THE EFFECT OF RESPIRATORY POISONS AND ANOXIA ON SIAMESE FIGHTING FISH IN RELATION TO LSD-25 REACTION

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I assume that the action of LSD-25 (or lysergic acid diethylamide) on man is well known by now, and I need not dwell on it. Whether or not it produces a psychosis that resembles schizophrenia is not a suitable topic for us at this time. However, I assume that it is rather important that we know, if possible, how LSD-25 and similar drugs work on man, even if we must work on lower animals to do so.

The work I shall discuss is still in progress at Cold Spring Harbor, and it is being done in collaboration with Mr. B. Weiss, who is a medical student in the College of Physicians and Surgeons at Columbia, and Mrs. M. O. Baron, who has been working with me for some years on the Siamese fighting fish (1,2,3,4).

Figure 1 illustrates the technique of studying Siamese fighting fish,

Figure 1. Characteristic position of Siamese fighting fish in LSD-25 compared with some other ergot compounds, 5µg/ml. of each compound in the outside liquid.
Figure 2. Characteristic positions of Siamese fighting fish from the same experiment illustrated in Figure 1.
using drugs of this type, and it also shows the characteristic reaction. The ergot drugs, such as ergotamine, dihydroergotamine, 2-brom-lyseric acid diethylamide, and ergonovine, do not alter the position of the fish in the way in which LSD-25 does. If you were watching the fish, you would see that these were swimming around fairly normally, but the fish under the influence of LSD-25 go into a quiescent state or stupor and show many other characteristics, such as kink in the tail, a change in pigmentation, barrel roll, and the Cartesian diver effect. They go up and down in an almost vertical position. This is very easily observed in 1 µg./ml. of LSD in the outside liquid. It is possible to detect about 0.1 µg. of LSD-25 in this way.

Figure 2 illustrates the same experiment at a different time. Again, the nose-up, tail-down position is seen, with the angle of the fish at the surface at about 45 degrees or more.

Figure 3 illustrates per cent response-time curves that are used as the basis of the bio-assay. These are data from about 240 fish. At about 15 minutes, 70 to 100 per cent of the fish are in the nose-up, tail-down position, with 1 µg./ml. in the outside liquid. Since quite a number of fish died in these experiments, we never reach 100 per cent reaction. This family of curves represents the bio-assay technique which we use routinely in all our experiments.

**Hoagland:** How do the points fit on those curves, Dr. Abramson? How are the curves drawn?

**Abramson:** The curves were freely drawn through the points illustrated in Figure 4, showing the raw data.

**Hoagland:** Are they log relationships or semilog plots?

**Abramson:** Semilog plots will straighten some of them out. That system weights the curve according to probability, at both ends, so you have a truer representation of the data, actually.
Abramson: We found that logarithmic plots didn’t help us in our thinking; that in extrapolating from these data to man, which we do all the time, the actual data were more helpful in our planning experiments on man. But I quite agree that if you were to plot them semilog, in many cases you would get a smoother curve.
**Respiratory Poisons on Siamese Fighting Fish**

**Figure 4.** Raw data upon which the smooth curves of Figure 3 are drawn.

_Marrazzi:_ You could get the slopes, which you can't do here.

_Abramson:_ I quite agree that there might be some advantage in plotting the slopes, but in our present approach it has not been so. We did that originally with all the data, but we found it more expedient for our purposes not to plot logarithmically.

Part of this presentation is a comparison of drugs unrelated to LSD, which act something like LSD. In that way, I hope to get at the mechanism by which LSD acts in the fish and in man. This introduction is merely for instructional purposes, to illustrate the way the bio-assays are
Figure 5. Effect of various concentrations of 1-methyl-LSD-25 on the Siamese fighting fish. Starting points of curves are first readings at 15 minutes.

carried out in the laboratories. We carry them out on the basis of the way the Siamese fighting fish reacts to LSD itself, as a function of time, at different concentrations of LSD in the outside liquid. We do not inject the fish with LSD; it's too much trouble. We get a lot of fairly accurate data by neglecting diffusion into the fish, for example.
If there is 0.1 \( \mu g/\text{ml.} \) of LSD-25 in the outside liquid under our conditions, we get a curve that will look something like this; that is, in 1 hour, about 50 per cent of the fish are nose-up, tail-down. We use
### TABLE I
Response Index of LSD, MLD, and ALD*

<table>
<thead>
<tr>
<th>Subject</th>
<th>LSD</th>
<th>MLD</th>
<th>ALD</th>
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<tr>
<td></td>
<td>Date</td>
<td>Dosage (µg)</td>
<td>Response Index</td>
</tr>
<tr>
<td>P.B.</td>
<td>5/25/56</td>
<td>35</td>
<td>.69</td>
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<td></td>
<td>2/8/57</td>
<td>35</td>
<td>.43</td>
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<tr>
<td>C.G.</td>
<td>5/25/56</td>
<td>35</td>
<td>.63</td>
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<td></td>
<td>2/8/57</td>
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<td>.71</td>
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<td>J.G.</td>
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<td>D.V.G.</td>
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*Response index = n/µg, where n equals the number of positive questionnaire responses.
more criteria for the bio-assay than that, but that is the criterion I am going to use in this presentation, and that is why these first figures are shown.

