LSD. Similar extracts from control animals are inactive, but by adding LSD to such extracts, an estimate could be made of the quantity of LSD present in the organs from LSD-treated animals. We have completed these data by using an LSD labeled with C^{14} in the side chain.

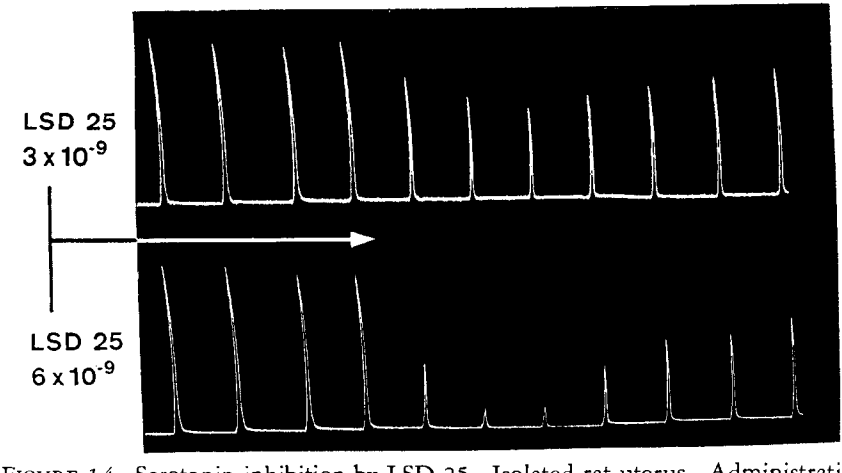


FIGURE 14. Serotonin-inhibition by LSD-25. Isolated rat uterus. Administration of serotonin (4×10^{-8}) every 10 minutes.

For serotonin and LSD, we have developed in our laboratory another test which has also proved very useful. Since serotonin is a substance also present on the thrombocytes of the blood and mainly responsible for the vasoconstrictor properties of the blood serum, we wanted a preparation for studying the antiserotonin potency of lysergic acid derivatives on the smooth muscles of the vascular wall. In the isolated perfused rabbit ear, for example, this can be done, but there is the disadvantage that these vessels are easily constricted by LSD itself. Therefore we tried other preparations and found the perfused rat kidney most useful. As shown in Figure 15, 2 μg. of serotonin produce a vasoconstriction which is diminished after adding 1 μg. of LSD, and in a second example even 10 μg. of serotonin are inhibited by 1 μg. of LSD. The

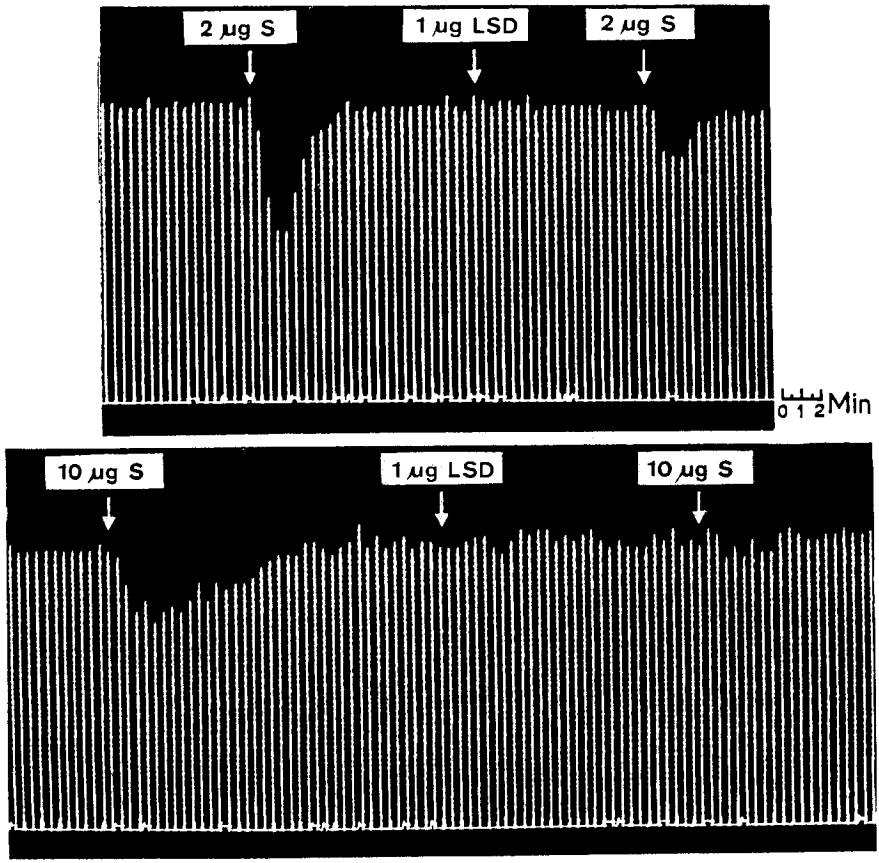


FIGURE 15. Effect of serotonin (S) and LSD-25 on the vessels of isolated perfused rat kidney.

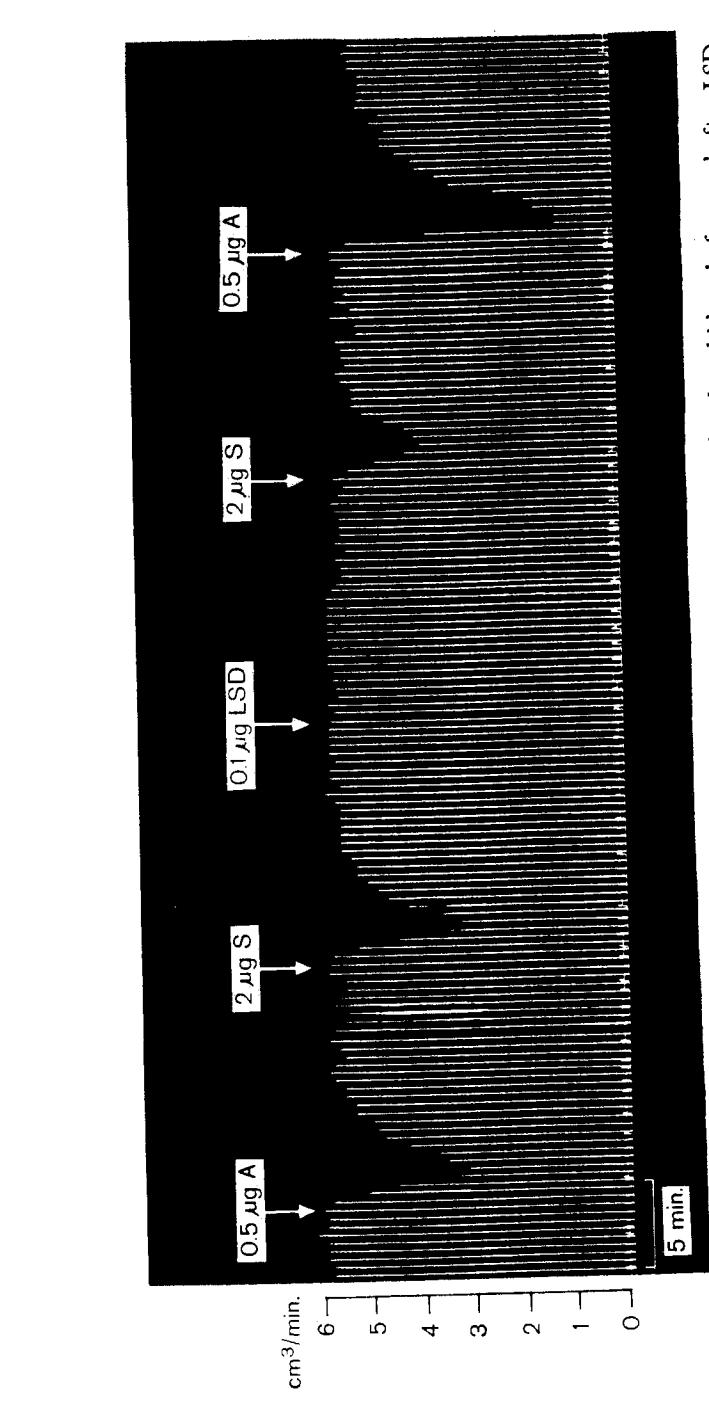


FIGURE 16. Effect of epinephrine (A) and serotonin (S) on the vessels of isolated perfused rat kidney before and after LSD.

recording system is a drop counter that writes every 30 seconds on an ordinate which is directly proportional to the number of drops during this time. The same preparation is also suitable to show that LSD is not an adrenergic blocking drug. Even in very large doses, LSD does not influence the action of epinephrine or norepinephrine. It seems important to mention this, because, since ergotoxin and ergotamine have been the prototypes of the adrenergic blocking drugs, there are some who think that all derivatives from ergot or from lysergic acid must have something to do with adrenergic blockade. For LSD, this is absolutely not the case. Even after extremely high doses of LSD, epinephrine effects are not inhibited. The contrary is observed, i.e., sensitization against epinephrine in LSD-treated animals. And also in isolated organs, such as this kidney preparation, the action of epinephrine is enhanced after LSD (Figure 16). Whereas the vasoconstriction caused by serotonin is diminished after LSD, the similar epinephrine effect is increased, and this sharp difference between the influence of LSD on serotonin and on epinephrine effects is also true for the intact animal.

Rinkel: Dr. Cerletti, you say that LSD does not antagonize the epinephrine effect. Of what animal are you speaking?

Cerletti: In all animals studied. It does not block epinephrine in rats, rabbits, cats, or dogs. These are the animals we have studied.

Rinkel: Are you familiar with the publication from the Royal Medical Faculty in Bagdad? (28)

Cerletti: Yes.

Rinkel: They say just the opposite. They say LSD inhibits and actually prevents not only the excitatory but also the inhibitory action of epinephrine, and they produce quite intriguing evidence for that.

Fremont-Smith: In what kind of animal and under what circumstances?

Hoagland: What preparations were used?

Rinkel: All kinds of preparations.

Cerletti: The main argument of these people was that the relaxing effect of epinephrine on the smooth muscle of the intestinal wall is inhibited by LSD. This type of epinephrine action is always more difficult to inhibit by adrenergic blocking agents than the excitatory effects of epinephrine. For example, dibenamine does not inhibit this epinephrine reaction. The high-molecular ergot alkaloids, however, can inhibit this relaxant effect of epinephrine as well as other epinephrine actions. But LSD is very different from these alkaloids and never produces the same effects.

Rinkel: The Royal Medical Faculty make a much wider statement. They also state that the excitatory action is inhibited, and they even go

so far as to say that when a lethal dose of epinephrine was given, the mortality of the animals was reduced when LSD was administered previously. Do you remember that?

Cerletti: I know that, but I do not think that this must be an argument in favor of an adrenergic blockade. If the LD-50 of epinephrine can be reduced by any drug, the conclusion should not be drawn from this fact only that the drug is an adrenergic blocking agent. In very high doses, LSD might constrict the pulmonary vessels and, in this way, interfere with the development of pulmonary edema produced by epinephrine. Perhaps in this way, the tolerance for epinephrine is increased a little bit, but that would not mean a specific blockade of epinephrine by LSD. If a real adrenergic blocking drug is used to protect animals against lethal epinephrine effects, the LD-50 of epinephrine can be multiplied in most instances several times.

