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THE IDENTIFICATION OF SMALL QUANTITIES OF HALLUCINATORY SUBSTANCES IN BODY FLUIDS WITH THE SPIDER TEST

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The term "hallucinogens" was introduced by Hoffer et al. (9) to characterize substances which produce hallucinations in man. The authors enumerate six substances as belonging to this group, namely mescaline, lysergic acid diethylamide (LSD), harmine, ibogaine, hashish, and adrenochrome.

No clear criteria exist which would be useful in the classification of the hallucinogens. Though many drugs like the barbiturates and alcohol may cause hallucinations if given to the right person at the right time in the right dose, they are not classified as hallucinogens. However, a substance like mescaline, if given in a dose of 400 mg., or 40 micrograms of LSD given subcutaneously invariably cause hallucinations (3, 20). Other substances are less reliable in this respect.

All of these drugs have effects in addition to their hallucinatory properties, particularly on the autonomic nervous system. These "side effects" might appear before, during, or after the phase of hallucinations and their relationship to the psychological symptoms is not known.

The psychological changes brought about by the hallucinogens are so subtle, the variations of symptoms from person to person so great, that it has never been possible to establish whether a substance invariably causes the same characteristic response pattern in all persons. It might well be that a preformed individual reaction pattern is released in each person. To put it differently: when a test is made and an unknown hallucinogen is given, it has not been possible to say which drug has been applied.

In recent years an hypothesis, to be elaborated below, has stimulated interest in the hallucinogens. The drugs have been used to produce so-called model psychoses in man. It has been observed repeatedly that the changes which appear after the application of these drugs have a striking similarity to acute deliria. These so-called symptomatic psychoses can become manifest during hunger, cachexia, fever, and

after cerebral lesions. They appear also as hallucinatory episodes in chronic schizophrenia (4). In the pharmacological experiment a distinct relationship between the application of the drug to the person and the appearance of psychological changes can be seen, while in the case of acute deliria the symptoms might appear in the course of a chronic disease without apparent reason. The similarity of symptoms has led a number of observers to suspect that in acute deliria a substance similar to one of the hallucinogens might be present in the patient's body (10). A pathological metabolic pathway in the body of the diseased person might lead to the production of an abnormal substance in quantities sufficient to cause hallucinations. Such an hypothesis can only be substantiated if an hallucinogenic compound is found to be present in the patient.

Peters and Witt (14) have described a biological test which allows identification of most of the hallucinatory and some other psychotropic drugs. This method is at the same time sensitive enough to allow identification of small quantities of substances. A brief description and enumeration of results to date are in order.

The method uses differences in web-building behavior of the spider *Zilla-x-notata Cl.* before and after drug application.

The particular spider was chosen because it is commonly found in Switzerland and Germany where the method was developed. McCook (11) mentions its habitat in the United States as New England, New York, and California. It is easy to catch and lives outside its web in a self-made tube of thread. This tube is connected with the center of the web by means of a signal thread which transmits vibrations and tension changes from the web to the forelegs of the spider (Fig. 1). Whenever *Zilla's* web is destroyed it will build a similar web in the same place close to its old nest. In contrast, the common garden spider (*Aranea diademata*), which lives in the center of its web, falls down when the web is destroyed, and builds a new web at a new location.

The following method is used to catch the spider and make it settle down in the laboratory: as soon as the characteristic web of *Zilla* has been discovered (outdoors near a wall or window) the spider is enticed to come into its web. This can be done by throwing a fly into the mesh or by means of touching the threads with a vibrating tuning fork. While the animal looks around for the prey the signal thread is disengaged from the nest and attached to a little paper bag. When the disappointed spider now leaves the web, it proceeds along the signal thread directly into the paper bag. A new nest found in that way will be accepted by the spider without difficulty. The web is now cut and the spider brought back into the laboratory in the paper bag, which is fastened in an upper corner of a wooden frame measuring 15 x 15