Hoagland: And these crosses and circles are drugs that are conjugates of LSD, but they don’t give the same curves, and you are comparing them?

Abramson: No, they give approximately the same shaped curve. ALD at 10 µg./ml. is weaker, probably corresponding to 1 µg./ml. of LSD here. With 1 µg./ml. of LSD, all the fish usually go up to the surface in about 10 minutes; nine or ten fish are nose-up, tail-down, and stuporous, with periods of excitement. If there is more than 1 µg./ml. in the outside liquid, the solution studied has to be diluted if the reference curves in Figure 3 are to be used.

Bain: Does this mean that the acetyl LSD is actually weaker, or just more slowly absorbed by the fish?

Abramson: If we take a 4-hour criterion, and the fact that the curve has reached that level, it can be either weaker or more slowly absorbed; we have no data on that. But it acts very much like LSD; it is indistinguishable with our methods. We don’t have data on a more quantitative comparison on fish, but this led us to study it in man, which is somewhat more difficult. Table 1 summarizes these data on man.

Leake: Of course, it is not only the rate of absorption that may be different, but also the rate of destruction; that can be more rapid.

Abramson: That is correct. I am told, though, by the members of a large pharmaceutical house that they don’t believe that the acetyl group would be hydrolyzed off or destroyed any more rapidly.

Bain: If it is hydrolyzed off, you get the parent compound back. So there ought to be a big jump in activity, if that is the mode of destruction.

Abramson: That’s right; but, in man, on MLD-41 and ALD-52, the data are much more like LSD itself. These are really just a proving ground for the data on man.

Gerard: Your response to Dr. Hoagland, that the conjugates of LSD give approximately the same shaped curve, destroyed my initial perception that the LSD curves were included not merely for dose comparisons but, more explicitly, to bring out that curves are not the same shape. Your answer to Dr. Marrazzi seemed to be that a semilog plot, to straighten out the LSD, would give power-function curves for the ALD.

Abramson: I would agree that you might have power-function curves; yet, we can’t run 200 fish for ALD.

Gerard: Do you regard those curves as of the same family? The crosses look like a straight line, the full circles look like a straight line, and the others don’t look like anything.
Abramson: I’ll let you be the judge of that. I think there are so many factors involved, such as those brought out by Dr. Bain, for instance, that I have been content to look at the data, as I mentioned, and not draw any conclusions as to rate of diffusion or rate of excretion.

Gerard: I’m just asking about the looks of the curves. You would say they belong to the same family?

Abramson: I would say, in general, that they belonged to the same family.

Marazzi: But the slopes are different.

Abramson: I would say, if you wanted to study 200 to 300 standardized fish, that you might get a curve worth analyzing that way.

Robins: In connection with what Dr. Gerard asked, Dr. Abramson, are there compounds unrelated to LSD which will do something similar?

Abramson: That is the purpose of the presentation.

Robins: The reason I asked that was because of the shape of the curve. It was a little different, and if no unrelated compound does it—

Abramson: I’ll take that up shortly.

Fremont-Smith: How many fish are represented by each dot or cross?

Abramson: Each of these, of course, is approximately one experiment. Each dot usually represents 10 fish, and each series of experiments involves about 100 or 150 fish.

Hoagland: So your percentage is based on 10 fish?

Abramson: Yes. The percentage is usually based on 10 in a single experiment, though there is occasionally a death.

Grenell: Do you always have the same number of fish per jar?

Abramson: Not always. Sometimes the fish die, or we run out of them.

Grenell: But do you start out with the intent of having the same number of fish?

Abramson: Yes, but it depends on the experiment. I will now come to the main purpose of the experiment. We have recently been studying the effects on the Siamese fighting fish of potassium cyanide, sodium azide, hydrogen sulfide, hydrazine, and oxygen lack, among other respiration inhibitors. In addition, we have been studying oxidation reduction systems. We have not as yet studied carbon monoxide.