Magoun: Dr. Cerletti, do you think this generalization could be carried over into the action of LSD on the central nervous system? As you know, Paul Dell's (29) and Marthe Vogt's (30) observations suggest an adrenergic mechanism of excitation of the brain stem, and I wonder if any of the central actions of LSD were proposed on the basis of adrenergic blockade, and whether your conclusion would apply centrally? Are you going to talk about that later, Dr. Sherwood?

Sherwood: What I have to say has some bearing on that, but as to the point you are raising, it is norepinephrine, not epinephrine in the brain stem, and it is practically coextensive with the brain stem reticulum. But that applies also to serotonin. Have you seen the paper by Amin, Crawford, and Gaddum (31)?

Cerletti: Yes. I think that at least from the classical pharmacological point of view, LSD must be considered a very specific serotonin antagonist, but not an adrenergic blocking agent.

Marrazzi: You mean peripherally.

Cerletti: Yes. In pharmacological terms, adrenergic blockade is always limited to the periphery. No one has yet cleared up the situation in the central nervous system concerning such an adrenergic blockade.

Fremont-Smith: Isn't it also fair to say that this sense of pharmacologic blockade really applies only to certain systems which have been studied, and from this, we generalize to a totality? But, actually, in the intestine, as I pointed out, it may not operate in the same way. In fact, it may work in a slightly different way, and we must introduce our concept of relativity into all these generalized reactions, which are only generalized when we extrapolate, and, actually, no pharmaceutical agent has been tested in every system of the organism.

Cerletti: Yes. I am absolutely of the opinion that we cannot say that

LSD does not interfere, perhaps with epinephrine and norepinephrine in the central nervous system.

Fremont-Smith: Or in some peripheral organs.

Cerletti: Or in some peripheral organs, too. But what for the pharmacologist is the classical layout of an adrenergic blockade cannot be demonstrated for LSD, in contrast to many other ergot preparations which all contain lysergic acid, and are classical types of the so-called adrenolytic drugs.

Fremont-Smith: We are trying to introduce relativity into classical biology, as well as into classical physics.

Seevers: Have you any evidence as to whether this is a competitive type of antagonism? Can this be reversed by more serotonin, for example?

Cerletti: On the uterus it can be done, but less so in the isolated kidney. Recently, Gaddum (32) published quite an extensive paper on different serotonin antagonists, and I think he proposed in this connection not to speak about competitive inhibition, etc., but of surmountable or insurmountable inhibition. I have not yet studied all the details of this paper, but in any case, for the uterus it can be shown that by increasing the dose of serotonin after LSD the original response can again be arrived at. There is an equilibrium between the two antagonists, and the blockade is surmountable. For the isolated intestine, the situation is rather different. The serotonin response of this preparation is much less sensitive to the antagonizing effect of LSD. On the other side, a self-blocking action of serotonin can be shown on the ileum; after a large initial dose, subsequent applications of serotonin are without effect.

Elmadjian: How long does it take for the muscle in that uterus preparation of the rat to contract from its base to its maximum?

Cerletti: Only a few seconds.

Elmadjian: Only a few seconds and then an immediate rise?

Cerletti: Yes. I would say, perhaps from 1 to 2 seconds and maximally 3.

Seevers: I would like to ask also, have you seen any evidence of tachyphylaxis in this particular preparation?

Cerletti: In the uterus preparation, there is no tachyphylaxis, and it is the same with the isolated perfused rat kidney. But a strong tachyphylaxis is observed in the isolated intestine. Gaddum (33) is of the opinion that the serotonin receptor in the rat uterus and the serotonin receptor in the gastrointestinal wall are different. He thinks that the receptor for serotonin in the gastrointestinal wall is a neuroreceptor, let us say, something at the level of the intramural ganglia in the intestinal tract,

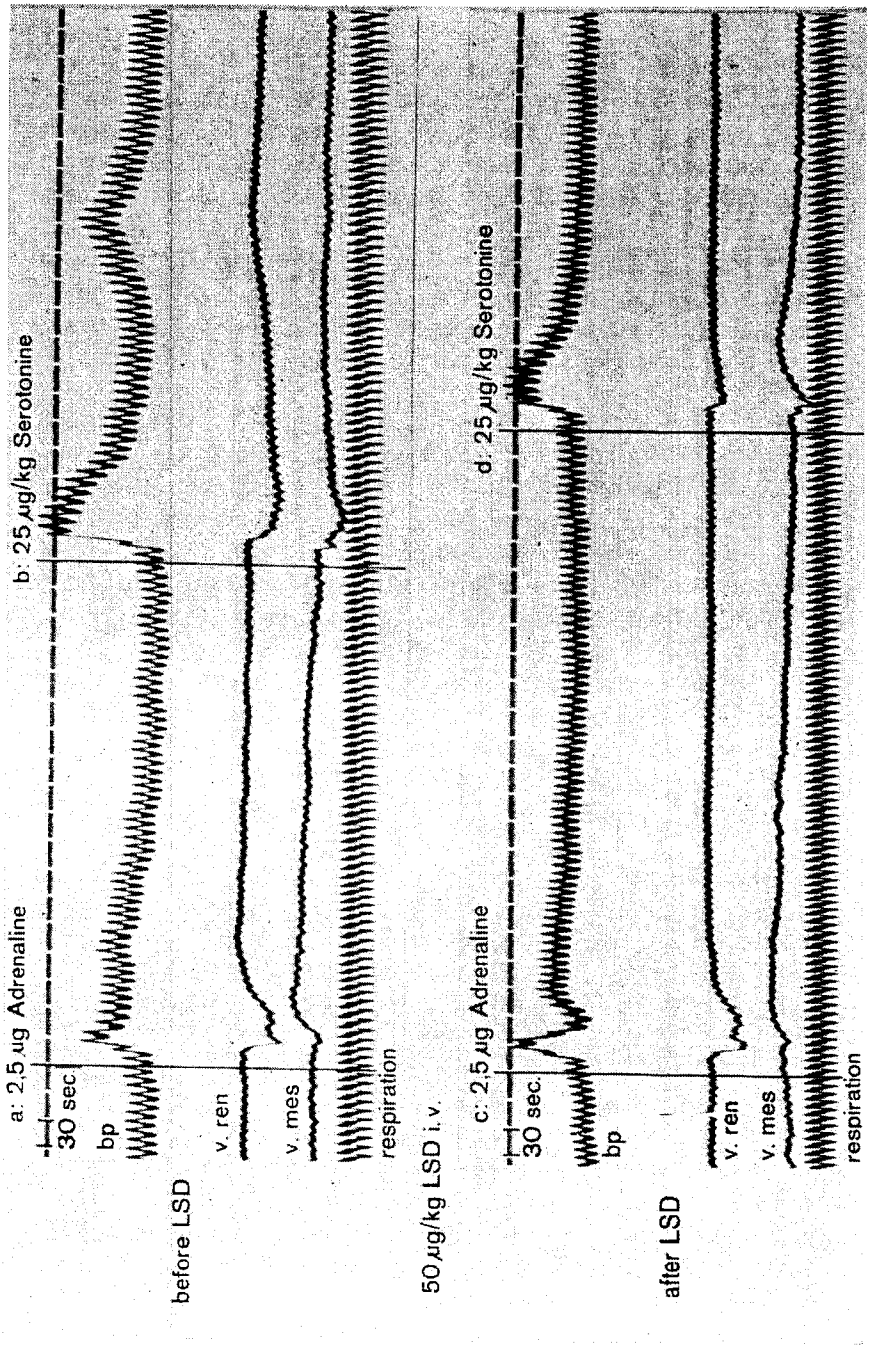


FIGURE 17. Effect of serotonin before and after LSD. (Epinephrine and serotonin are injected in the aorta thoracica.)

whereas, in the uterus, it is a peripheral receptor at the level of the single contractile element. This is a very interesting point. Differences of serotonin receptors exist also in other parts of the body. If, for example, serotonin is injected intravenously in the cat, afferent nerve endings in the chest, mainly pulmonary receptors, but also receptors in the coronary system are stimulated. In this way, several reflexes, mediated to a great part by the vagus nerves, are activated and produce bradycardia, fall in blood pressure, and respiratory arrest. This serotonin effect, which looks quite similar to the action of veratridine, must be distinguished from the peripheral vasoconstrictor effect which, in the cat, can only be shown by intra-aortic injections of serotonin. Whereas tachyphylaxis plays no role in this last action, i.e., the serotonin-vasoconstriction, it may be observed for the reflex stimulating effect of intravenously injected serotonin. In addition, this reflex action is not blocked by LSD. I think the nature of these two types of serotonin-sensitive receptors is a very interesting problem, although many things are not yet quite clear.

Leake: Except that it seems to bear upon whether or not the serotonin or the LSD may be fixed in irreversible combination with the receptor, in the one case, and in the reversible situation in the other. And then, the question of competitive or insurmountable inhibition; if there is a reversible combination, then it would depend on mass action, apparently.

Cerletti: I forgot to mention that, according to Gaddum (33), cocaine is quite effective in inhibiting the serotonin response of the ileum of the guinea pig but not the effect on the rat uterus. It is interesting, in this connection, to note that the vascular and respiratory reflex effects of serotonin, which are not inhibited by LSD, can be blocked by local anesthetics.

Figure 17* gives an example of an antiserotonin effect of LSD in the intact animal, i.e, in the cat. As already mentioned, intravenous injections of serotonin cannot be used because of the reflex stimulation which completely overshadows all other actions, and which, besides this, is not counteracted by LSD. To prevent serotonin from reaching the receptors in the chest, we inject it into the aorta. In this way, we obtain an increase in blood pressure caused by the peripheral vasoconstriction produced by serotonin. The renal and mesenteric blood flow are decreased. After pretreatment of the animal with LSD, it is quite obvious that the different effects of serotonin are changed; the blood pressure and the blood flow response to serotonin are reduced after LSD.

In this preparation, epinephrine is not inhibited by LSD. The rise in blood pressure after epinephrine is the same or may even be increased

*For adrenaline read epinephrine and for serotonine, serotonin.

