in the gray matter of the spinal cord and that 5-HIAA formed from 5-HT in that tissue has to diffuse across peripherally located white matter and the pia-glial membrane to reach the perfusate, in what seems to be a time-consuming process.

Our experiments exclude the possibility that 5-HIAA in the spinal fluid derives from the cisternal fluid (Fig. 1) or blood (see above) and show that changes of 5-HIAA in the spinal cord are followed by similar changes of 5-HIAA in perfusate of spinal subarachnoid space (Fig 2). We conclude, therefore, that 5-HIAA in the spinal fluid derives from the spinal cord itself and reflects metabolic changes of 5-HT in the spinal tissue. Consequently, we suggest that the concentration of 5-HIAA in the spinal fluid of patients (*2*) cannot give insight into metabolism of 5-HT in the brain as previously supposed (*4*). However, the concentration of 5-HIAA in the cerebral fluid may reflect brain 5-HT metabolism. Despite promising indications that a correlation between the concentrations of 5-HIAA in the brain and cerebral fluid exist (*3*), a clear-cut relationship, like that of 5-HIAA in the spinal cord and in perfusate (Fig. 2), is still lacking.

Our experimental model of spinal cord superfusion seems to be suitable for investigation of the origin of other substances present in the spinal fluid and spinal cord. In view of the general use of spinal fluid analysis for diagnostic purposes in medicine such investigations may be not only of academic but also of practical significance.

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2. Instead of the general term cerebrospinal fluid, more specific names are used in this report: cerebral (ventricular or cisternal) fluid and spinal fluid. Cerebral fluid is obtained by puncture of brain ventricles (ventricular fluid) or the cisterna magna (cisternal fluid). Spinal fluid, located in spinal subarachnoid space, is obtained in man by lumbar puncture. Because of the relatively small volume of this fluid in the cat, before taking a sample of spinal fluid an extradural ligature of the spinal cord at the T_{11} segment was performed to avoid insufflation of cerebral fluid into the syringe under negative pressure.
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Decreased Systolic Blood Pressure through Operant Conditioning Techniques in Patients with Essential Hypertension

Abstract. *Operant conditioning–feedback techniques were employed to lower systolic blood pressure in seven patients with essential hypertension. In five of the patients, meaningful decreases of systolic blood pressure were obtained in the laboratory, ranging from 16 to 34 millimeters of mercury. The therapeutic value of such techniques remains to be established.*

Arterial blood pressure in animals can be made to rise and fall predictably when environmental stimuli are scheduled according to variations in blood pressure (*1, 2*). Further, unanesthetized squirrel monkeys with behaviorally induced hypertension can be trained by operant conditioning techniques to lower their mean arterial blood pressure to control levels (*2*). Normotensive human subjects can also be trained to raise and lower arterial systolic and diastolic blood pressure by the use of similar procedures (*3*). The present report describes the lowering of systolic arterial blood pressure through operant conditioning–feedback techniques in seven patients with essential hypertension.

The diagnosis of essential hypertension was established by exclusion of the known causes of hypertension. The patients had moderate or severe hypertension. All had complete medical evaluations, including renal arteriography in patients Nos. 2 and 6. The patients were ambulatory and were attending the Hypertension Clinic of the Boston City Hospital. The average age of the patients was 47.9 years (Table 1). There were five males and two females. Six of the seven were taking antihypertensive medications. Medications were not altered during the experimental sessions, and all patients had maintained constant medication regimens for at least 2 weeks prior to any laboratory sessions. Informed consent was obtained from each patient. They were told they would be paid $5.00 per session to come to the behavioral laboratory and have their blood pressure measured automatically for approximately 1 hour while they sat quietly. They were also informed that no other medications or invasive techniques would be employed and that the procedures might be of value in lowering their blood pressure.