On the basis of our data on probably several hundred fish, we now think that potassium cyanide acts on the Siamese fighting fish in a way which is very similar to that of LSD-25. Although our experiments are not complete, it would also appear that sodium azide is even more powerful than potassium cyanide in producing the nose-up, tail-down position so characteristic of LSD-25.

Leake: Is that comparison on a molal basis?
Abramson: I will come to that. I haven't given you the quantitative data, but that is a very good point.

Marrazzi: Does the similarity include the Cartesian diver effect?

Abramson: Yes, but it does not include the kink in the tail, as I shall point out. There are differences, and these differences are very important from the point of view of studying the effect of these drugs on a vertebrate.

Thus far, hydroxylamine and hydrazine sulfate, in much higher concentrations, were not as effective as potassium cyanide. Dr. Magoun, I believe, asked at the 1956 Conference whether or not the oxygen had anything to do with this, and I am prepared to say from preliminary experiments that it does; not alone the oxygen, but the whole oxidation process. Anoxia will make the fish go to the surface in the nose-up, tail-down position, and so will asphyxia. I have done some experiments recently on asphyxia, and the process is reversible.

Elliott: How about an anesthetic?

Abramson: Anesthetics are quite different. None of those that we have used produces this effect.

Alles: In what position is the fish when it is dead?

Abramson: They usually go to the bottom, many dying nose-up, tail-down, as a result of an overdose of potassium cyanide.

For the purpose of this study, four criteria were used: the nose-up, tail-down position at the surface, as shown in Figure 1; fish at the surface but approximately parallel to the surface; fish at the bottom; and kink in the tail. We happen to like those four criteria. Some other laboratory, I am quite sure, would pick out four others, one of which might be pigmentation. But they would probably include the nose-up, tail-down position, with the body at an angle of approximately 45 degrees or more.

A rather important finding was the distinction between potassium cyanide and LSD-25: the kink in the tail was not observed with potassium cyanide. Observations were always made every 15 or 30 minutes, and in a typical experiment, ten fish were usually exposed to the drug. The temperature of the water was between 77° and 80°F.

Next, I would like to discuss potassium cyanide, because of its very great importance in the study of enzyme systems. The concentration of potassium cyanide is analogous to those used to obtain readily observable LSD effects; that is, 1 μg./ml. in the outside liquid. As shown in Figure 7, the fish act in general as if they were under LSD-25, rising to the surface and assuming a nose-up, tail-down position. Compare KCN in Figure 7 with LSD-25. The fish under LSD-25 are nose-up, tail-down, at the surface of the liquid. The fish here have probably been exposed.
for about 15 to 20 minutes. There is a dead fish on the bottom of two jars. The typical Cartesian diver effect, in which the fish assumes a vertical position in a semistupor, is well illustrated in the cyanide jar.

Fremont-Smith: And then sinks?
Abramson: He may sink.
Marrazzi: And then rises?
Abramson: You see, these fish (KCN) look more like LSD than the LSD itself. They are vertical at the surface, and the angle is greater than 45 degrees. But there are differences which are discerned in double-blind tests conducted by the team running these assays routinely. My research assistant, who has had the most experience, can distinguish between the potassium cyanide and LSD quite readily.

Elliott: Do you use 1 mg. did you say?
Abramson: No, 1 µg./ml. in the outside liquid.
Elliott: Was it neutralized?
Abramson: No.
Elliott: That would make about 1.5 millimolar or maybe a little less?
Abramson: That's right.
Elliott: So you don't feel that the pH would be different?
Abramson: The pH might be involved. We usually measure the pH, and if it is much below 6, or much above 7, we neutralize it.

Leake: But that wasn't the same molar concentration equivalent, then, between KCN and the LSD?
Abramson: No, it is not.
Leake: What is the range of difference?
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Abramson: I would say that the molecular weight of the LSD is approximately five times that of the potassium cyanide; so the molarity of the potassium cyanide is about five times that of LSD.

Leake: Then, in a comparison of the two drugs, that would be a quantitative distinction?

Abramson: I don't believe so, because we have other factors such as oxidation rate, diffusion, etc. I feel that it is of the same order of magnitude.

Cantoni: Isn't it much less than that?

Elliott: The correct figure for the cyanide concentration is 15 micromolar, 15x10^-8 M.

Abramson: I have something else in this connection. Sodium azide is effective at a lower concentration. Actually 1 μg./ml. of KCN is not needed. This effect also occurs with less than half the concentration. Figure 7 is a good representation of the phenomena.

Robins: Is that a fifty-fold difference you are counting when you're doing this?

Abramson: No, a five-fold.

Robins: Is it 0.1 μg./ml. of LSD that will do this?