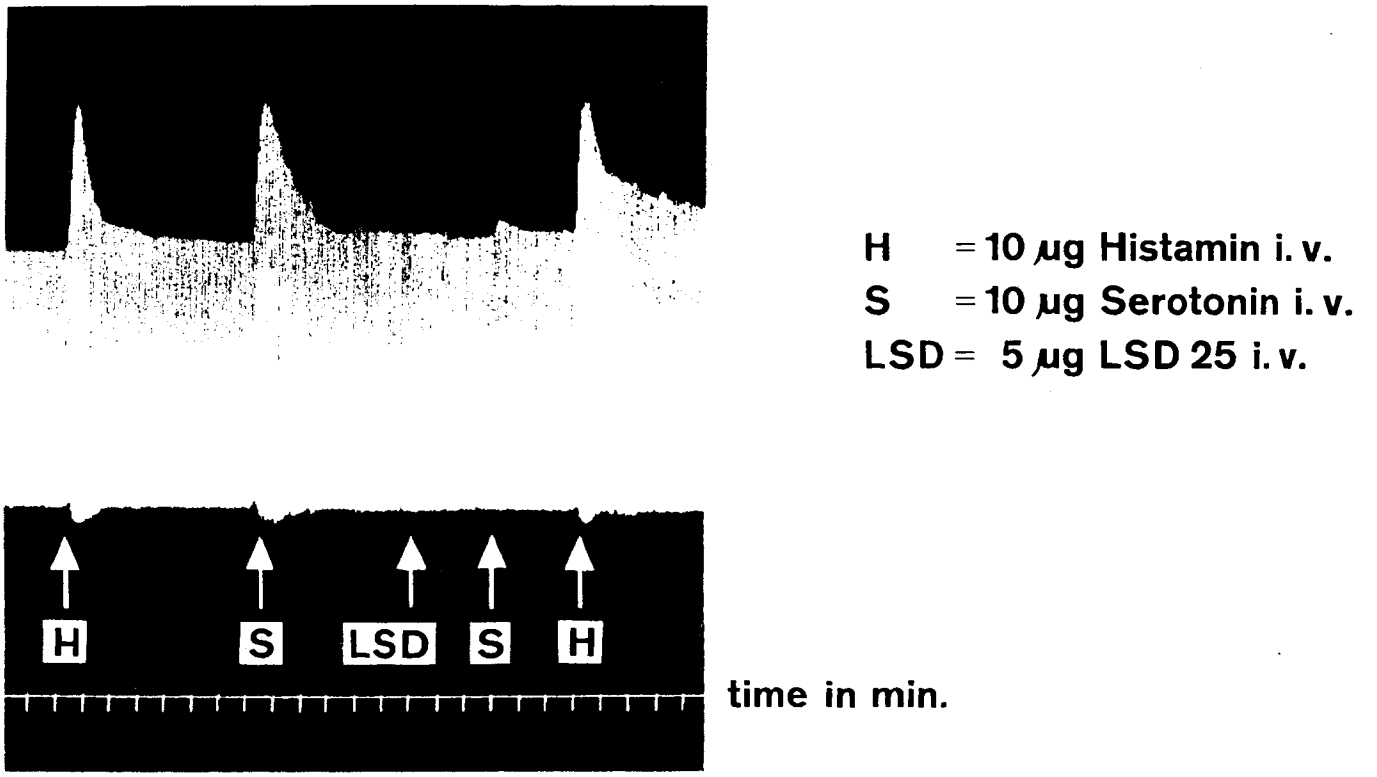


FIGURE 18. Specific inhibition of serotonin-induced bronchoconstriction by LSD-25 in the guinea pig.

after LSD. And similar observations can be made on the blood flow curves. With serotonin, however, a clear antagonism of LSD becomes manifest in this experiment on the whole animal. One point, however, is different from the experiments in isolated organs, and that is the dose ratio of LSD and serotonin. In the intact animal, the dose of LSD necessary to inhibit serotonin effects is similar to the dose of serotonin, whereas on isolated organs a dose of LSD from 10 to 20 times smaller than that of serotonin is sufficient.

Seevers: Have you tried mescaline against serotonin?

Cerletti: No; we have not tried mescaline. Another approach to test serotonin inhibition by LSD in the intact animal is to measure bronchoconstriction produced by serotonin. Serotonin has a constricting effect also on the bronchial muscles. In guinea pigs, therefore, the experimental asthma produced with histamine can also be evoked with serotonin. By using the method developed by Konzett and Rossler (34), to measure changes of the bronchial muscular tone, records such as the one shown in Figure 18 are obtained. The animal is ventilated by a constant amount of air delivered by a respiratory pump and only the overflow of air, which does not enter the lungs, is recorded. If the capacity of the bronchial system decreases, because of spasm, then increased overflow results. Histamine, which in the guinea pig is a very strong constrictor of the bronchial muscles, gives a typical response, but serotonin, in the same total dose of 10 μg. is similarly active. After injection of only 5 μg. of LSD, a specific block of the serotonin effect can be seen, whereas histamine is not influenced. With large doses of LSD a nonspecific inhibition of histamine is sometimes possible, but this can never mean that LSD would also be an antihistaminic.

Brazier: How long does that effect last?

Cerletti: You cannot see it in the Figure 18, but it lasts about half an hour to one hour.

Brazier: If that is a continuous experiment, haven't you some histamine effect as well?

Cerletti: This is a continuous experiment on the intact animal, and we must repeat the injections of serotonin from time to time in order to see how long it takes for the reactions to come back to the original value.

Cleghorn: When do you inject another dose?

Cerletti: Let's say we inject every 10 minutes.

Magoun: Have you applied the same maneuver here as with the others? Do isomers of LSD without psychic functions have that same effect?

Cerletti: Yes; isomers have also been tried.

Magoun: So, again, this does not seem to be a clue which would enable us to explore the peculiar psychic effects of LSD centrally? Do you draw this conclusion?

Cerletti: Woolley (35, 36) has promoted a serotonin hypothesis for the LSD effects. It is true that the isomers of LSD are not active as serotonin antagonists.

Magoun: They are not?

Cerletti: No.

Magoun: So this is in a different category from your pyrogenic and vasomotor changes; this may be an area where you can move into the central nervous system, with profit?

Cerletti: Yes, perhaps. This was indeed a way which seemed very promising, but the more results we obtained the more the situation was repeated in which the compounds that were very similar to LSD in serotonin inhibition were without psychic effects.

Abramson: Dr. Cerletti, Dr. M. Jarvik and I (37) found that the Siamese fighting fish responded to LSD in spite of the presence of serotonin; however, snails reacted differently. Several species of snails respond to LSD-25, but our data are mainly concerned with *Ambularia cuprina.* This species is known in pet shops as the Mystery Snail and is readily obtainable. Shortly after immersion in solutions of distilled water containing 1 μg./ml. of LSD-25, the snail opens its operculum, extrudes its tentacles, proboscis, and gastropod. It will be observed that the motion of the gastropod is abnormal, consisting of a rather wild,

FIGURE 19. Two snails, having been taken out of their bath of LSD-25 (1 μg./ml.) demonstrate the disorganized, wavelike motion as evidenced by the serrated rim of the gastropods. Shells of untreated snails remain closed. Reprinted, by permission, from Abramson, H. A., and Jarvik, M. E.: Lysergic acid diethylamide (LSD-25). IX. Effect on snails. *J. Psychol.* 40, *337 (1955).*

undulating, waving, muscular movement which prevents the snail from adhering to any surface (Figure 19). Normal response to gentle tactile stimulation is lost, but the snail will close its shell when exposed to a more vigorous stimulation, only to reopen again within a brief period. This disorganized movement has been observed to last for 36 hours, after which the snail usually dies. This prolonged action is similar to the prolonged response by *Betta splendens* and it persists after the snail is removed to fresh water or to open air. Quite a number of the snails die rapidly in higher concentrations. After immersion for 2 hours in solutions containing 0.1 μg./ml. of LSD-25, the same phenomenon occurs. After immersion for 4 hours in solutions containing 0.01 μg./ml. of LSD-25, the shells may remain open but the snails are relatively immobile. The fantastic, enveloping, persistent, wavelike motion of the gastropod is very different from the distorted, stuporous state of the *Betta splendens* although the Bettas, as mentioned, show a preliminary, short excitation phase. Serotonin stopped the vigorous undulation of the LSD-25 reaction with closure of the operculum, but after a few minutes the snails opened their shells again and resumed their LSD-25 stimulated activity with possibly some minor alterations which could be described only with slow motion pictures. Do you have any explanation for this serotonin effect in the intact animal (Figure 20)?

Cerletti: That is similar to the results I will show you in the next figures. To avoid confusion, I would like first to discuss the antagonism of LSD against serotonin. The next step will be to discuss whether serotonin can block LSD. Some three or four years ago we also used a fish, *Lebistes reticulatus*, or the "guppy," for testing the effects of LSD. We found that the best way to control the effect of LSD in this fish was to observe the activation of the melanophores. The female guppy has practically no color and looks like glass. It is interesting to see how dark this fish becomes under the influence of LSD. The intensity of reaction depends on the dose and, therefore, this test can also be worked out on a quantitative basis. Increasing doses of LSD produce first poikilocytic forms of these melanophores, and later on, a so-called spiderlike distribution of the pigment (Figure 21). If the effect of LSD is controlled under the microscope, a rather good dose-response curve can be plotted. This fish test was used as a simple tool for controlling LSD and to differentiate it from other compounds. Later, we tried to influence the melanophore effect of LSD with serotonin, and we succeeded.

This melanophore response is the only typical example known to me where the antagonism can also be reversed, i.e., where LSD can be blocked by serotonin. When we first tried to block the LSD action by serotonin, we were disappointed, as in many other cases, because it did

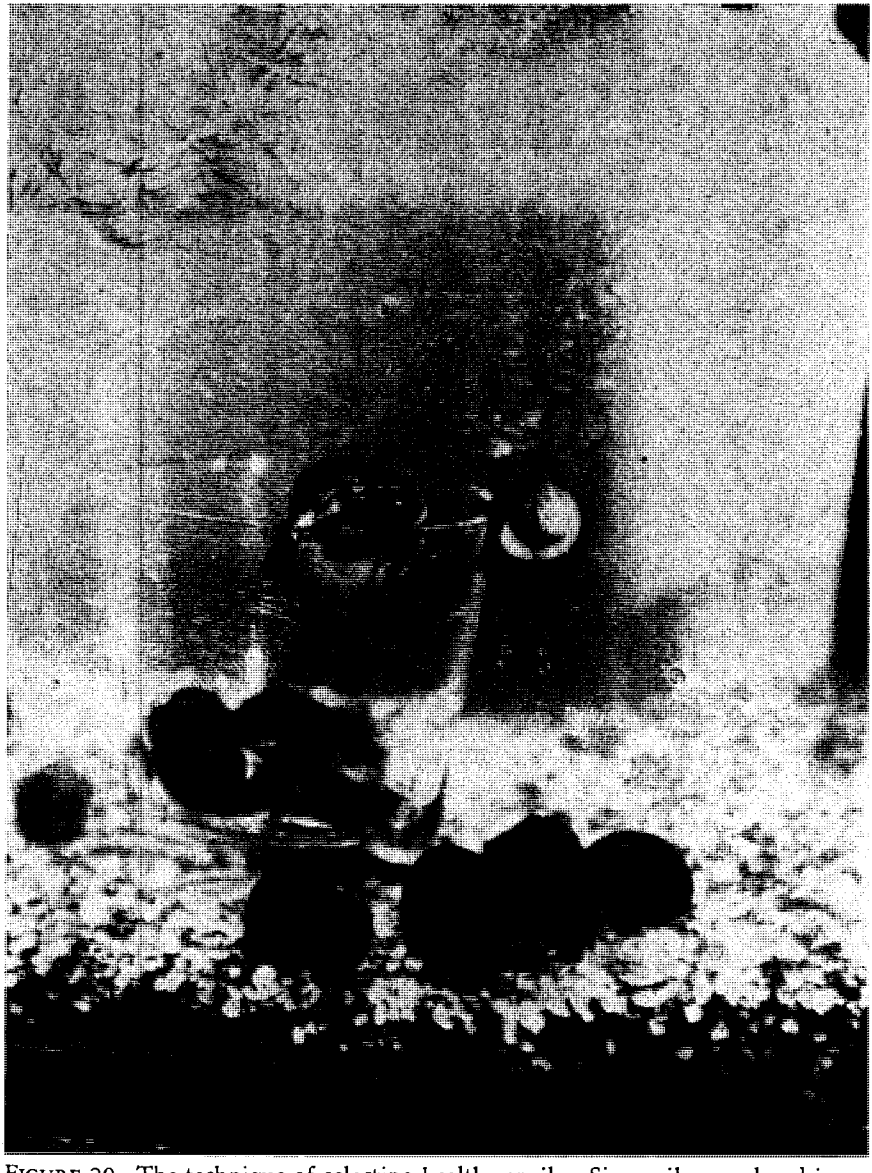


FIGURE 20. The technique of selecting healthy snails: Six snails are placed in an underwater vessel. Only those snails that climb out within an hour are used. Reprinted, by permission, from Abramson, H. A., and Jarvik, M. E.: Lysergic acid diethylamide (LSD-25). IX. Effect on snails. *J. Psychol.* 40, *337 (1955).*

not work here either. But later, we realized that serotonin must act on the fish for a full hour and that it must be given in a very high dose;