Median systolic blood pressure was recorded by use of an automated constant cuff-pressure system (*3*). A standard, 13-cm wide blood pressure cuff was wrapped around the left arm and inflated to a given pressure by a regulated, low-pressure, compressed-air source. The cuff was connected by plastic tubing to the air-filled chamber of a Statham P23Db strain gauge pressure transducer. The electrical output of the strain gauge was recorded on one channel of a Beckman type RM polygraph. The output of a crystal microphone, placed under the cuff and over the brachial artery, recorded Korotkoff sounds on a second channel of the polygraph. The electrocardiogram was recorded on a third channel. By setting the cuff at a constant pressure, close to systolic blood pressure, increases or decreases in systolic pressure with each heart beat relative to the cuff pressure could be ascertained. When cuff pressure exceeded brachial artery systolic pressure, no Korotkoff sound was produced; when cuff pressure was

Table 1. Patient characteristics and effects of operant conditioning on systolic blood pressure.

Patient No.	Age (yr)	Sex	Antihypertensive medications administered throughout the study		No. of control sessions	No. of conditioning sessions	Median systolic blood pressure (mm-Hg)		
			Medication	Amount (mg/day)			Last five control sessions	Last five conditioning sessions	Conditioning minus control
1	30	M	None		5	8	139.6	136.1	− 3.5
2	49	F	Spironolactone Methyl dopa Guanethidine	100 1500 30	5	33	213.3	179.5	−33.8
3	52	M	Methyl dopa	500	5	22	162.3	133.1	−29.2
4	54	M	Chlorothiazide Spironolactone Methyl dopa	1000 100 1500	16	34	166.9	150.4	−16.5
5	44	M	Chlorothiazide	1000	15	31	157.8	141.7	−16.1
6	53	F	Chlorothiazide Spironolactone Methyl dopa	1000 100 1000	15	12	165.7	166.6	+ 0.9
7	53	M	Hydrochlorothiazide Spironolactone Methyl dopa	100 100 1000	15	12	149.0	131.7	−17.3
Mean	47.9				10.9	21.7	164.9	148.4	−16.5

less than brachial artery systolic pressure, a Korotkoff sound was present. During each trial, the cuff was inflated for 50 consecutive heart beats (recorded automatically from the electrocardiogram) and then deflated. The presence or absence of a Korotkoff sound was noted within 300 msec after the R-wave of the electrocardiogram. Median systolic blood pressure during the trial was equal to cuff pressure when 14 to 36 Korotkoff sounds per cycle of 50 heart beats were present (*3, 4*). If less than 14 Korotkoff sounds were present, indicating cuff pressure exceeded systolic arterial pressure for most of the trial, the cuff pressure was decreased by 4 mm-Hg for the next cycle. If more than 36 Korotkoff sounds were present, indicating cuff pressure was lower than arterial pressure for most of the trial, the cuff pressure was increased by 4 mm-Hg. Thus, median systolic pressure was tracked throughout each session.

The patients were studied on consecutive weekdays. During all sessions, median systolic blood pressure was measured for 30 trials. Between trials, the cuff was deflated for 30 to 45 seconds. There were 5 to 16 control sessions for each patient during which median systolic blood pressure was recorded with no feedback or reinforcement of lowered systolic pressure. Thus, the control pressures represented median systolic blood pressure of between 7,500 and 22,500 heart beats.

In each of the following conditioning sessions, the first five trials had no reinforcement presented. However, in the subsequent 25 conditioning trials, relatively lowered systolic pressure, indicated by the absence of a Korotkoff sound, was fed back to the patient by presentation of a 100-msec flash of light and a simultaneous 100-msec tone of moderate intensity. The patients were told that the tone and light were desirable and they should try to make them appear. As a reward, after each 20 presentations of tones and lights, a photographic slide, equivalent to $0.05, was shown for 5 seconds. The slides consisted of scenic pictures and reminders of the amount of money earned. The conditioning sessions continued until no reductions in blood pressure occurred in five consecutive sessions.

Blood pressures did not change within the first five control sessions. In the four patients with 15 or 16 control sessions, no decreases in blood pressure were noted after the initial five control sessions (*5*). Average median systolic blood pressure during the last five control sessions in the seven patients was 164.9 mm-Hg (Table 1). Pressures in the last five conditioning sessions were used as an index of the effectiveness of training. During these sessions, average median systolic blood pressure was 148.4 mm-Hg ($P < .02$) (*6*). In the individual patients, systolic blood pressure decreased 3.5, 33.8, 29.2, 16.5, 16.1, 0, and 17.3 mm-Hg.