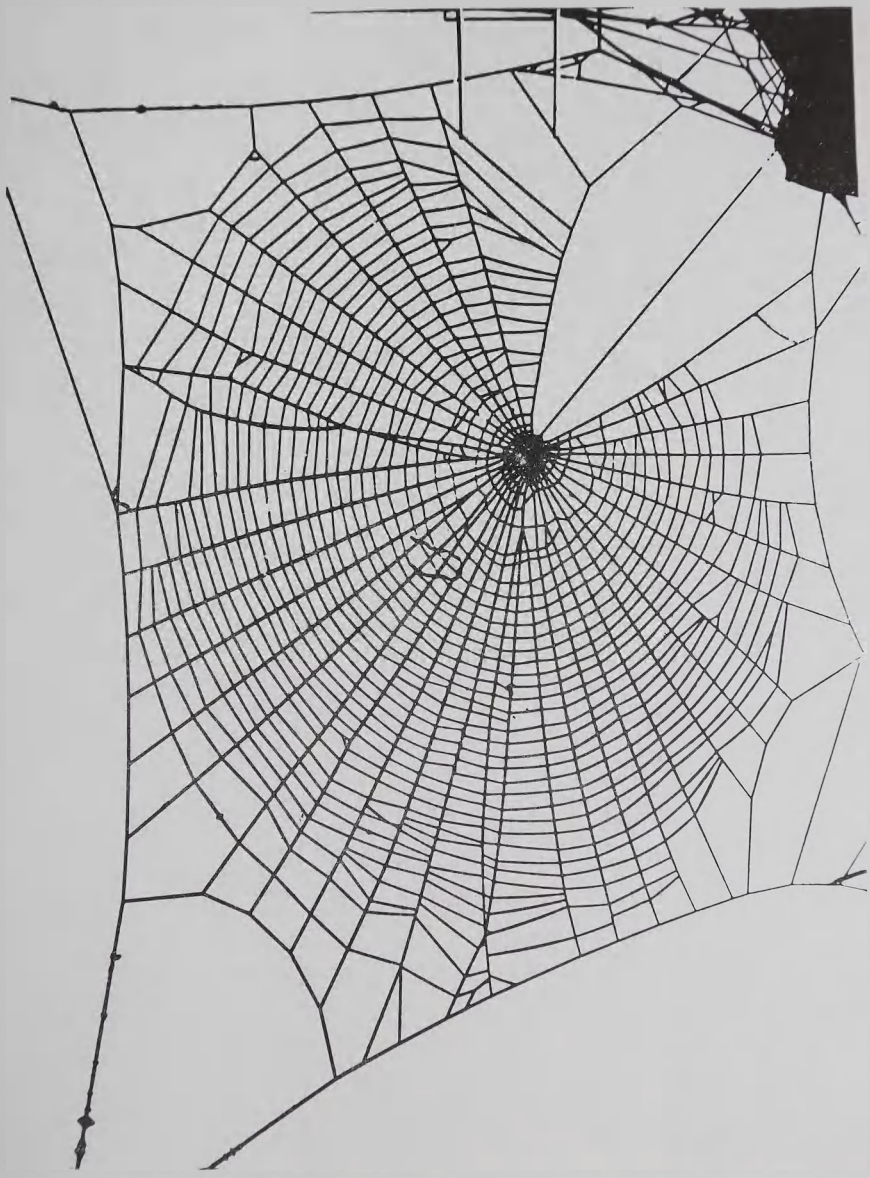

Fig. 1. Characteristic web of spider zilla-x-notata Cl. Thirty-six radii connect frame with hub; catching spiral leaves one sector free; signal thread divides free sector into two and connects spider in house at right upper corner via hub with all parts of web.

inches. If conditions are favorable and if the existing web is destroyed, a new web will be spun in this frame each night.

