tone, evaporating a suitable aliquot, and chromatographing in the same manner. However, since the free cholesterol in the adrenal gland is relatively very low, one must use a large aliquot and dilute the ester fraction after chromatography if the free cholesterol level is desired.

Although the ester fraction can be determined by using the method of Brown *et al.* (*3*), the free adrenal cholesterol is accompanied by turbidity, which interferes slightly with this method. Turbidity is not observed with a modified Lieberman-Burchard procedure. Because of its sensitivity, the modified Tschugaeff reaction of Hanel and Dam (*4*) may be better suited to the determination of free cholesterol in adrenal tissue after chromatography on silicic acid.

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21 December 1956

Actions of d-Lysergic Acid Diethylamide and Its 2-Bromo Derivative on Heart of Venus mercenaria

Physiological evidence indicates that the inhibitor nerves of the heart of the mollusk, *Venus mercenaria,* are cholinergic in nature (*1*), and that the activity of the excitor nerves is mediated by 5-hydroxytryptamine (5-HT, serotonin) (*2*). *d*-Lysergic acid diethylamide (LSD) was found to be an antagonist of serotonin on certain mollusk hearts under the conditions of the experiments (*3*). An early report that LSD was an effective antagonist on the *Venus* heart was later modified (*4*) when it became clear that LSD had a marked excitor action on this heart. This action persisted for long periods of washing during which a maximum amplitude of beat obscured the action of large doses of serotonin. It was stated that on the *Venus* heart, LSD acts as an essentially irreversible analog of serotonin (*4*). Recently, Shaw and Woolley (*5*) confirmed this observation.

The importance of a proper understanding of the fundamental mode of action of LSD prompts us to report further our earlier studies with LSD and our more recent observations of the action of 2-bromo-lysergic acid diethylamide (Bol-148, bromo-LSD) on the *Venus* heart (*6*). At a concentration of $10^{-6}M$, both serotonin and LSD produce a nearly maximal increase in amplitude in less than 10 minutes. After a heart has been washed for a few minutes, it recovers from serotonin; but after many hours of washing a heart that has been treated with LSD may still be greatly excited. No way has yet been found, including washing at a high *p*H, to restore quickly an LSD-excited heart. At concentrations below $10^{-9}M$, serotonin seldom excites the isolated *Venus* heart. If hearts are allowed to remain in a 10-ml bath of $10^{-10}M$ LSD, they are maximally excited in 1.5 to 2 hours. At a $10^{-16}M$ concentration of LSD, up to 3 hours may be required for the heart to adsorb an amount of LSD that produces near maximal excitation. Axelrod *et al* (*7*), from studies of tissue distribution, calculate that LSD exerts its characteristic effect in man at a level of 0.0003 μg/g of brain tissue. The *Venus* heart responds maximally at a tissue concentration that must be below this, for 10 ml of $10^{-16}M$ LSD contains only 602,000 molecules.

An important problem not yet resolved is whether the "LSD psychosis" results from central blocking of serotonin, or from a serotoninlike action of LSD, or for other reasons. 2-Bromo-lysergic acid diethylamide may prove useful in helping to solve this problem. Cerletti and Rothlin (*8*) found bromo-LSD to be a more effective antagonist of serotonin than LSD at a number of sites in mammals. This blocking action was highly specific, and they saw no signs of antihistamine, antiadrenaline or antiacetylcholine action. Certain of these observations have been amply confirmed and extended (*9*). Cerletti and Rothlin, however, failed to find any indication of an abnormal psychic disturbance produced by doses of bromo-LSD even 20 times as great as effective doses of LSD. They concluded that their results with bromo-LSD make it difficult to correlate the psychic effects of LSD with its antiserotonin property. The interesting observation has now been made by Ginzel and Mayer-Gross (*10*) that bromo-LSD, when it is administered 1 or 2 days before LSD, abolishes or greatly reduces the LSD psychosis without, by itself, having significant central action even in 2- to 3-mg amounts.

On the *Venus* heart, bromo-LSD is an effective antagonist of serotonin. On some hearts, high concentrations (10^{-4} to $10^{-5}M$) have a weak stimulating action resembling that produced by LSD, while on others there is no apparent effect. However, after treatment of hearts with bromo-LSD in concentrations in the range of 10^{-4} to $10^{-6}M$ for 1 hour or longer, the excitor action of a molar concentration of serotonin one-tenth as great is completely blocked. It is of further interest that previous exposure of a *Venus* heart to bromo-LSD abolishes or greatly reduces the excitor action of LSD that is subsequently applied. For example, on some hearts, pretreatment with $10^{-4}M$ bromo-LSD may completely prevent the otherwise marked excitor action of $10^{-6}M$ LSD.