Abramson: No, it takes a longer time to do it. The time element has to be considered.

Elliott: The cyanide would be very freshly made up?

Abramson: It is freshly made up or kept in the deep freeze.

Elliott: Because if you make up cyanide and neutralize it, you can drink it the next day.

Abramson: I think that you can drink cyanide at this concentration, anyway, can't you?

Elliott: Probably. But I'm just wondering whether, if you kept it at all, it might not be the cyanide that had the effect.

Abramson: I do feel that we really don't know whether or not it is the cyanide, but we get the same results if we make it up fresh and do it at once.

Elliott: Yes, but you might have ammonium formate.

Abramson: I am quite sure that we must have some ammonium formate, because I assume that reaction starts at once, doesn't it?

Elliott: I guess so. I haven't done it myself.

Abramson: It must have a reaction velocity. That is one of the impurities that is formed. But I don't think we have much ammonium formate when we make it up and use it within an hour, do we?

Elliott: I guess you still have some cyanide there, anyway.

Abramson: Yes, I think so.
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Abood: In Chicago, there is enough copper in our water to react with all that.

Abramson: We use distilled water, but I am sure that there is some copper in our distilled water. Certain differences between the cyanide and the LSD response, such as the kinking of the tail, are readily observed. The excitatory stage does not occur as readily under KCN. LSD-25 stimulates the fish, but if the concentration of KCN is increased above 2 to 3 μg./ml. in the outside liquid, the fish become very excited, and often most of them die. The fish are more quiescent under potassium cyanide, and their stupor is much more pronounced. If the fish are disturbed and moved into other parts of the containing vessel by shaking, they do not usually rise to the top as soon as those under LSD, but under anoxia they do so immediately. It is very interesting to see the rapid clustering of the fish at the top, both under anoxia and asphyxia. However, the display of fins exposed to 1 μg./ml. of KCN is more evident, and there is greater swelling in the gill region. Apparently, the KCN is utilized more rapidly than LSD; or, as Dr. Elliott pointed out, it might be destroyed more rapidly, since the fish when transferred to fresh water recover faster from the KCN than they do from LSD-25, which lasts a very long time. Mrs. Baron, who works with the fish, says they appear to be healthier after they have been exposed to KCN than they do after LSD.

In connection with Dr. Elliott’s point, an old batch of KCN that we have apparently is better than a newer batch.

Hoagland: Better in the sense that it has more action?

Abramson: Yes, better in that it has more action per unit weight. The fish respond more to a smaller concentration, but we have some discrepancies which probably have to do with impurities, stabilization, etc., which I don’t think we can solve at this stage of our experiments.

To continue: I would like to discuss the effect of other oxidation inhibitors. Hydrogen sulfide at high concentrations, that is, 100 μg./ml., was lethal, but the surviving fish assumed a nose-up, tail-down position at the surface. I mentioned that hydroxylamine, thus far, has been without effect. Sodium azide, although more toxic than potassium cyanide, seems to be closer in its strength to that of LSD itself, and very fine curves were obtained. Figure 8 illustrates an experiment showing the effect of sodium azide. A negative result with hydrazine sulfate is given for comparison. We have not as yet studied carbon monoxide. I have already mentioned that anoxia and asphyxia produced very similar responses. As far as oxidation-reduction indicators and other dyes are concerned, we have done so few experiments that I really can’t give any systematic description, but I will talk about them in a moment.
Seevers: What do you mean by asphyxia? Carbon dioxide?
Abramson: Yes. I am distinguishing between anoxia and asphyxia in the following way: In anoxia, the carbon dioxide is not allowed to accumulate; in asphyxia, the carbon dioxide remains in the system, with decreasing oxygen supply also.
Seevers: Have you tried carbon dioxide alone?
Abramson: Not yet.
Pfeiffer: How many days does it take in a stoppered bottle?
Abramson: With ten fish in a volume of 250 ml. distilled water, and a volume of air at the top of about 32 ml., it takes about 12 hours. In fact, I recently started an experiment at about 6 p.m. but had to stop it at midnight because no anoxia effects were observed.
Pfeiffer: Betta splendens is a fighting fish. Why don’t they kill each other, the way they’re supposed to...
Abramson: These are juvenile forms. The adults do attack and kill each other, but the juvenile forms rarely do.
Hoagland: Are these all males?
Abramson: No, they are males and females. It is very hard when they are in the juvenile form to tell which is male and which is female.
Elliott: Do these fish show any particular orientation to light? Could this be a perversion of their orientation to light?
Abramson: We have not studied heliotropism systematically in the fish. However, our illumination is a fluorescent lamp, and we use polaroid film with a Weston rating of 400 to take these pictures.
Bond: Are they much more active in the light?
Abramson: Yes.
Bond: Our experience is that they have been much more active in the light.
Abramson: If floodlights are turned on, the fish are very active. I have also taken motion pictures with fluorescent lighting, and it does not influence their activity. Dr. Evans (1,2) made the first observations on these fish with me some years ago, and we were both very much impressed with the stimulating effect of light on the fish. Therefore, we usually keep the illumination as low as possible.
Leake: What is the status of the swim bladder in these fish?
Abramson: You have raised a very important question, but we have not studied it.
Hoagland: Do these particular fish have swim bladders?
Abramson: Yes, they do.
Leake: If I remember correctly, it was Hall (5) who showed, some 30 years ago, the sensitivity of the swim bladder to variations in oxygen content of water.
Abramson: Minor changes in oxygen do not affect the fish, apparently. We studied that very simply by varying the pressure within a closed vessel, and we got no changes.