The method of catching described above indicates that the spider is an animal in which the sense of touch and vibration is predominant. The whole web may be regarded as an extended organ of touch, because the slightest change in its tension or a light vibration in the area of the net is transmitted via radii and signal thread to the spider. Predominance of the sense of touch and a poor sense of vision make spiders unable to discern between vibrations caused by a tuning fork and those caused by an insect. This was discovered by Boys in 1880 (6) and has since been used as a simple means of enticing spiders to come out of their house into the web. The animals recognize their error only when — expecting a fly — they bite into the metal of the tuning fork (12).

To prove the high specialization of the sense of touch Baltzer (2) performed another experiment: he showed that even an extremely hungry spider would not touch a fly that passed directly under its mouth if the fly's movements were not transmitted through a web. A spider sitting on a table or other solid surface would never attack its prey. Finally, Peters (13) eliminated the function of the eyes of some spiders by covering them with black lacquer. After this treatment the animals showed no change in behavior; they even built normal webs.

Thus it was shown that web-building as well as feeding is probably determined by the sense of touch alone. The spider measures distances by walking straight along a thread (21), it probes the tension of a thread by pulling it, and it orients the shape of the web according to gravity. The latter was shown by Peters (13), who turned the frame with the spider during the web-building. The web normally has its longer axis in the vertical direction. After it had been turned ninety degrees the spider added on in its new position until it was again longer in the vertical axis.

One of the purposes of the web is doubtless to bring the animal in touch with as much space as possible with as few threads as possible. But a web which could not catch and hold the insects which touch it would be useless. It actually provides the spider with all its food. This means that hunger is probably one of the stimuli that starts a spider on the web-building process. It has often been mentioned that satiated spiders will temporarily reduce the scale and frequency of their web-building, but no systematic investigation of this has been made. However, light and temperature also have a bearing on frequency of web-building. Spronk (19) put his spiders into a dark thermostat and found that web-building started during falls of tem-

perature or at the nightly temperature minimum. Witt (25) showed that constant temperature and constant light reduced web-building activity; a group of spiders kept at temperatures and light which changed with day and night rhythm built significantly more webs than any of the controls. Under optimal light and temperature conditions, constant feeding with 1–3 drosophila flies a day (the quantity adjusted to the size of spider) produced regular new webs every day from each individual.

The animals have to go undisturbed through all phases of catching the insect before they will accept the booty and suck its contents. With an ingenious method, Wolff (27) has imitated the movements, surface and taste of the prey so that the spider will drink various solutions without even suspecting that anything is amiss. Sugar was found to disguise nearly every strange drug, its taste proving highly attractive to spiders. Drugs were therefore dissolved in sugar water, injected into the empty abdomen of house flies, thrown into the web, and made to vibrate by means of a tuning fork. The spider would then come out of its house, find the lure, wrap it up, bite it and drink part of the contents. After the spider has finished drinking, the rest of the fly abdomen can be taken out and weighed, the difference in weight before and after drinking indicating the amount that has been ingested by the spider. By knowing the concentration of the substance in the fluid, the amount of drug that was actually taken up by the spider can easily be calculated.

Inhalation of volatile drugs has also been tried successfully (7, 18). However, it is difficult to give drugs by injection, because the chitinous outer membrane of the spider is irremediably damaged by the needle. By using a microsyringe and very fine needles, Wolff and Hempel (27) were able to reduce the mortality of spiders after injection to about 50 per cent.

It has already been mentioned that there is a certain time at which webs are built. This time is constant for all spiders working on the same day under the same conditions, and it changes with the seasonal changes of sunrise. If we know how long it takes for a certain drug to reach its peak effect in spiders, we can apply it just long enough before web-building time so as to allow maximum effects to be seen in the web. On the other hand, by applying a certain drug at different times on different days, the onset, course and end of the effect of a certain drug can be determined.