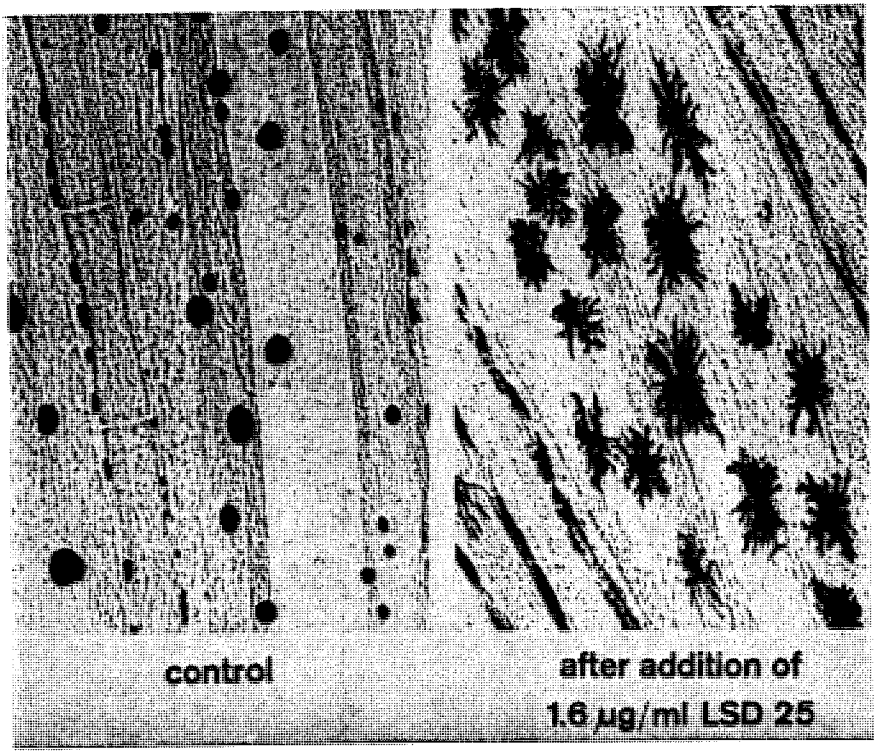


FIGURE 21. Melanophore reaction to LSD-25 in fish. *(Lebistes reticulatus* ♀*)* Reprinted, by permission, from Cerletti, A., and Berde, B.: Die Wirkung von D-Lysergsäure-diäthylamid (LSD-25) und 5-Oxytryptamin auf die Chromatophoren von *Poecilia reticulatus. Experientia* 11, *312 (1955).*

under these conditions the melanophore effect of LSD will be completely blocked.

Bard: Is that isolated skin or is that the whole fish?

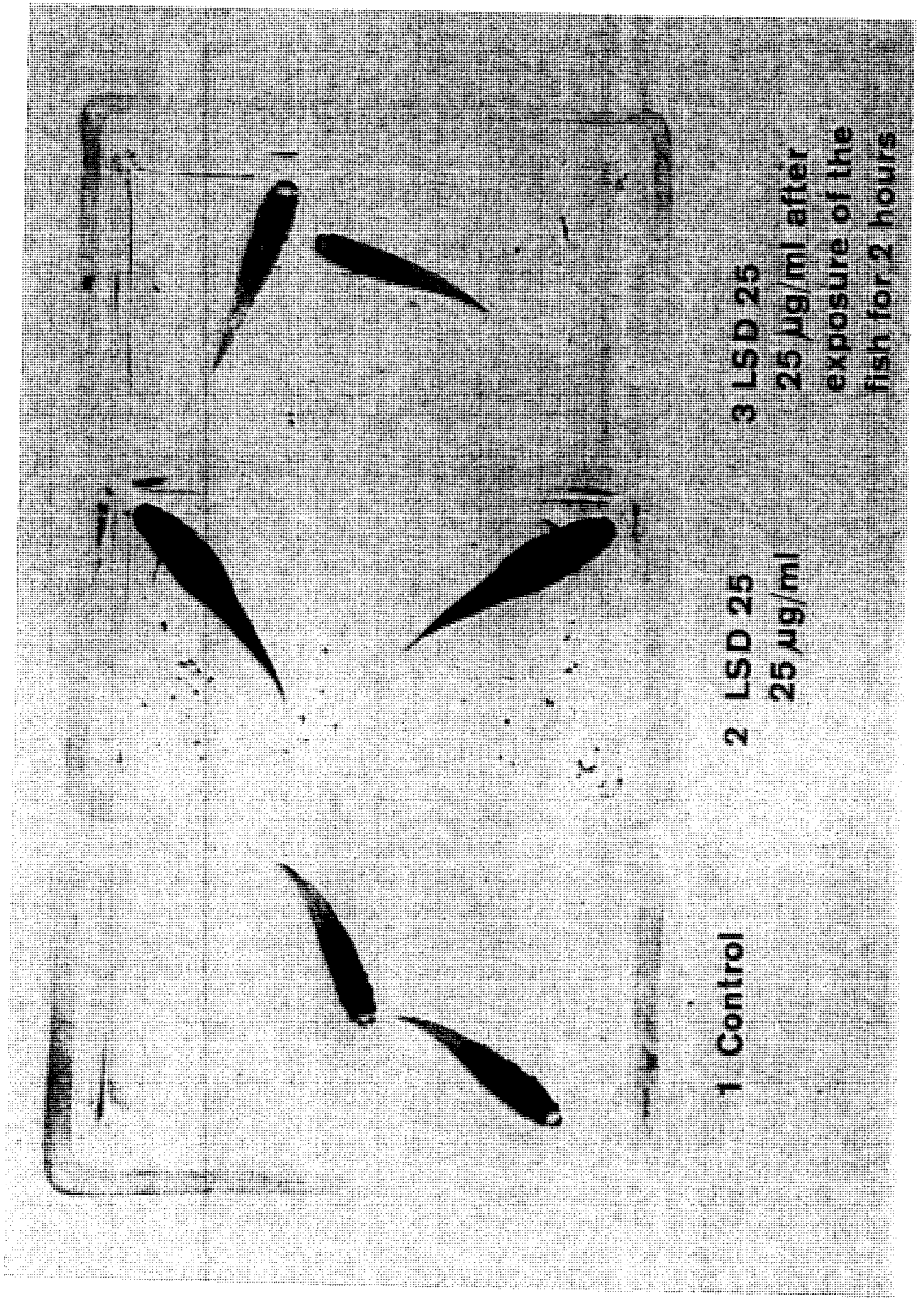
Cerletti: This is isolated skin.

Hoagland: This type of response in the skin is what you would expect with epinephrine, isn't it?

Cerletti: In our experiment, if we add epinephrine, we observe nothing of this sort; we observe the contrary.

Hoagland: There is constriction? Yes; I see; that is right. One would expect contraction of melanophores in fish with increased epinephrine activity.

Cerletti: My opinion is that in this type of animal serotonin could play a physiological role in controlling the state of the melanophores. As a hypothesis, I would say that by the LSD-blocking of serotonin which, perhaps, is one of the substances responsible for the contraction of the


1 Control
2 LSD 25
25 µg/ml
3 LSD 25
25 µg/ml after
exposure of the
fish for 2 hours

melanophores, there is a melanophore expansion. We can now reverse and try to inhibit the effect of LSD by serotonin, and this we did. Figure 22 shows the result. With 25 μg./ml. of LSD, a very strong melanophore activation can be seen. Two fish, which have been exposed for 2 hours to a high concentration of serotonin, no longer respond to this same, rather high LSD concentration. Serotonin alone produces no change. This is a very simple, but clear example of this reversed antagonism. I do not know if it has any importance. For example, clinicians have tried using serotonin to block the effects of LSD in man, but as far as I know, it does not seem to be clear whether serotonin can influence the effects of LSD.

Sherwood: It was shown by Gaddum that he was able to abolish the effects of LSD by taking serotonin (32).*

Hoagland: What LSD effects?

Sherwood: The mental effects.

Abramson: Were these double blind experiments?

Sherwood: No.

Marrazzi: When does the ability of these melanophores to expand return after administration of serotonin?

Cerletti: I cannot remember that for the moment. It is possible that this has not yet been studied.

Marrazzi: With such high doses, one wonders whether or not one is dealing with the specific action.

Cerletti: Yes; but it is the only example for demonstrating that at least in principle a serotonin block of LSD is possible. Otherwise, we have not obtained it. For instance, we could not block by serotonin the increase of body temperature produced by LSD. Or, if we measure the pupillary dilation produced by LSD in mice, it is not influenced by serotonin, at least not by a pretreatment with single doses in acute experiments. Whereas we can demonstrate in many different ways the inhibition of serotonin, we have no evidence for the contrary except this one example I showed you.

Abramson: Would you be willing to discuss the difference between the response of the snail and that of the fish to serotonin?

Cerletti: I have no experience with snails. The fish used in our experi-

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FIGURE 22. Inhibition of LSD-25-effect by serotonin. Reprinted, by permission, from Cerletti, A., and Berde, B.: Die Wirkung von D-Lysergsäure-diäthylamid (LSD-25) und 5-Oxytryptamin auf die Chromatophoren von *Poecilia reticulatus*. *Experientia* 11, *312 (1955)*.

*Gaddum, J. H.: Personal communication, 1954.