Hoagland: Yes, but the swim bladder does work in concentrating oxygen. Any toxic agent that might affect cell respiration and permeability might be expected to be reflected in the ability of the bladder to concentrate oxygen. You might have a very sensitive indicator here, in terms of the fish's orientation up and down, of the action of the swim bladder mechanism.

Abramson: Yes, I think the swim bladder is involved. The fish that have been stimulated can swim around and fight; that is, if the fish under LSD are stimulated, they go into combat, but only for a short period. Then they resume their nose-up, tail-down position and the stuporous state. Of course, they may be killed by a fish that has not been treated with the drug.

Figure 9 illustrates an experiment with an oxidizing agent which is toxic—potassium permanganate—and shows that a substance which probably produces gill injury causes some of the fish to go up to the surface, but their action is very different.

Methylene blue in a concentration of 100 µg./ml. produced a suggestive LSD reaction. Bindschedler's green produced a noticeable excitatory period in the fish, and 20 µg./ml. produced the nose-up, tail-down position, with no kink in the tail, in a reasonable time.

We studied other oxidation-reduction indicators; and we have found that gentian violet also produced similar effects. I must be frank and say that we don't have enough data yet on the oxidation-reduction systems, and I am not sure that we should go further with them. I do feel, however, that the data which we are presently compiling on the oxidase and catalase inhibitors—that is, the potassium cyanide, the

Figure 9. A typical reaction of fish brought to surface by potassium permanganate. Note absence of nose-up, tail-down position.
sodium azide, and hydrazine—will be rather significant in understanding the action of LSD on the fish.

Leake: It seems that these fish were not able to detoxify potassium cyanide to the extent that mammals can. They show an effect with low concentration, yet they recover quickly when removed from it. How does the effect occur with potassium cyanide?

Abramson: It comes on in approximately the same time.

Leake: As LSD?

Abramson: Approximately the same as LSD, yes.

Fremont-Smith: You mean, in 10 to 20 minutes?

Abramson: Yes.

Leake: Do any of these fish die from this concentration of potassium cyanide?

Abramson: The death rate from low concentrations of potassium cyanide is not striking. The death rate from sodium azide is.

It has been often observed that human subjects under the influence of comparatively small doses of LSD-25 become confused and show other symptoms associated with anoxia. One of my subjects, who was a navigator during World War II, has taken LSD many times and reiterates that LSD reactions resemble those of anoxia. The effects of LSD-25 in man, therefore, are not incompatible with the assumption that LSD-25 in fish and in man act by poisoning some parts of the cytochrome oxidase, peroxidase, catalase, or other metal enzyme systems.

Although we have not yet had access to the data dealing with the effects of nonlethal doses of potassium cyanide on the mental state of man, the available data in the literature indicate that an exogenous psychosis, with loss of reality sense, is produced with nonlethal doses of potassium cyanide. Indeed, potassium cyanide has been used in the therapy of schizophrenia, apparently to stimulate the respiratory enzymes. It is worth while, on the basis of these data, to explore the possibility that not only may the LSD psychosis in man be connected with the poisoning of special oxidative enzymes, but also that schizophrenia may be connected with a similar process in which the respiratory enzymes of the brain are not functioning adequately. Perhaps shock therapy, nonspecifically, stimulates the cytochrome oxidase and/or related metal enzyme systems in the brain. It is hoped that with this type of theoretical consideration the schizophrenic process in man may ultimately be modified chemically in the direction of recovery.