Only if the sequence of events in the spinning of a web is known can its changes be interpreted. Much work has been done to establish this sequence and to analyze the influences which determine special patterns and proportions in a web. Descriptions can be found in Wiehle

(23), Peters (13), Tilquin (21), Savory (17), Witt (25); for the present purpose it is sufficient to remember that at first a Y-structure is made. This Y is suspended in a frame which consists of particularly strong threads. In the subsequent building period the spider connects the center or hub with the frame by means of a great number of radii (Fig. 2). Finally, it fills in the thin thread of the sticky spiral, beginning at the outside and climbing from radius to radius until it gets near the hub. This spiral in its regularity and proportions is the most sensitive reagent to drugs (24).

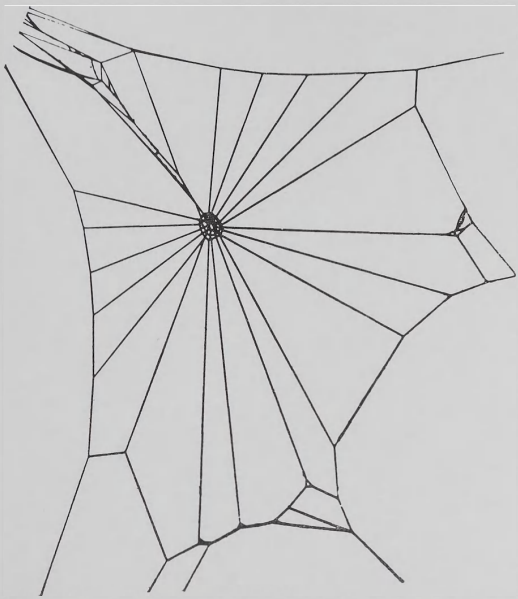

Fig. 2. Unfinished web of spider zilla-x-notata Cl. Frame, hub, and 18 radii have been built. Note house of spider in upper left corner.

The complete web is a fine record of the spider's movements during a certain period of time — normally about 20 to 30 minutes. By measuring and analyzing the web we actually measure the spider's movements. The movements again depend on the animal's ability to coordinate and translate incoming stimuli into outgoing signals. We measure possible lesions of all these functions in the web. The great number and complexity of functions involved in web-building is probably one of the reasons why relatively small doses of many drugs affect the web pattern.

A fresh web can hardly be seen and can easily be torn. It must therefore be made visible and preserved. In order to achieve the first purpose, little vessels with ammonia and hydrochloric acid are placed under the web. The rising fumes combine in the air and turn into ammonium chloride, forming a fine white film on the threads. We have now achieved good visibility without distortion. The pattern of each web is preserved on photographic film. The white threads stand out beautifully against a black background if light is directed onto the web from both sides. Care must be taken that the camera stands parallel to the web so that proportions are not distorted. With the help of enlarged or projected films, comparison of different webs built on different days by the same individual with or without drug influence can be made.

Statistical methods are used for evaluation of drug effects on web-building behavior. On any one day, some of the spiders receive the drug at a certain time; the others get nothing or only sugar water. The following two mornings the webs of all animals are again photographed,