Serotonin appears to be a normal regulatory neurohumor of the *Venus* heart. This heart is extremely sensitive to LSD, which has an excitor action resembling that of serotonin. Unlike serotonin, however, the action of LSD is very slowly reversed by washing. Bromo-LSD antagonizes the actions of both serotonin and LSD on the *Venus* heart. These several actions and interactions appear to parallel rather closely those seen in the mammalian central nervous system.

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13 September 1956

Technique for Behavioral Analysis of Human Observing

The monitoring of a display (for example, a search radar) by human beings raises problems of considerable practical and theoretical interest. In general, the probability of detection of a signal varies directly with the signal rate; is a function of the temporal arrangement of the signals; and, in the case of low signal rates, varies inversely with the duration of the monitoring task. Such monitoring situations are badly in need of a descriptive behavioral analysis that would permit isolation of the variables which control the behavior underlying the probability of signal detection.

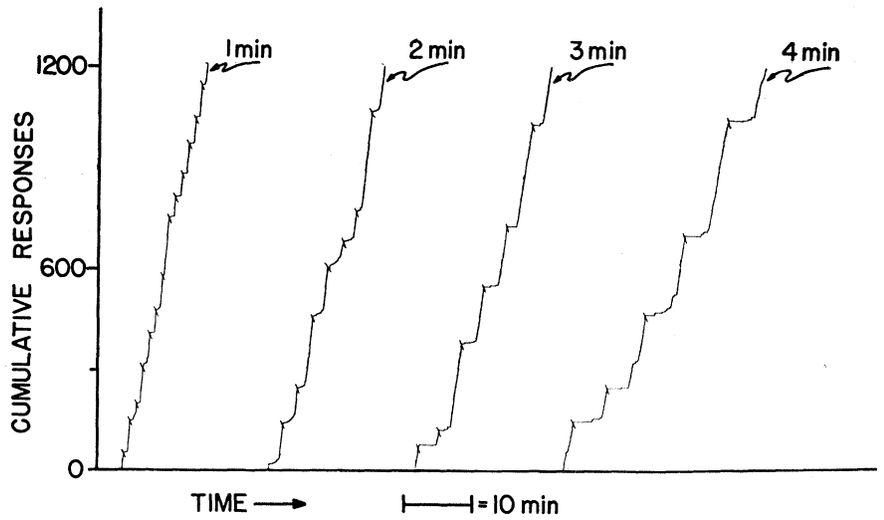

Fig. 1. Cumulative response records for 1-, 2-, 3-, and 4-minute fixed-interval schedules of pointer detections. Detections are indicated by lines cutting across the records.

It is here assumed that a detection can result only after the operator has provided the responses of "looking at" or "orienting to" the display. In other words, certain responses, here called *observing* responses, must be made in order that a signal can be observed and reported.

The present study serves to illustrate (i) that the techniques employed in operant conditioning of animals (see Skinner, *1*) can be useful in analyzing these observing responses and (ii) that the behavioral principles that have emerged from animal laboratories show promise of being rather directly applicable to the observing behavior of human beings. The proposed approach assumes that observing responses are controlled by detections of the signals for which the individual searches, and that this control is in the nature of reinforcement paralleling exactly the effect of food reinforcement upon operant responses that have been demonstrated with animals (*2*).

To pursue this analysis, human subjects were provided a task in which the observing response was easily recorded. Working in a dark room, they were required to observe and report deflections of a pointer on a dial that could be seen only after they had pressed a key which provided a brief flash of light, thereby illuminating the face of the dial. When the subject pressed the key (that is, emitted an observing response), the light flashed for a period of 0.07 sec, even if the subject held the key down. Thus, he had to release and redepress the key to obtain another look at the dial. When he observed a pointer deflection, he reported it by pressing another key that reset the pointer. The pointer remained deflected until this key was pressed.

Each subject was instructed that his only aim should be to make as many detections as he could and to reset the pointer as rapidly as possible. At the end of each session, he was informed of the number of detections made and the average time per detection. He was not informed that the experimenter was in any way concerned with the frequency with which he flashed the light. Actually, however, cumulative response records were made of his responses on the light-flashing key.

Deflections of the pointer were scheduled in many different ways analogous to the scheduling of food reinforcement with animals. Several of these have been successfully used and have provided data closely paralleling those found with more conventional reinforcement.

The control exerted by the detections of the pointer deflections can be illustrated by what has been termed a fixed-interval schedule. On this schedule, the pointer deflection, (or reinforcement) would occur at some set period of time after the last deflection. Five navy enlisted men serving as subjects were placed on fixed-interval schedules beginning with a ½-minute interval, after which the interval was gradually increased in blocks of eight 40-minute sessions to 1 minute, 2 minutes, 3 minutes, and finally to 4 minutes.