Leake: This reminds me of the kind of thinking that we were doing many years ago in relation to morphine, when we tried to show some of the analogies between the action of morphine and an interference with various forms of intracellular oxidation. Loewenhart (6,7) studied the
action of cyanides, particularly in connection with stimulation of the central nervous system, even under conditions where the blood pressure or cerebrospinal fluid pressure was so great as to have knocked out all functioning of the central nervous system. For example, even in instances where the blood pressure can be thrown up high enough to stop the activity of the respiratory center or any other center of that sort, relatively small amounts of sodium cyanide, injected intravenously, can act as a very strong stimulant to the respiratory center.

Once I was subject in an experiment in which I was lying blindfolded on a couch, and sodium cyanide was injected in me intravenously, so that I wouldn't know when it was going in.

Abramson: About 6 mg.?

Leake: It was somewhere around that. If the injection was slow enough, there was practically no effect at all. Subjectively, I was conscious of no change whatsoever. But if the rate of injection of sodium cyanide would exceed the rate at which detoxification would occur in the body, the first reaction would be an increase in the respiratory rate, of which I was not conscious. That, however, could be demonstrated on the kymographic tracing.

Hoagland: There is the measure for blood flow, too.

Leake: Yes, or for circulation time.

Hoagland: Circulation time is what I mean.

Leake: But the rate of detoxification of sodium cyanide seems to me to be an extremely important factor in the mechanism of its action. However, the validity of an analogy which stretches some sort of interference with an oxidative process into the mechanism of the action of a drug like LSD is dependent upon the extent of correspondence between LSD effects and those experimentally produced by some controlled interference with some known oxidative enzyme system. We had the same difficulty with morphine, and I don't think that it has been further explored.

Abramson: We have done some preliminary experiments on enzyme systems in vitro, and we have not been able to set up those systems showing an LSD metal enzyme reaction. But I have had no experience with cytochrome oxidase.

Hoagland: I believe that there have been studies of effects of LSD in vitro on the cytochrome oxidase system. I can't recall who did them, but I think the conclusion was that there is no particular evidence that LSD had any effect on the system in physiological amounts.

Key: Dr. Abramson, as I understand it, your thesis is that cyanide and azide produce in these fish behavioral effects suggestive of LSD?

Abramson: Very closely so.
Kety: And, therefore, you speculate that perhaps the LSD acts by interfering with oxidative metabolism?
Abramson: Yes.
Kety: And perhaps even schizophrenia. That much evidence, of course, is not entirely compelling because you may have a whole sequence of chemical processes involved in mental function, among the very first of which is the oxidative process, which leads to a dozen others. Different substances may interfere with different phenomena all along the line, producing the same end result. The mere fact that behavioral results are the same is, of course, no indication of identity of the site of action.

Furthermore, it might be worth while to cite some data which are not exactly compatible with that hypothesis, although they do not completely disprove it. With regard to LSD in the hallucinating man, Sokoloff and his associates (8) measured the over-all oxygen consumption of the brain and found that it is not significantly different from that in the normal state. A long time ago, we measured the over-all oxygen consumption of the brain in schizophrenia (9) and found it not significantly different from the normal state. Neither of these findings rules out the possibility of a highly localized effect on some area of the brain.

But since the oxidative process is one which is general throughout all cells in the body, if something hits cells like a sledge hammer the way cyanide does, by interfering with oxidation, it would be odd that its effects would be as selective and delicate as those of LSD and that at the height of its hallucinogenic effect there wasn't any over-all diminution in the oxygen consumption in the brain.

Gerard: Another point, Dr. Abramson. You asked me about the cytochrome oxidase, and this work reminded me of the early thirties when, on just such an hypothesis, we were trying methylene blue injections in schizophrenics, as well as thionine and other oxygen-substituting or electron-transporting molecules. There was a decreased oxygen consumption of schizophrenic brain, especially in layers 4 and 6, as I remember. The dyes produced some very interesting autonomic reactions, but didn't do much to the psychosis.

Abramson: Although LSD is called an hallucinogen, it really rarely produces hallucinations with low doses in the light. Whether or not the hallucinations are similar to those observed in psychosis is questionable. More important are the feelings of depersonalization, withdrawal, confusion, and paranoid thinking that are produced by LSD.

I would like to emphasize that the important effects of LSD are obtained at very low concentrations where an autonomic storm would
not be expected to occur. There is very little present in the brain, and I would be very much surprised if anything that went on could be detected with the over-all oxygen consumption approach. I really feel that phenomena of that type occur in highly localized and specialized areas of the brain.

Kety: You ought to have more evidence for it instead of merely indicating that the evidence against it doesn't rule it out.