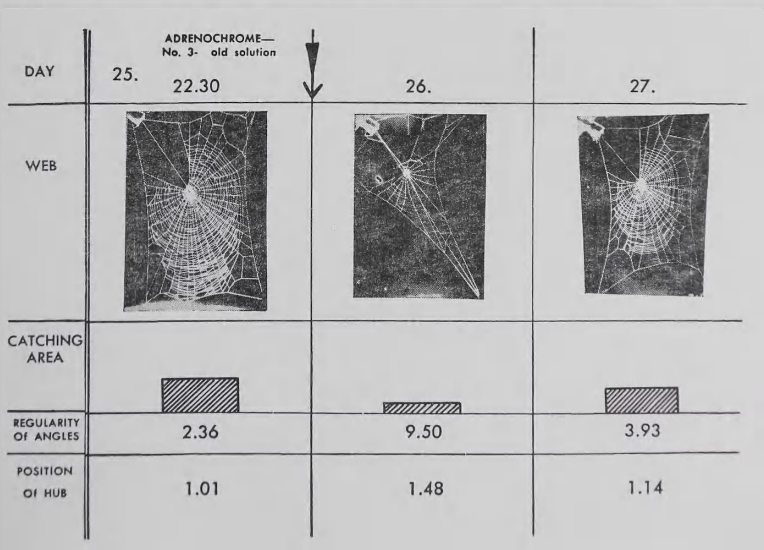

Fig. 3. Three different webs built by the same spider on three subsequent days; the drug was applied before the spider built the second web. Catching area, angles and position of hub were measured on the photographs and noted under each web (see text for further explanation). Note the similarity between web one and three and the abnormality of web two as a result of drug influence.

thus giving three photographs of three different webs per individual, one built before, one directly after, and one more than 24 hours after the drug administration (Fig. 3). Comparisons can be made among the three webs. Short-acting drugs, for instance, should cause a disturbance in the second web, while the first and third would be similar. By taking the mean figures of measurements made in the group of webs built under drug influence, we obtained reproducible effects of the drug independent of the individual. Comparing these with webs of spiders who built on the same days but did not receive any drug makes it possible to control for such influences as atmospheric changes.

A certain number of proportions, distances, and angles are known to recur fairly consistently in all webs, and standard deviations of these

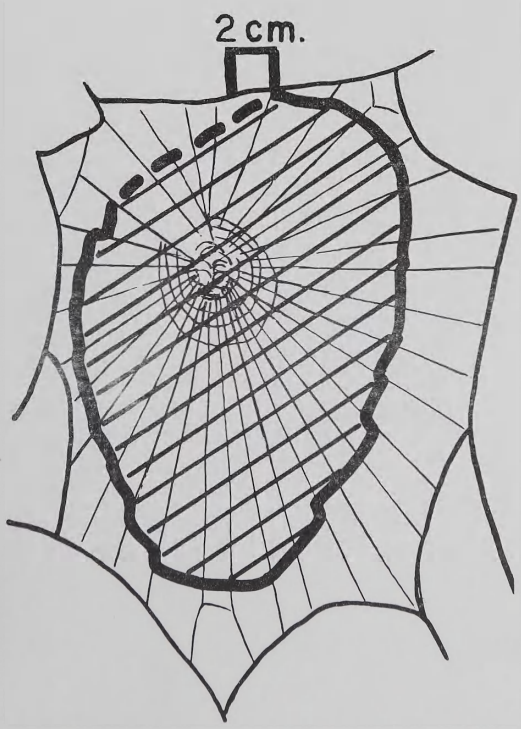

Fig. 4. The thick black line indicates the peripheral spiral thread which circumscribes the catching area. The "2 cm" mark on top was photographed together with the web so that original size can be calculated from the film.

figures have been calculated (25). Consistent deviations of one or more means have been found under the influence of certain drugs. The pattern of changed proportions is highly specific for each drug.

Measurements most frequently made in web photographs are:

1. The size of the catching area, measured with a planimeter along the peripheral turn of the spiral (Fig. 4). This area increases in size in the course of the spider's life, but is relatively stable from day to day.

2. The relationship between the horizontal and vertical diameter of the web. After drug application (Strychnine), changes have been observed in the value of the quotient of these two measures (27), as well as in the size of the standard deviations.

3. It has been observed that when the spider puts in radii it goes on doing this until it reaches the point where all angles are smaller in size than the sum of their two neighboring angles. After drug administration many oversized angles may be observed (Fig. 5). Their

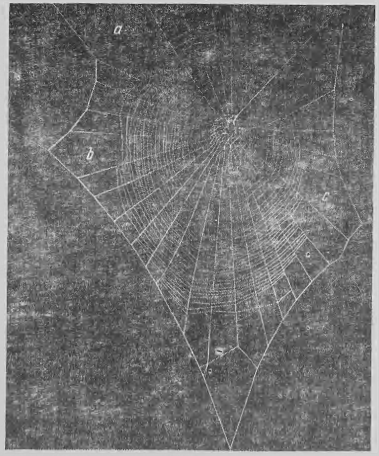

Fig. 5. In this web angles between radii at a, b, and c are called "oversized" because they are larger than the sum of their two neighboring angles.

number can be compared to the number of oversized angles in normal webs.