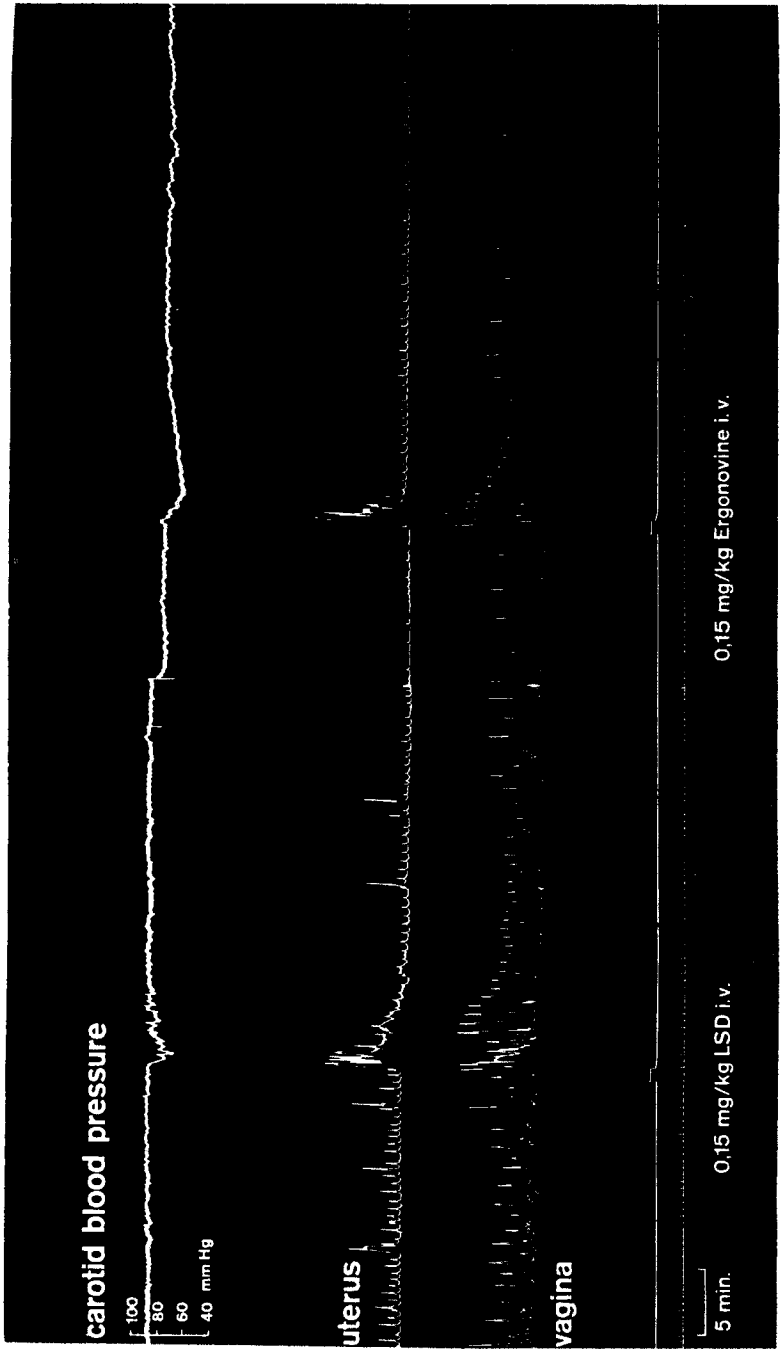

FIGURE 23. Oxytocic effect of LSD on the rabbit.

ments, the *Lebistes reticulatus*, shows with low concentrations of LSD, practically no effects except the melanophore reaction. If, in the presence of such a melanophore reaction, the dose of LSD is further increased, a narcotic-like action can be obtained: The fish makes only rare movements, and he may even lie on his side or in other strange positions, but this is an effect obtained only with higher doses of LSD.

Brazier: Did I understand you to say that you obtain this effect on the melanophores in the isolated skin?

Cerletti: No; the experiment was done on the whole animal. The fins and the skin were removed only for the microscopic control. We are now trying to produce the effect also in the isolated skin to see if it is a purely peripheral antagonism.

Leake: The narcotic action that you described for the fish after administration of LSD, then, is correlated with the reduction in activity of the Siamese fighting fish?

Abramson: Yes; there, we get a stupor lasting for days, at the surface of the liquid and a Cartesian diver effect is readily obtained with 1/10 μg./ml., provided the fish have been exposed a few hours.

Leake: This shows the importance of keeping in mind our semantic terms under these various different conditions. When one is thinking of the reduction of anxiety in fighting fish, or narcosis in fish, to carry that over to the human action of LSD might get us into difficulties, to return to Dr. Magoun's point. But I think if we can consider, in either case, a disorganization of the ordinary type of central nervous system control of the animal as influenced by LSD, then these points are reconcilable. But we must make sure that we keep our terms clear in a semantic sense as we go along.

Abramson: I fully agree with that position.

Cerletti: I think that this should be enough to show the situation when serotonin is used. On the question raised by Dr. Magoun as to whether LSD would produce psychotic-like symptoms merely because it is a potent serotonin antagonist, we have difficulty in understanding such a connection, because we found derivatives of LSD which are even stronger antagonists to serotonin but do not produce any psychotic symptoms. The two strongest compounds against serotonin, which we have tested according to the methods I have mentioned, are brom-LSD and acetyl-LSD. With brom substitution in the LSD molecule, up to twice the serotonin antagonistic activity of LSD results. But in all other respects, this compound is absolutely different from LSD. As I have already mentioned, LSD is quite a strong oxytocic drug. It has about the same potency when used on the rabbit uterus as ergonovine (Figure

23). Brom-LSD, however, has no more of a contracting effect on the uterus muscle.

As I showed previously, LSD, in a certain dose range, produces bradycardia and a fall in blood pressure. Brom-LSD, even in the highest tolerated dose, does not produce this fall in blood pressure. In man, brom-LSD has no LSD-like effects at all. We have tried up to 1 mg. and could observe no action, except in a few cases some vegetative symptoms and a certain sedative effect. This sedative property is only manifest after doses of more than 1 mg., and is something absolutely different from any LSD effect. But in one respect, brom-LSD is identical with LSD in that it blocks serotonin, and it blocks it even about one and a half to two times more than LSD. I think such a fact must be considered, if the Woolley hypothesis (35,36) is discussed.

Kety: Dr. Cerletti, would you agree that in order to use this information as very good evidence against Woolley's hypothesis, it would also have to be shown that these compounds are able to get across the blood-brain barrier?

Cerletti: Yes. We had indirect evidence, from the symptoms observed, that this compound also reaches the brain. If brom-LSD and LSD are injected in an animal in similar amounts and the brain extract is tested for serotonin antagonistic action, a similar activity in such an animal is obtained as with LSD.

Kety: You really have very good evidence, then.

Cerletti: Yes; I think this should be evidence enough that the blood-brain barrier is not the reason for the difference.

Cantoni: But I do not think that this kind of argument is really sound, because there are other examples in pharmacology of antagonisms which on theoretical grounds are consistent with, or lend support to, the Woolley hypothesis (38). For instance, as is well known, histamine has more than one pharmacological action. Among the antihistamine agents there are some which block more or less specifically the peripheral vascular effects and/or the smooth muscle stimulation without affecting the gastric secretory response. Others may block the effect of histamine on gastric secretion without affecting the response of the smooth muscle or the peripheral vascular response, so it does not seem to me necessary that all antiserotonin-like drugs should inhibit both the vegetative effects and what we call the psychic effects, if we assume that these two effects are different and not related.

EDITOR'S NOTE: Dr. Cantoni would like to add the following "afterthought" to his remarks at the conference:

> Probably a better analogy can be drawn from a consideration of the pharmacology of cholinergic blocking agents. As is well known, acetyl-

choline stimulates specific cellular receptors in skeletal muscle, in smooth muscle, and in ganglionic cells, and the cholinergic blocking agents can be divided into three groups, according to their ability to block the effect of acetylcholine specifically at each of these three sites. Thus, the curare-like drugs affect the cholinergic receptors of skeletal muscle, without blocking the effects of acetylcholine at the other sites. Likewise the cholinergic blockade caused by atropine and nicotine-like drugs exhibits a high degree of receptor specificity.

It is true that for the present it has not yet been established that these two effects of serotonin are not related, but if it is assumed that they are separate responses, then I would have no difficulty in imagining that there are two types of drugs, very closely related chemically, which have a different type of antagonism.

Cerletti: Then, you would say that we still can accept the hypothesis that the LSD effects are mediated by this antiserotonin action at the level of the central nervous system. But in this case, you also postulate that, in spite of the fact that brom-LSD reaches the brain and that it shows a peripheral antiserotonin effect, perhaps it does not have an antiserotonin effect in the central nervous system. This is a theoretical possibility. Like LSD, brom-LSD also inhibits the potentiation of barbiturates by serotonin.

Cantoni: Yes; because serotonin might have one action on the central nervous system, a psychic action, caused by some property of its molecules which are antagonized by LSD, and *not* by the brom-analog, and it has a peripheral effect on the smooth muscle of the uterus, because of some other property which *is* antagonized by the brom-lysergic acid analog.

Cerletti: But since the whole hypothesis is based on a chemical similarity, for example, that the serotonin molecule can be projected in the LSD formula, it is very difficult to understand why both compounds should be antagonists in the periphery and behave in such a different manner, as far as serotonin is concerned, in the central nervous system.

Cantoni: No; I do not think it is so terribly difficult, because it is possible to visualize differences. You are introducing the brom in the 1 position, or a methyl in the nitrogen, and it could be assumed that the serotonin in the brain does not act by these two positions, but that in the periphery, it does. It is completely speculative, I admit, and there is no experimental basis for it yet, but on theoretical grounds, I think it is sound.

Cerletti: I would like to point out some difficulties for this working hypothesis on serotonin which arise with derivatives of LSD. There is another compound which is substituted in another place.

Marrazzi: There have been so many references to the hypothesis of

Woolley and Shaw (36,39) that, in all fairness, we should admit for the record that they have a second and diametrically opposite hypothesis, namely, that LSD is an amine-oxidase inhibitor and therefore preserves serotonin, and the psychic effects are caused by an excess of serotonin.

Cerletti: Yes; but for this, the evidence is reversed. I would like to mention another serotonin antagonist of the LSD series, the acetyl-LSD, which is substituted in position 1. In most tests, this compound was also more active than LSD, but it has not yet been tested in human subjects. It would be very good if, in the future, someone would analyze the acetyl-LSD, because it may be very similar to LSD in man also.

For the hypothesis of a serotonin excess as basis for the LSD-psychosis, it will be important to consider the activity of LSD as an inhibitor of the amino-oxidase. We have studied in our laboratory the mono-amino-oxidase of the liver of different animal species, but it was impossible to show any effect of LSD on this enzyme. We have used marsilid (iproniazid), which is a very specific inhibitor of the mono-amino-oxidase, and have compared it with LSD, but LSD was not effective. We also tried to test the mono-amino-oxidase from animals which were injected with very high doses of LSD, and there was no change.

Quastel: Do you remember what the substrate was?

Cerletti: One time, the substrate was just serotonin. Another time, it was tyramine.

Quastel: Was this done with liver, but not with brain tissue?

Cerletti: As far as I remember, only liver was used, and perhaps kidney.

Quastel: But this was not done with brain tissue?

Cerletti: No, not with brain tissue.

Quastel: Why didn't you try it on the nervous system?

Cerletti: The work is only under way and not yet finished. But, to my knowledge, no one has proved any activity of LSD on amino-oxidase. It was just a theoretical implication made by certain authors.

Hoagland: Dr. Cerletti, would you discuss the metabolism of this substance?

Cerletti: Yes; that is the next point. Figure 24 shows the results we obtained by following the distribution of LSD in the body, using the serotonin-blocking effect as an assay procedure. The LSD was injected intravenously and, after certain time intervals, the LSD content in the blood, liver, and brain, was estimated by testing the extracts of blood and the different tissues on the isolated rat uterus for antiserotonin activity.