Hoagland: I'd like to make a suggestion here. It deals with the idea of rate-limiting processes and differs from measuring total oxygen consumption. Suppose you have a series of reactions, as one does have, in connection with substrate metabolism, finally involving the cytochrome oxidase system. There are a number of enzymatically controlled steps involved in electron transfer and in removal of hydrogen. You have a number of steps involved here, the rates of which may differ in the system, so that one may be slow with respect to the others. If a drug modifies the relative rates of the intermediate steps, this is the type of thing you may be talking about, so that the actual rate-limiting process may be shifted, but the total, over-all oxygen consumption may not be changed appreciably.

Work that we did a number of years ago (10) showed that the activation energies in a chain of reactions can be predictably shifted by specific enzyme inhibitors. The slowest pacemaker step can be identified in terms of its activation energy. If cyanide is used, for example, a shift in the relative reaction rates can be obtained with the cytochrome system the slowest, yielding by temperature analysis its characteristic activation energy in vivo. This type of thing can be done here also. We did that with cyanide years ago. The enzyme system extracted from the beef heart showed the same rate as that found for a number of in vivo rates of rhythms of activities. This might be worth doing here to get activation energies and see whether one can get a shift actually characteristic of the cytochrome system, as we did with cyanide. In our experiments, the total oxygen consumption was not appreciably shifted. The activating energy of the over-all pace-making system was shifted.

Abramson: It is a very good idea.

Leake: With regard to the gross over-all symptoms seen as reflecting sensitivity at some local point, I want to point out that the respiratory center is very sensitive indeed to disturbances in any sort of an oxidative picture. Is there any shift that you could note in the respiratory activity of these fish?

Abramson: We don't have any method at present for studying them, although there are changes in the gills.

Leake: But you can observe the gill action, can you not?
Abramson: We don't have equipment for getting slow-motion pictures of them. But in man in certain subjects there is a change in the respiratory pattern.

Leake: With LSD?

Abramson: Yes, with LSD.

Leake: What is that change?

Abramson: Deep sighs, deep breaths, just as if cyanide had been injected to measure the circulation time.

Cantoni: But doesn't cyanide act on the carotid sinus?

Abramson: It is supposed to.

Cantoni: That is the effect about which Dr. Leake was talking; when it is injected very slowly, the sinus is not stimulated, but when it is injected fast enough, the sinus is stimulated.

Abramson: But somebody has done this very striking experiment in which potassium cyanide or sodium cyanide was injected in the carotid artery of a dog, and he has found that the venous blood on the opposite side was red. Do you remember who did that? A little bit of cyanide in the dog brain cuts out the oxygen uptake.

Fremont-Smith: Or increases the rate of flow. There has been just a hint of a suggestion that because the approach we are discussing resembles approaches made 20 or 18 or 15 years ago that were unsuccessful, this would therefore probably be unsuccessful. There are many reasons why any approach to schizophrenia is likely to be unsuccessful at the present time. But the history of scientific advance shows that there is no reason to feel that because a similar approach was made by somewhat different methods 20 years ago, this current approach is likely to fail. This kind of reasoning has been shown again and again to be invalid.

Leake: There is another point here in connection with schizophrenia which perhaps is anomalous in this discussion; that is, the administration of carbon dioxide to a schizophrenic patient will frequently result in clarification of the whole picture, at least for a few moments, until the carbon dioxide blood tension is reduced to average.

Fremont-Smith: It increases the rate of blood flow more than anything you could give to the brain. I have done experiments in which I gave CO₂ to patients and found that the retinal veins become bright red, and this, in humans, corresponds to animal experiments. If dilatation of the arterioles of the brain, increase in the rate of blood flow, and the delivery of whatever oxygen may be present is desired, CO₂ is the thing to do it. This could be the explanation of the stimulating effect of CO₂.

Leake: But there is another aspect of it; namely, that these same
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**TABLE II**

Comparison of Tolerance Production by MTDA 1-48 and BOL 1-48
concentrations which produce this effect in schizophrenic patients are preceded by anesthesia. As we showed in 1928 (11,12,13), and as Loevenhurt, Lorenz, and Waters (14) have also shown, carbon dioxide in concentrations of 20 to 30 percent in oxygen is anesthetic to animals and man.

Hoagland: We should remember also that giving schizophrenics oxygen isn't therapeutically beneficial.

Marchetti: There is a pertinent example of Dr. Kety's objection to merely stating the hypothesis without positive evidence. In anticholinesterase poisoning, for example, there are transient mental effects. The argument ran, perhaps, that this is the way LSD acts. Thompson, Tickner, and Webster (15) studied this very carefully and found that LSD does not have an action of any real significance on cerebral cholinesterase; so the objection is a very real one. An hypothesis can be constructed, but more positive evidence than the analogy is needed.