4. The size of neighboring angles between radii can be compared by calculating their quotient. If the angles are similar in size, the

quotient is one or near one. The means of all such quotients measured around the whole web should be near one in a regular web, higher or lower in drug webs.

5. The hub of the web may shift its position under the influence of drugs. This can be established by comparing the distance from nest to hub (a) to the distance from the hub to the opposite side of the web (b) in photographs taken before and after drug application (Fig. 6).

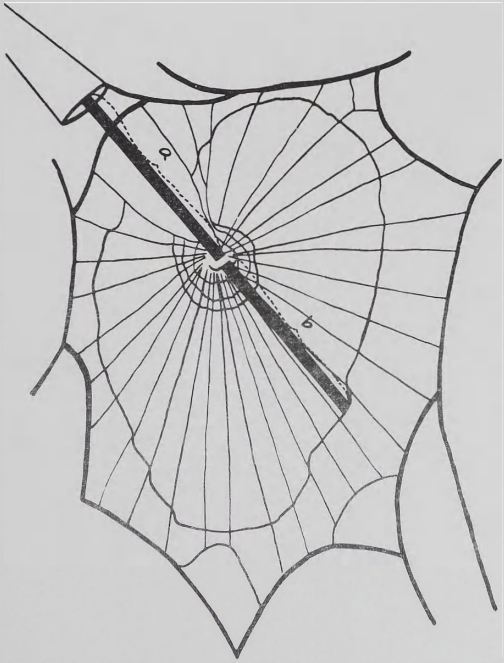

Fig. 6. Position of hub in web is calculated by dividing the length of (a) through that of (b).

6. The spiral is the finest indicator of the influence of any drug on the spider. Normally the distance between its turns decreases logarithmically from the periphery to the center (13) (Figs. 7 and 8). Slight disturbances can become manifest in the greater variation of distance from turn to turn (Pervitin, 15), while heavy disturbances lead to strangely deformed spirals with several centers or heavy digressions from the right direction (scopolamine, 27).

7. Finally, the frequency of web-building can be evaluated for each

spider. It has, for instance, been noted that a tranquilizer (chlorpromazine) interrupts the web-building totally for one or several days, the length of the resting period depending on the dose (8). There are physiological interruptions in web-building, like the periods of molting, which can be kept under control.

As soon as the method was published, investigations were started in a number of laboratories, applying the technique to problems in the chemistry of schizophrenia. Particular attention was given to the questions of whether the spider test could detect small amounts of hal-

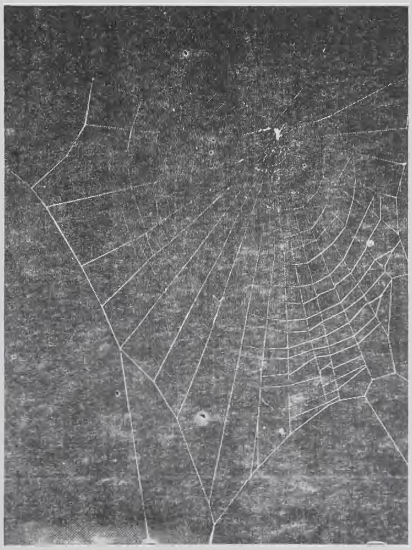

Fig. 7. Web built under the influence of mescaline; note increasing distance between turns as spiral approaches hub. Compare with Fig. 1.

lucinatory substances in the body fluids of mental patients, and what effects these substances have on behavior. That the spider test could detect the presence of behavior disturbing substances in the urine and that these substances could be recovered was suggested by two preliminary observations.