It is clear that this is not absolute evidence for the presence of LSD. But we always made controls with extracts from untreated animals,

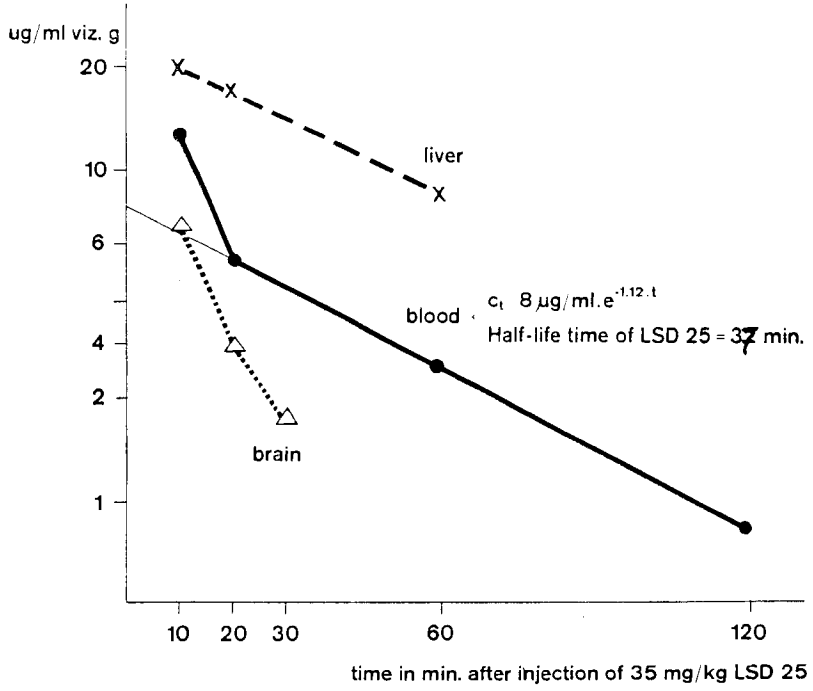

FIGURE 24. LSD-25 distribution in blood and tissues of the mouse (biological assay of LSD-25 by means of the antiserotonin test in the rat uterus.) Reprinted, by permission, from Lanz, U., Cerletti, A., and Rothlin, E.: Über die Verteilung des Lysergsäurediäthylamids in Organismus. *Helvet. physiol. et pharmacol. acta* 13, *207 (1955)*.

which usually have no significant antiserotonin activity. The values we found are indicated as µg. of LSD per ml. of blood, or per gm. of tissue. LSD was injected intravenously in mice in a dose of 35 mg./kg. or about 700 µg. total dose. During the first 2 hours a curve is obtained in the blood, which shows a half-life time of LSD of 37 minutes. It is very important that by this method LSD can be shown to be present in the brain also, and especially high concentrations are found in the liver.

Quastel: These are isolated tissues, are they?

Cerletti: No; these are the tissues removed from the animal after a given time interval. These are average values of several animals, one series of animals being sacrificed 10 minutes after the injection, another series after 20 minutes, and so on.

Quastel: I see; it is not the effect of the isolated tissue on the drug.

Cerletti: No; that is LSD injected intravenously into the animal, and later blood, liver, and brain are removed, extracted, and tested for anti-

serotonin activity, which is expressed as μg. of LSD present in one ml. of blood or in one gm. of tissue.

Elmadjian: What is the procedure of extraction from tissues?

Cerletti: The procedure is to grind the tissue and to extract with diluted tartaric acid.

Kety: Dr. Cerletti, would you agree that those curves are not necessarily LSD, but LSD and/or any of its degradation products which may have an antiserotonin effect?

Cerletti: Yes; these curves do not represent LSD, but they do represent the antiserotonin activity of the extracts, expressed in terms of LSD/ml. This amount of LSD must be added to an extract of an untreated animal for obtaining the same effect. We have still other evidence that it is really LSD that we are measuring, at least in the beginning, because we studied the LSD distribution with radioactive LSD also.

Rinkel: Dr. Cerletti, how did you identify the substance as LSD?

Cerletti: We did not identify it at the time of this experiment, but later a chemical identification was possible.

Rinkel: I understand; later on, you did identify that it was LSD?

Cerletti: Yes.

Rinkel: How did you identify it?

Cerletti: We pooled the extracts from several livers and gave them to our chemists. They are now able to identify very small quantities of LSD by combining paper chromatography and the different fluorescence and color reactions. They could actually isolate LSD from these extracts, but, as I say, this is possible only in the first 20 or 30 minutes. Later, the situation is more complicated, because there are metabolites which, in some respects, are similar but in others are different from LSD.

Abramson: I would like to confirm Dr. Cerletti's observation. At Cold Spring Harbor, Dr. Geronimus and I (40) have been breaking up tissues with various methods, and we have been using the Siamese fighting fish as a test object. For instance, in one experiment LSD-25 in an amount of 40 μg./gm. of body weight was injected into the femoral veins of three young rats. The rats were killed after 90 minutes and their brains and livers ground in distilled water in a Potter homogenizer. The resulting suspensions were assayed by a Siamese fighting fish titration in which groups of three fish were exposed to 1 to 1 serial dilutions in which the end points of the test titrations were equated with the end point of a similar LSD-in-water titration. The titers of LSD-25 in the brain and in the liver were very roughly 4 and 16 μg./gm., respectively. Controls for the effect of normal tissue alone and with added LSD-25, as well as a titration for LSD-25 in the blood for the test animals, were also run in this particular experiment. These

controls suggested only one modification of the figures that I have just cited, and that is that they have a tendency to be low. Both homogenized brain and liver apparently tend to bind or to destroy some of the LSD-25 added to them.

Hoagland: Are you going to report something on C^{14} labeled LSD?

Cerletti: Yes. We are absolutely of the opinion that, as stated by Dr. Kety, if only the antiserotonin activity were tested, and we know that many other compounds are very active, it might be found that LSD is metabolized to an antiserotonin compound which cannot be distinguished by the biological assay.

I would like to present the results which were obtained in our laboratories with this labeled LSD, the same LSD as was used by Dr. Hodge's group in Rochester, and I believe Dr. Hoagland used some.

Hoagland: Yes; you sent us some, too.

Cerletti: As has already been published by Dr. Hodge's group (41), very small amounts of radioactivity appear in the expired air, or in the urine or the feces for 12 hours after the administration of LSD. I think maximally from 6 to 8 per cent of the total dose injected is eliminated from the body.

Rinkel: I am familiar with Dr. C. Hodge's* work because I initiated it and kept up with its progress. The maximum amount of radioactivity was found inside the intestine and the smallest amount in the brain; a relatively large amount was also found in the liver and kidney.

Cerletti: This is labeled diethylamine, and nothing is labeled in the ring structures, but, fortunately, this side chain is not easily split off and, therefore, after 12 hours the radioactivity is still related to a lysergic acid derivative.

Marrazzi: Did I understand you to say that the Siamese fighting fish are not sensitive to derivatives of LSD-25?

Abramson: Not in the same way. The one that is closest in effect is LAE-32. But Dr. L. T. Evans, Dr. L. H. Geronimus, and I have been able to distinguish between LAE-32 (the monoethylamide) and LSD-25 with the Siamese fighting fish (Figures 25 and 26). But we have tested BOL-148 and other similar compounds which are fairly different. We have not tested acetyl or the oxymethyl derivatives yet.

Cerletti: Without entering into all of the details, the main results from our laboratory can be summarized as follows (Figure 27): After the intravenous injection of C^{14} labeled LSD, the radioactivity disappears quite rapidly from the blood. The half-life time of radioactivity

*Professor of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

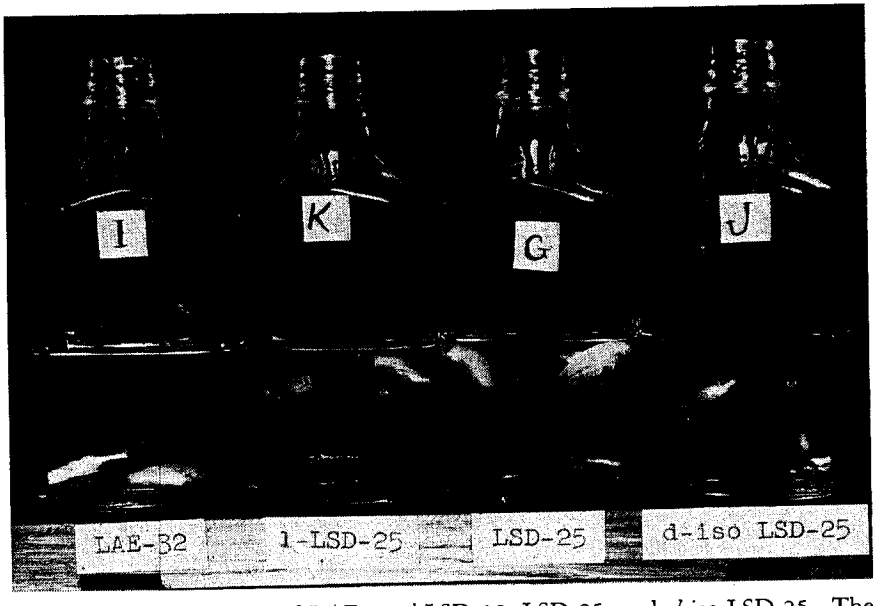

FIGURE 25. Comparison of LAE-32, *l*-LSD-25, LSD-25, and *d*-iso-LSD-25. The characteristic angular position on the surface clearly shows the effects of LSD-25 and its derivatives which are markedly different.

in the blood is less than what we found with our biological assay method. On the other side, the findings are similar in that the liver also shows the highest content of radioactivity. The spleen, kidney, and pancreas have quite high concentrations. If we assume an absolute homogeneous distribution of the radioactivity in the whole animal, we would arrive at a level of 40 counts per min. and per mg. It is interesting to note that the skin and muscle are below this level, whereas the liver, kidney, adrenal glands, pancreas, and spleen are above. The curve of the radioactivity of the small intestine increases very rapidly.

Fremont-Smith: The contents or the wall?

Cerletti: The contents and the wall. Actually, the curve gives the total amount, but if we separate, then we have more than 90 per cent in the contents and only a small amount in the wall.

After about 2 hours, the radioactivity in the duodenum reaches a maximum and after that time it begins to decrease. The deeper parts of the gastrointestinal tract show a similar increase, but at a later time. This is because the radioactivity reaches the intestines via bile secretion. By cannulizating the choledochus, this can be clearly demonstrated.

Rinkel: Dr. Cerletti, were those experiments done with the isotopes Dr. Hodge sent you, or were they done with your own radioactive LSD?