Abramson: I don't deny that we have to do more experiments. As a matter of fact, these experiments were begun in July, 1957.

Hoagland: Have you done anything with the other psychosomimetic agents in which we are interested?

Abramson: No. We are only working, and only plan to work for the present, with LSD-25 and its derivatives, such as the methyl derivative. We now have the dimethyl compound, which is very interesting.

Hoagland: Dimethyl tryptamine, dimethyl serotonin, and that sort of thing?

Abramson: No, the lysergic acid dimethyl amide instead of the 1-methyl. One finding that may be pertinent is that if we administer the MLD, that is, the 1-methyl derivative, to a nonpsychotic individual in increasing doses for about 10 days, we can essentially completely protect that man against 150 μg. of LSD taken orally. Since MLD is relatively nontoxic in man, its threshold being about three times that of LSD, we can administer large doses of MLD at home and have the subject return for LSD and be protected (16). (See Table II which illustrates this cross-tolerance phenomenon.)

Elliot: Can you protect him by repeated low doses or LSD?

Abramson: We have tried that, but we never achieved a complete protection, such as Dr. Isbell has reported with psychopaths at Lexington.

Pfeifer: We have. The dose should be spread out four times a day, starting with 4 μg., 4 times a day, up to 50 μg. Then we challenge with 100 or 150 μg., and there is no effect from the 100 or 150 μg.

Abramson: We have not succeeded in doing that.

Freemont-Smith: Do you use the same system that they use?
Abramson: Not exactly, but somewhat similar. For example, we have given 100 µg every day for 6 days. This should produce tolerance, but we always have a residual LSD effect, as indicated by the questionnaire results presented to this group previously.

Seevers: I would like to point out that the critical factor in tolerance development is the length of the dosing interval. If a drug-free interval is allowed, or administration is spaced so that the drug is present in minimal concentrations, both the rate and degree of tolerance development are greatly reduced. If rapid and complete tolerance is desired, it is necessary to administer the drugs at intervals frequent enough to exceed the detoxication rate, so that there is an overlap of pharmacological effect from one dose to the next.

Fremont-Smith: That is an interesting point, because you are suggesting that your questionnaire technique is a more sensitive method of showing an effect of LSD. But unless the same dosage and the same interval is used that has been used here, your test is not comparable.

Abramson: We did use small doses.

Fremont-Smith: I mean the same dosage, with the same intervals, in the light of what Dr. Seevers says about the tolerance. It would be quite interesting to repeat that.

Abramson: I am quite prepared to believe that LSD should produce complete tolerance. We just haven't been lucky enough to hit upon the technique as Dr. Pfeffer says he has.

Fremont-Smith: It would still be interesting to know whether or not your questionnaire would detect a residual effect, using that dosage under discussion. It would be very nice to repeat it exactly.

Abramson: Yes, it would.

Marrazzi: But your very important finding that you just described is a protection against LSD by an inactive substance, MLD.

Abramson: That's right. I did know that LSD almost protected against itself.

Marrazzi: But that may be an entirely different mechanism. That is an effect of tolerance.

Abramson: But this is tolerance, too. This is production of tolerance by the one method.

Marrazzi: But not in the technical sense. This is protection by an inert substance.

Abramson: It is only essentially inert, because of the way we use it.

Kety: But the same phenomenon has been produced by BOL (2-brom-LSD).

Abramson: It is true that BOL will produce some tolerance, but it
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is not more than one-third that of MLD. We have quantitative evidence of that. MLD is at least three times as potent, as Table II shows.

Elliott: I would like to take up the question of the cytochrome system. I feel rather strongly that one cannot pin anything on the cytochrome oxidase system without some other type of more direct evidence. I protest inwardly at the suggestion that the excitatory states are due to a stimulation of the cytochrome system. This, to my mind, is very hard to imagine, especially because an excitatory condition can arise and be explained under all sorts of conditions. Anoxia, particularly, is one condition which is known to cause excitation in the early stages, before depression sets in. That can be perfectly reasonably explained on the basis of anoxic depolarization, partial depolarization of cells, so that they would be more excitable. There is simply no reason at all, in my opinion, to postulate any stimulatory reaction on an enzyme system.

Hoagland: I am a little unclear as to just how the excitatory action was postulated.

Abramson: I wasn't aware that I said that. I said the fish were poisoned.

Elliott: You were explaining some transitory hyperactive states.

Abramson: I think I know where the misunderstanding conceivably might have arisen. I felt that incidental to the poisoning of the system, there is very often a period of stimulation preceding; I believe this is commonly taught.

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