In one instance mescaline was added to a urine specimen. In the usual extraction process from 50 to 70 per cent of the mescaline was recovered.

At another time the urine of a patient had clearly shown the presence of a substance which disturbed the spider's web-building. A thorough

check showed that the patient had received 0.75 mgm. scopolamine on the previous evening. This finding was regarded as an indication that a substance like scopolamine, if present in the patient, could be recovered in the urine and identified by the spider test.

The following summarizes the procedure and results of two groups

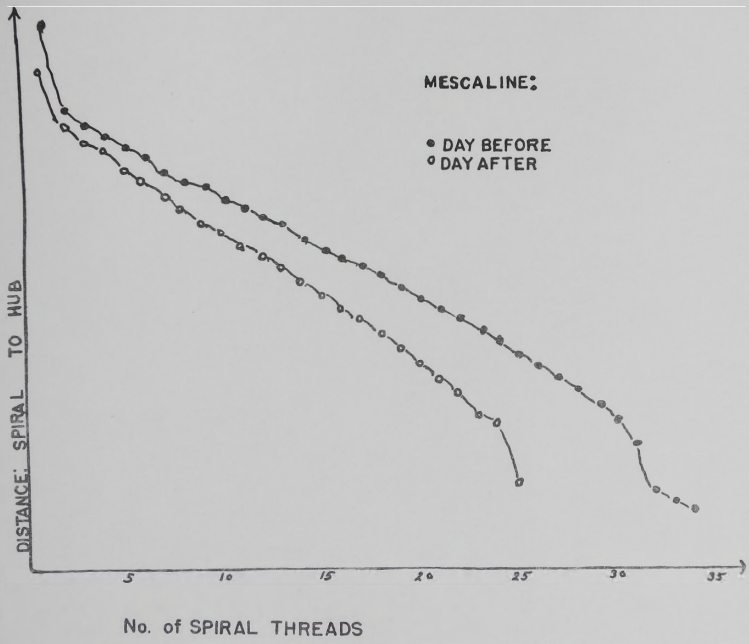

Fig. 8. Graphic comparison of distances between turns of spiral of normal and mescaline web built by the same spider. The graph shows the greater steepness of the spiral near the hub after mescaline. Compare with Fig. 7.

of experiments. The first was done by the author in cooperation with Dr. Manfred Bleuler, head of the department of psychiatry at the University of Zurich, and Dr. Rolf Weber, biochemist at the Theodor Kocher Institute of Medical Research at Bern (26). Dr. Bleuler chose from among his patients three subjects and two controls who had the same diet. Patient Number One was a chronic alcoholic with hallucinations; Number Two showed a symptomatic psychosis after an accident which had occurred two years earlier — he was semicomatose with hallucinations; Number Three had shown a sudden marked de-

terioration and had received a diagnosis of chronic schizophrenia with hallucinations.

Having established that the patients had not had any drug for 24 hours or more, we waited for their acute hallucinations and then took about 500 ml. of urine. The fresh urine was immediately frozen in order to avoid deterioration of labile substances. By means of ion exchange resins and other extraction methods, the urine was concentrated to 400 mgm. of solids consisting mainly of basic amines. It is interesting to note at this point that the urine of the three patients yielded between 200 and 400 mgm. of dry substances, while the urine of the control persons yielded about 30 mgm. substances each. Directly before the substance was given to the spiders it was dissolved in sugar water and a quantity corresponding to 1/150 of the total fed to each spider 6 or 11 hours before the time of web-building.