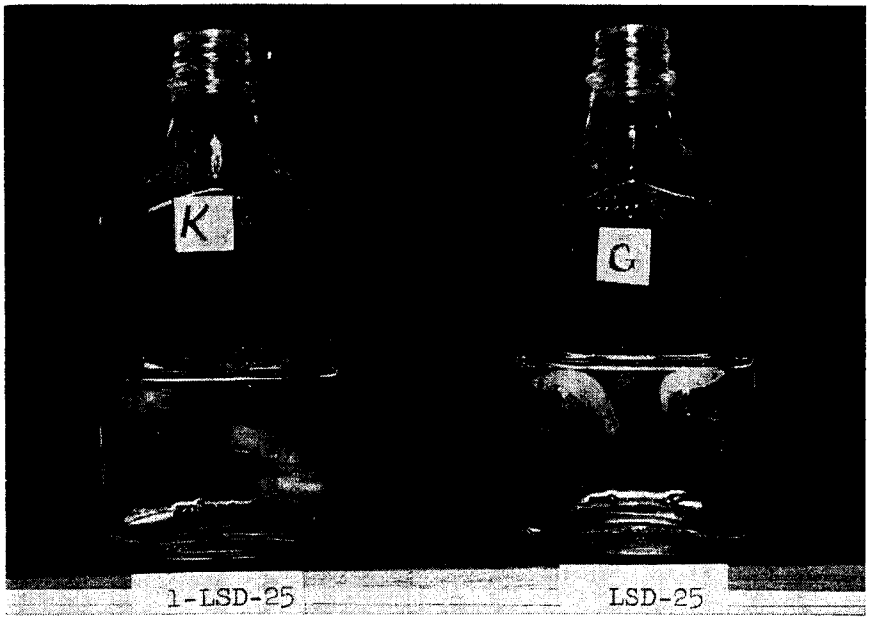

FIGURE 26. A comparison of the marked difference between the dextro- and levo-isomers of LSD-25, also showing angular position on the surface and the twist in the body of fish treated with the dextro-isomer.

Cerletti: This work was done with the first batch of radioactive LSD, which was prepared from acetonitrile and sent to us by Dr. Hodge's group.

Rinkel: Dr. Hodge used the radioactive material you sent him, and then he sent you radioactive isotopes which were much stronger than the ones you sent him. I wonder whether those experiments were done with the isotopes he sent you or with your own.

Cerletti: The first preparation was, perhaps, not strong enough, and we were asked to prepare a stronger one, if we could be supplied with a stronger acetonitrile. This acetonitrile eventually arrived, but it had taken such a long time to obtain the export permit from the United States that it had deteriorated quite a bit; however, a new batch of labeled LSD is being prepared now.

Hoagland: The specific activity was adequate. That is the real point.

Cerletti: Yes.

Kety: Do you know what the specific activity was, or what this represents in dosage of LSD?

Cerletti: This represents an injection of 50 μg. of radioactive LSD per animal.

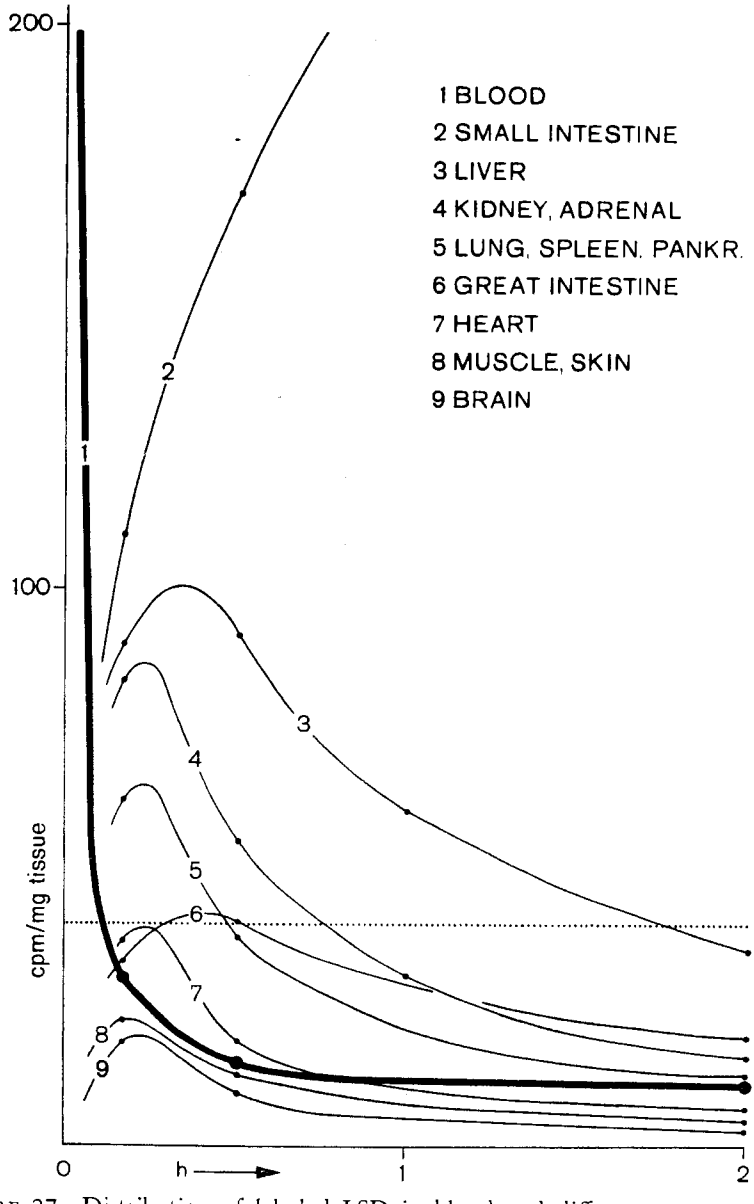

FIGURE 27. Distribution of labeled LSD in blood and different organs of the mouse after intravenous injection of 50 μg. total dose. The counts per minute and per milligram (cpm/mg) are plotted against time. The dotted horizontal line at 40 cpm/mg would represent the level of an equal distribution of radioactivity over the whole body. Reprinted, by permission, from Stoll, A., Rothlin, E., Rutschmann, J., and Schalch, W. R.: Distribution and fate of 14C-labeled lysergic acid diethylamide (LSD-25) in the animal body. *Experentia* **11**, *396 (1955)*.

Kety: In other words, a comparable dose to that which is of human interest?

Rinkel: No, not at all!

Kety: It was much bigger?

Hoagland: Yes; it was much bigger. It was many thousand times more. This is a big dose.

Cerletti: We must always consider what animal is being used. This work was done on mice, and the tolerance of the mouse for LSD is extremely high. The LD-50 of LSD in the mouse is 60 or 70 mg./kg. and, therefore 50 μg./mouse can be considered a small dose. If we used the rabbit, a similar dose would have been impossible. I think the very big differences from species to species should not be forgotten.

Rinkel: Dr. Hodge used rats and he used 1 mg./kg. of body weight; that was much too weak.

Unna: In the mouse, the dose is 50 mg./kg./mouse?

Cerletti: Yes; but 50 μg./animal is, for this species, a relatively small dose.

Hoagland: Did you obtain data on the brain?

Cerletti: The brain data obtained with labeled LSD are similar to the ones I showed you before with our bio-assay method. The values in the brain are always below the blood concentration. The ratio of LSD in the blood, brain, and liver, as found by bio-assay, is very similar to the results obtained with the C^{14}-material.

I have some very recent data showing that the activity in the intestines after 1 hour, and even more after 2 hours, is no more attributable to LSD. At least 90 per cent is caused by something different. However, it is still a lysergic acid derivative with the labeled side chain, but with changed physical properties. I hope that it will be possible to isolate this derivative and to make an analysis of the biological activity.

Rinkel: Dr. Cerletti, Dr. Hodge also found at least two distinct LSD metabolites, and I believe both were still radioactive.

Cerletti: Yes; that is also the case here, and I think the radioactivity of the metabolites proves that the diethylamide has not been split off. On the other side, the compounds give the color and fluorescence reaction which are typical of the lysergic acid molecule. They are, however, different from LSD in the chromatographic behavior and in solubility. It will be very interesting to follow up these things now.

Rinkel: I believe it is important to mention, in regard to what we have just discussed, that similar observations were made with radioactive mescaline. You are probably familiar with the work which was done at the Max Planck Institute (42-49).

Cerletti: Yes.

Rinkel: There it was found that mescaline disappeared from the mouse brain within 30 minutes, while the highest concentration of mescaline was found in the liver and in the kidneys. I wonder if you would say a few words about these experiments because there seems to be some similarity.

Cerletti: As far as I remember those papers, there would be a fundamental difference, because the authors have shown that the mescaline effects seem not to be correlated with the presence of mescaline in the brain. They have hypothesized that from mescaline another compound or a protein-mescaline complex is formed in the liver, and that only this produces the symptoms. If we consider what I have told you about the vegetative symptoms, I believe that in the first 30 to 60 minutes all that can be observed in animals is caused directly by LSD. A motion picture that I have shows that the dog, even after a very high dose of LSD recovers so rapidly from the motor and vegetative effects that after only 60 minutes he is quite well, and it would be difficult to tell that he is very intoxicated by LSD. In human subjects the effects of LSD appear more slowly. Even if it is injected intravenously, some time elapses before there is any effect.

Hoch: I would support what you just said about the radioactive property of mescaline. On the other hand, we did some experiments, introducing mescal and ordinary LSD, intraspinally, and found the action of both to be instantaneous. There resulted the whole psychotic picture which otherwise would be obtained after a half hour or so.

Sherwood: Do you inject this intraspinally or intrathecally? Do you inject it into the cord itself?

Hoch: Into the cord, yes, intraspinally. Even though it was introduced into the cord and not intrathecally, the psychotic picture with both mescal and with LSD appeared almost immediately. We have no explanation.

Fremont-Smith: This is in man?

Hoch: Yes, in man. Incidentally, both compounds introduced this way, are highly toxic, and there are all the vegetative manifestations which are described for both drugs. Also the psychotic manifestations in both drugs come on very rapidly. It is interesting that the intensity of the psychotic manifestations or the vegetative symptoms produced by *d*-lysergic acid diethylamide are not stronger or as strong as when introduced intravenously. On the other hand, the intensity of the manifestations with mescaline is greater if it is introduced intraspinally rather than intravenously.

Kety: Dr. Hoch, were these drugs injected into the parenchyma of the spine or into the subarachnoid space around the spinal cord?

Hoch: Into the subarachnoid space around the spinal cord; the patient had a lumbar puncture and then LSD and mescaline were introduced similarly as other substances are introduced into the spinal fluid.

Fremont-Smith: Into the spinal fluid?

Hoch: Yes, into the spinal fluid.

Kety: Isn't it true, Dr. Fremont-Smith, that the rate of diffusion of the substance from the spinal fluid up into the subarachnoid space around the cerebral hemisphere is relatively slow?

Fremont-Smith: I believe it is, but it depends a little upon the pressure relationships; it can be sucked out and gotten up, but I think that the evidence of past years is that the material that is put in without marked disturbance of pressure relationships in the lumbar region will take a long time to reach the system.

Hoch: That is right, and that is why we have no explanation of why these substances introduced in this way should have such a rapid effect, or a more rapid effect than if they were introduced intravenously. Actually, it should not be more rapid.

Fremont-Smith: In trying to understand how it was possible for drugs injected, shall we say, into the ventricle, to act as rapidly as they do, I remember being very much impressed by an observation that Tracy Putnam, formerly of Columbia University College of Physicians and Surgeons, made a great many years ago. He was injecting fluorescein intravenously into a cat and I happened to be watching, under the microscope, the area where the choroid plexus protruded, from the lateral ventricles, or from the fourth ventricle on the foramen of Luschka. I had the opportunity to see the meningeal vessels and not only around the veins but also around the arteries there was an immediate collar of fluorescein, showing that fluorescein came out of arteries and veins. What I argued with myself was: If a molecule as large as fluorescein can diffuse out through the arterial wall in the meninges, then in the small arteries, couldn't the reverse take place? And couldn't material injected into the ventricle diffuse into small arterial walls and be carried immediately to the capillary bed, in the brain stem or in the medulla, or around adjacent to the ventricle, which is served by these small arteries, and couldn't a very prompt effect be obtained that way?