The data from portions of cumulative response records for one subject at each interval are presented in Fig. 1. Each curve is a segment of the record from the last session which the subject had on the indicated interval. The curves are displaced along the horizontal axis. Each time the subject pressed the key that flashed the light (that is, emitted an observing response) the recorder pen moved a very small step upward while the paper moved slowly to the left. Thus the slope of the line provides a direct indication of the rate of observing responses. Flat portions, then, indicate periods of no observing responses. Reports of detections are marked by the lines cutting across the curves. It should be noticed that after each detection observing responses cease for a while; then, after some time has elapsed, responding resumes in an accelerated manner, providing a scallop-shaped record of the observing rate. These scalloped records are also characteristic of animals working for food reinforcement on fixed-interval schedules.

A further demonstration of the nature of the control exerted by detections can be seen by examining what happens to the observing response rate after no more pointer deflections occur (that is, during extinction). In Fig. 2 data are presented for the same subject reported in Fig. 1. However, the subject first received three pointer deflections on the 4-minute schedule on which his behavior had been maintained for eight sessions, and then no further pointer deflections occurred. Following the detections, the typical scalloped records are seen; when deflections no longer occur, the high rate of observing continues for a time and then rather rapidly drops off. Again this finding is analogous to that obtained by using food reinforcement on animals and demonstrates that the observing rate is controlled by the detection of the signal for which the observer searches.

These and other results suggest that a behavioral analysis is possible and that observing behavior is controlled by the detections in the same manner that instrumental responses in animals are controlled by food reinforcement. If so, the findings of classical attention studies,

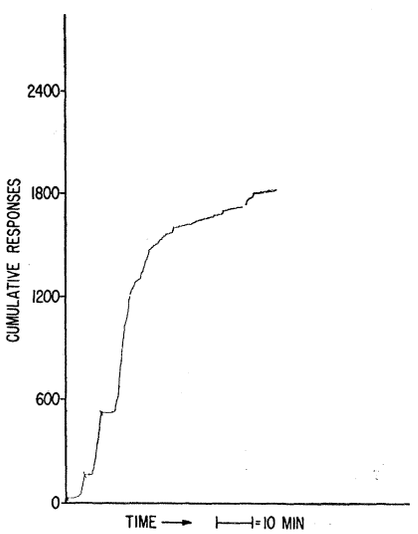

Fig. 2. Cumulative response record showing effect of withholding pointer deflections following fixed-interval schedule. After three detections (indicated by lines cutting across the record) no further pointer deflections occurred.

such as the decrement found in prolonged search for infrequent signals, would be the result of the reinforcement schedules involved. In addition, it should be possible to develop and adopt schedules that would mold the observing behavior into the form desired for practical purposes, making possible the engineering of monitoring and inspection tasks so that the operator's behavior is molded in a fashion that would provide superior man-machine systems.

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19 December 1956

Synthesis of Amino Acids by Beta Radiation

A recent paper (*1*) from this laboratory described a method of synthesizing oxalic acid by subjecting aqueous inorganic bicarbonate solutions to ionizing radiation. Acetic acid and inorganic acetates in aqueous solutions, upon irradiation, produce di- and tricarboxylic acids (*2*). The present investigation shows that ammonium acetate, on exposure to β-radiation in water solutions, forms, in addition to di- and tricarboxylic acids, small amounts of glycine, aspartic acid, and an unknown amino acid believed to be diaminosuccinic acid. Glycine would be the initial amino acid formed, whereas both of the aminosuccinic acids would be secondary ones. This is postulated because of the ease of formation of succinic acid and its homologs from the acetic acid moiety; that is, the active site is the alpha hydrogen to the carboxyl group.

The following sequence of reactions may be applicable to the formation of the observed products. Ammonium salts of organic acids, being salts of weak acids and bases, undergo hydrolysis reactions in the presence of water.

$$CH_3COONH_4 + H_2O \rightarrow NH_4OH + CH_3COOH \quad (1)$$

$$NH_4OH \rightarrow NH_3 + H_2O \quad (2)$$

Irradiation of aqueous solutions of ammonium acetate produces the following reactions (*3*).