In the second group of experiments (16), the urine of a great number of patients was collected over many days, cooled and chemically separated into several fractions. This was done in the Basel Psychiatric Hospital by Dr. Georgi and co-workers under the assumptions that the illnesses of the patients were basically similar and that they had similar chronic metabolic disturbances. This increased sample enabled us to feed far more than 1/150 of the content of the urine to each spider; one assumed consequence of the increased dose was an increase in the amount of the hypothetical substances contained in the dose which each spider received. One difficulty with this procedure is the assumption that the same substance was present in all patients all the time with or without manifest hallucinations.

The spider test revealed no difference between the urine of the controls and the patients' urines in either experiment (26, 16). These results do not support the recent set of optimistic statements appearing in the press and popular magazines. The most recent of these appeared in *Time* magazine of May 6, 1957 and in a United Press release, in connection with the work of Nicholas A. Bercel. I quote: "Spiders fed with serum taken from patients suffering from the catatonic form of schizophrenia . . . seem to become catatonic too . . . the webs they spin are like the last vestiges of ragged lace. The spider's reaction, like that of human volunteers injected with schizophrenic serum (*Time*, May 14) shows that this disease is associated with a disorder in blood chemistry."

Certainly we may state that the urine of the three patients after their hallucinations contained smaller or no amounts of the known hallucinatory substances or substances of similar activity, than those known to disturb the spider's web-building. It is an advantage of the method that we can extend our statement to substances of similar ac-

tivity. It is a disadvantage that inactivated breakdown products of the active substances would not have been detected. A comparison of the minimal effective doses of certain substances in man to the dose that would have been detectable with the spider test is made in Table 1.

TABLE 1

Drug	Minimal effective dose * in gamma individual		The amount in gamma that would have been detected in the urine with the spider test **
	In spider	In man	
D-Lysergic Acid Diethylamide	0.03	10	9
Chlorpromazine	1.0	37 500	300
Adrenochrome (9)	4	500	1 200
Scopolamine	5	500	1 500
Pervitin	10	5 500	3 000
Strychnine	30	2 000	9 000
Xylopropamine (22)	40	2 500	12 000
Nembutal	40	50 000	12 000
Mescaline	100	400 000	30 000
Caffeine	100	100 000	30 000

* This dose is a rough approximation because the minimal effective dose varies considerably from one individual to the next, it depends on the criterion used for evaluaton, and it has never been clearly established for some of the drugs. The table gives the lowest dose which has shown an effect.

** The figures in this column are the figures in column 1 times 300. If we assume (according to the model experiment with mescaline) that 50 per cent of any amine that was in the original urine is still in the extract, and if we take into consideration the drinking of 1/150 of the total extract by each spider, a change in web-building behavior through the extract would have been caused by 1/300 of the quantity that was in the total urine. Each figure in column three that is smaller than the corresponding figure in column two shows that the minimal effective dose in man was not in the patients' urine.

Assuming that the patients in their severe toxic state contained more of the hypothetical substance than the minimal effective dose, considerable amounts might have been excreted and would have been identified by the spider test. This was clearly not the case.

The major aims of this paper were to present a new biological test and to apply that test to pertinent issues in the chemistry of schizophrenia. The evidence so far leads to confidence in the general technique. With regard to the essentially negative finding from the urine of patients and its relationship to the hypothesis of toxicity, the following questions come to mind.

1. Is it necessary to postulate a toxic substance or could we not just as well assume lack of a normal substance or substances? This is reminiscent of the serotonin hypothesis (28).

2. Is this hypothetical substance evenly distributed over all extracellular water of the body and is it excreted in the urine?

   a. Could it be produced and destroyed locally like acetylcholine?
   b. Could it be excreted in the urine in inert form?
   c. Could it be excreted in the urine but not extracted by our method of preparation?

d. Could it be active only as long as it is bound to proteins? (Mescaline, 5)

e. Could it be a substance that is active in man but not in spiders?

These are only a few points that must be taken into consideration when interpreting our results. However, at the moment, there seems to be only one way to test the hypothesis, namely, to go on looking for the substance in urine, blood and maybe cerebrospinal fluid of many patients at many different times.

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