Is it conceivable here, particularly with the longitudinal arteries running up and down, that your material goes into the arteries leading directly into the central nervous system?

Sherwood: We have some information on that. Bhawe* working at the National Institute for Medical Research at Mill Hill, has examined,

*Bhawe, W. B.: Personal communication, 1955.

during the last few months, the disappearance of intraventricularly injected histamine in cats and dogs. Histamine is used because it is a substance which can easily be assayed biologically. He injected 500 μg. Although in the dog none appeared in the lumbar theca, some did appear in this region in cat, when tested about one hour after injection. In order to detect histamine in larger amounts in the lumbar theca, it is necessary to "pump", i.e., to push and pull with syringes. Dye injected into the ventricle, for example methylene blue, stays certainly for from one half hour to an hour, mainly in the third ventricle and the first part of the iter. Further, in some experiments which Feldberg, Malcolm, and I,* have done in the anesthetized cat, and where the dura had been opened, thus establishing a pressure gradient, the intraventricular injection of small doses of *d*-tubocurarine (+tubocurarine) was not followed by the appearance of appreciable amounts of that drug on the surface of the hemisphere. Cerebrospinal fluid was collected from the cortex first with filter paper and then with a very fine sucker tube. With a biological assay method which is capable of detecting +tubocurarine in a dilution of 1 μg./5 ml., no +tubocurarine appeared over the surface of the brain.†

Fremont-Smith: Showing very slow circulation.

Sherwood: That was in about three experiments.

Fremont-Smith: Was there any indication of the apparent pressure in the blood vessels and, hence, into the brain substance?

Sherwood: We have no information on the blood vessels or on the blood circulation.

Hoch: In animal experiments, mescal appears on the surface.

Fremont-Smith: Without dislocating the pressure?

Hoch: Without dislocating the pressure.

Sherwood: What volume did you use, Dr. Hoch?

Hoch: In the experiments I know of, mescaline was actually introduced in a much higher concentration.

Fremont-Smith: What volume?

Hoch: From 5 to 10 ml.

Fremont-Smith: That is enormous. That is bound to dislocate all the pressure relationships.

Hoch: Yes, but it was also produced later on with 1- or 2-ml. volume.

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*Feldberg, W., Malcolm, J. L., and Sherwood, S. L.: Paper in preparation.

†Further experiments have shown that detectable amounts of +tubocurarine do appear on the surface after intraventricular injection of 300 μg. but not after intraventricular injection of from 15 μg. to 50 μg. which is a convulsive dose; the effects of topical application of +tubocurarine are, in many aspects, entirely different from the intraventricular mode of administration.

Sherwood: But the volumes we used for our injections were never more than 0.3 ml., and usually they were 0.1; or 0.2; or 0.25.

Fremont-Smith: I think this is the point; if you introduce anything of this volume, you dislocate the pressure relationships, and the recovery of the material in another place is very little indication of what would happen under normal circumstances.

Hoch: Yes.

Sherwood: The cat's ventricle is very small. It contains probably not more than 2.5 to 3.0 ml. total volume.

Hoagland: Some data collected in our laboratory on the accumulation in the brain of C^{14} labeled LSD sent us by the Sandoz Company may be of interest. Seven rats were each injected with 100 μg. of LSD labeled on the diethylamine with a specific activity of 8 μg. per mg. The animals were killed by exsanguination 30 minutes post-injection. The brains were removed and were dissected into cerebellum, brain stem, cerebral cortex, thalamus, and hypothalamus. Each of these brain areas from the seven rats was then pooled. Livers were also removed and pooled. Counts were made using a gasometric technique on each of the pooled samples after converting the carbon of the samples to CO_2. The following figures show the distribution expressed as percentages of theoretically equal distribution throughout the body: hypothalamus, 16; cerebellum, 26; brain stem, 17; cortex, 31; thalamus, 28; and liver, 135. This distribution appears to parallel the vascularity of the brain somewhat. From your data, Dr. Cerletti, it is clear that we should have killed animals earlier, perhaps 5 or 10 minutes post-injection instead of after 30 minutes, in order to have obtained more counts for each of the samples.

Leake: Wouldn't you say, though, that from the total amount of data that has been given on it, LSD seems to localize in the reticuloendothelial system?

Hoagland: Yes; I think so.

Seevers: Dr. Cerletti, I have a question concerning bile excretion. Do you have evidence that most of this is limited to bile?

Cerletti: Exclusively. All that we get in the gastrointestinal tract comes from the bile. It cannot be distributed by the blood. We made sections of the intestine and we could follow the radioactivity from the upper part of the duodenum downward. By a bile fistula, this is shown directly. I think that Dr. Hodge has recovered from the bile 70 per cent of the total injected dose within 2 hours.

Seevers: Is it a pure substance or is it a glucuronide? Do you know whether it occurs as a glucuronide?

Cerletti: This is not known. It has not yet been analyzed.

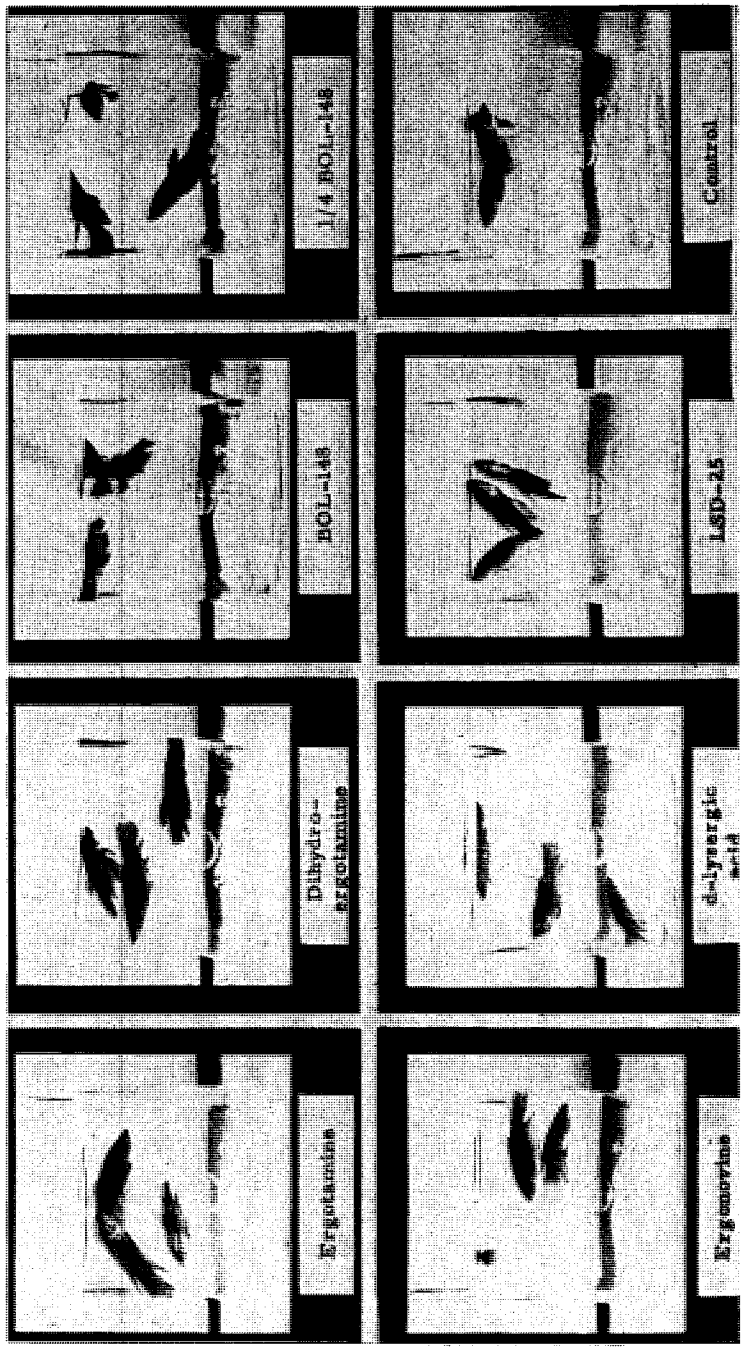

FIGURE 28. A comparison of the effect of 5 milliequivalents of lysergic acid and some of its derivatives on Siamese fighting fish. Only LSD-25 produces the typical angulation at the surface.

Leake: Then, relatively little goes out in the urine?

Cerletti: In the urine, after 12 hours, I think about 2 per cent of the total activity has been found. In the feces, also 2 or 3 per cent are excreted, and as expired CO_2 about the same amount leaves the body, so that the figure for the total excretion amounts to from 7 to 8 per cent within 12 hours. In contrast to this, after 2 hours from 60 to 70 per cent is already in the gastrointestinal tract, and after 12 hours this value may be higher. I will check what you said, Dr. Hoagland. The highest values of the radioactivity in the brain were found after 10 minutes, and by the biological assay the same observation was made.

Hoagland: Yes; we certainly should have used a shorter time interval.

Cerletti: You should have killed the animals earlier.

Kety: In relation to the data presented by Dr. Hoagland, he has mentioned two reservations: The first is that he did not know what percentage of the LSD that he had measured was in the blood vessel and what percentage was in the brain. My comment on that is that the LSD must have been largely in the brain tissue since the blood makes up only from 2 to 3 per cent of the total, and Dr. Hoagland's values for brain were greatly in excess of that quantity which could be accounted for by the blood content. The other reservation that he mentioned was that he did not know to what extent his data might have been the result of differences in local blood flow. I should point out that if one waits 30 minutes, as he did, then blood flow is no longer a limiting factor in the concentration developing in the brain. By that time, with a freely diffusible substance, even the slowest areas of the brain would have come to complete equilibrium. If one had taken these data at the end of one minute, then the distribution would have been highly sensitive to blood flow. The differential distribution after 30 minutes is more likely to be limited by local differences in permeability, solubility, affinity, or utilization of the drug.

Leake: It would seem to me that these figures are all indices of chemical uptake by particular tissues, and their distribution in respect to the reticuloendothelial system and other tissues is simply of the general nonspecific type for compounds of that sort, as Dr. Marrazzi has pointed out; so I don't think there is anything particular to be gained by further studies of distribution of LSD.

Hoagland: Yes; I think that is true.

Abramson: I would like to show the specificity of the action of LSD-25 compared with some other ergot compounds that I have not as yet mentioned. I said that I thought Dr. Cerletti's data were rather significant. Figure 28 compares the typical LSD-25 response in the fish, nose up and tail down, and many of the other phenomena. A dose of

5 μg./ml. was given and observations were made an hour afterward. BOL-148, incidentally, is extremely toxic; it kills fish but as I have mentioned, it does not act in the same way on snails. BOL-148, which is the brom-diethylamide, closes the shells of the snails, whereas LSD-25 forces them open and keeps them so. There are pigment changes in the fish which Dr. Evans has studied very carefully, and I hope that he will report on them in detail some time in the future. Once this phenomenon has been seen, it is unmistakable.

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