$$NH_3 \text{—}\wedge\wedge\wedge\wedge\rightarrow NH_2\cdot + H\cdot \quad (3)$$

$$H_2O \text{—}\wedge\wedge\wedge\wedge\rightarrow HO\cdot + H\cdot \quad (4)$$

$$CH_3COONH_4 + OH \rightarrow H_2O + \cdot CH_2COONH_4 \quad (5)$$

Combining Eqs. 3, 4, and 5, one obtains

$$\cdot CH_2COONH_4 + \cdot NH_2 \rightarrow NH_2CH_2COONH_4 \text{ glycine salt} \quad (6)$$

$$2 \cdot CH_2COONH_4 \rightarrow \begin{matrix} CH_2\text{-}COONH_4 \\ | \\ CH_2\text{-}COONH_4 \end{matrix} \quad (7)$$

$$\begin{matrix} CH_2\text{-}COONH_4 \\ | \\ CH_2\text{-}COONH_4 \end{matrix} + \cdot OH \rightarrow \cdot CHCOONH_4 + H_2O \quad (8)$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad CH_2COONH_4$$

$$\cdot NH_2 + \cdot CHCOONH_4 \rightarrow NH_2CHCOONH_4 \quad (9)$$
$$\qquad\qquad\quad |$$
$$\qquad\quad CH_2COONH_4$$

aspartic acid salt

A sequence of reactions similar to those of Eqs. 8 and 9 may also be written to postulate the formation of diaminosuccinic acid.

Two concentrations, 1 and 2.5 percent, of ammonium acetate were exposed to various dosages of β-radiation by means of a 2-Mev van de Graaff electron accelerator. The solutions were analyzed for amino acid content by a modification of Levy's (*4*) method for quantitative paper chromatography determination by measuring the absorbancy of the material eluted from the spots (*5*).

In the irradiation of ammonium acetate solutions, two aqueous concentrations of ammonium acetate (Merck reagent) were employed—namely, 10.002 g of ammonium acetate per liter and 25.006 g/lit. Approximately 200 ml of each of these solutions was placed in heat-sealed polyethylene bags. Six bags of each solution were subjected to β-radiation at 2, 10, 20, 30, and 50 Mrep in a van de Graff electron accelerator.

In order to separate amino acids from nonamino acids, 143 ml of wet Dowex-50 resin, 20–50 mesh, in the acid form, was poured into a glass tube 42 mm wide and 240 mm long. The resulting column or bed of resin was treated with 500 ml of 2*M* ammonium hydroxide, then with 500 ml of distilled water, and then with 500 ml of 2*M* hydrochloric acid. The column was then washed with distilled water until the effluent reached the *p*H of the distilled water.

Two hundred milliliters of the irradiated solution was pipetted into a separatory funnel above the column and allowed to drop slowly into the column as solution was withdrawn from the bottom. When addition was complete, the column was washed with approximately 300 ml of distilled water or until the effluent was of the same *p*H as the tap water. It was demonstrated with a known mixture that acetic and succinic acids were recovered quantitatively in a total volume of 500 to 600 ml of water, including that in which the sample was dissolved. This effluent was set aside for analysis for the nonamino acids.

The amino acids were displaced from the column by washing with 500 ml of 2*M* ammonium hydroxide. The effluent was collected in 10-ml fractions, and aliquots of the fractions were spotted on Whatman No. 1 filter paper and heated for 5 minutes to drive off ammonia. The paper was sprayed with ninhydrin solution (50 ml of 0.1-percent ninhydrin in ethanol, 2 ml of collidine, and 15 ml of glacial acetic acid). The ninhydrin-positive fractions were combined, care being taken to transfer the solutions quantitatively.

The solution containing the amino acids was evaporated to dryness at room temperature in a Roto-Vap. The residue was dissolved *in situ* in 5 ml of a 2-percent sodium bicarbonate solution. To this solution was added 10 ml of alcohol containing 0.101g of 2,4-dinitrofluorobenzene. The total volume of solution was 14.8 ml. A bright yellow solution resulted immediately. The flask was swirled from time to time to insure complete contact of the solution with an insoluble residue in the flask that was assumed to be from the ion-exchange resin. This residue does not contain amino acid, since 99.5 percent of a known mixture of amino acids was recovered when it was carried through the entire analytic procedure. In this run, the residue was still present after the reaction with 2,4-dinitrofluorobenzene was complete. The solution was

Table 1. Amino acids resulting from the β-irradiation of aqueous ammonium acetate.

Dosage (Mrep)	Quantity of amino acid from 2.5% sol'n. (μg)			Quantity of amino acid from 1% sol'n. (μg)		
	Glycine	Aspartic acid	Unknown*	Glycine	Aspartic acid	Unknown*
2	†					
10	33	23				
20	658	266		672	422	125
30	1400	985	360	1030	940	361
50	2200	1872	550	1720	1850	1079

* Unknown calculated as diaminosuccinic acid using $E_{365} = 15{,}500$.
† Where blanks appear, the quantity of amino acid was not measurable by the method employed.
k glycine = 1.03; k aspartic = 0.99; k unknown = 1.000 (calculated as diaminosuccinic acid).