Systolic blood pressure did not change significantly within each control session. However, it decreased an average of 4.8 mm-Hg ($P < .001$) (*6*) within each conditioning session. This within-session decrease is equivalent to that observed in normotensive subjects with similar training (*3*). In the two patients with little or no decrease in systolic blood presure, patient No. 1 did not have elevated systolic blood pressure, while patient No. 6 had renal artery stenosis. No consistent changes in heart rate were present in any of the patients during the blood pressure changes.

Elevated arterial blood pressure increases the risk of coronary artery disease and cerebrovascular accidents (*7*). This increased risk is lessened by lowering blood pressure (*8*). At the present time, the means of lowering blood pressure are pharmacological or surgical or both. In the present experiments, systolic blood pressure could be decreased by operant conditioning techniques in six of seven patients with essential hypertension. Since the decrease in systolic pressure was measured only in the laboratory and no consistent measurements were made outside the laboratory, the usefulness of such methods in the therapeutic management of hypertension remains to be evaluated (*9–12*).

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4. Simultaneous intraarterial studies have confirmed that median systolic blood pressure measured by this cuff system is equal to median intraarterial systolic blood pressure (unpublished data from our laboratory).
5. Separate analyses of variance were computed for the initial five control sessions in all patients and for differences between the initial five and final five control sessions in four patients. Although possible, it is unlikely that more than 15 or 16 control sessions would yield significantly lower systolic blood pressures.
6. The data were treated as a three-factor repeated measures experiment with two levels of the first factor (control-conditioning); five levels of the second factor (sessions); and 25 levels of the third factor (trials). For the significant main effect of control-conditioning, d.f. = 1/6; for the significant control-conditioning × trial interaction, d.f. = 24/144. Computed by the Biomed 08V Analysis of Variance Program on an IBM 360 computer.
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10. We acknowledge the competent technical contributions of Miss Barbara R. Marzetta.
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12. A preliminary report of these experiments was presented at the May 1971 meeting of the American Society for Clinical Investigation.

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Cell Dissociation: Univalent Antibodies as a Possible Alternative to Proteolytic Enzymes

Abstract. *Univalent antibody fragments directed against special membrane antigens dissociate multicellular bodies of the cellular slime mold* Dictyostelium discoideum *completely into single cells. This provides a gentle method for cell dissociation and demonstrates that a nonenzyme protein can disintegrate a tissue by binding to specific cell surface sites.*

Proteolytic enzymes have been shown to be the most effective agents for dissociating tissues into single cells (*1*). Enzymes or other macromolecules may interfere with cell-to-cell binding in two ways, either by sterically blocking or by degrading cell surface sites. It is open to question whether enzymatic activity is absolutely essential for a protein to dissociate cells. Therefore we tried nonenzyme proteins which specifically bind to certain cell surface structures. Univalent antibody fragments (Fab) prepared by papain digestion of immunoglobulin G (see *2*) were selected because of their specificity, because of their relatively low molecular weight (50,000) which restricts their direct action to a small target area, and because each Fab molecule binds to only one antigenic site. Furthermore, use of Fab avoids cell agglutination and other side effects of complete antibody action which follow from cross-linking of cell-membrane structures (*3*).

As a test tissue, fragmented multicellular bodies of the cellular slime mold *Dictyostelium discoideum* were used. In the "slug" stage of this organism, cells are intimately associated forming a tissue that can be dissociated only partially by ethylenediaminetetraacetic acid (*4*), and by proteolytic enzymes only in presence of 2,3-dimercaptopropanol or other sulfhydryl compounds (*5*, *6*). Earlier (*7*, *8*), we reported that, in *D. discoideum*, cell aggregation that precedes slug formation can be completely inhibited by Fab directed against special membrane sites. These sites are thought to be identical with structures that participate in cell adhesion.

The aggregation-inhibiting Fab was prepared from an antiserum produced by immunizing rabbits with a particulate fraction of aggregation-competent *D. discoideum* cells. To ascertain whether this Fab dissociates the slug tissue, slugs were cut into pieces of approximately 50 μm in diameter and incubated in barbital buffer, *p*H 7.3, containing 32 mg of Fab per milliliter (*8*). Dissociation was observed in 10-μl samples spread on a cover slip and covered with a thin, O_2-permeable Teflon mem-

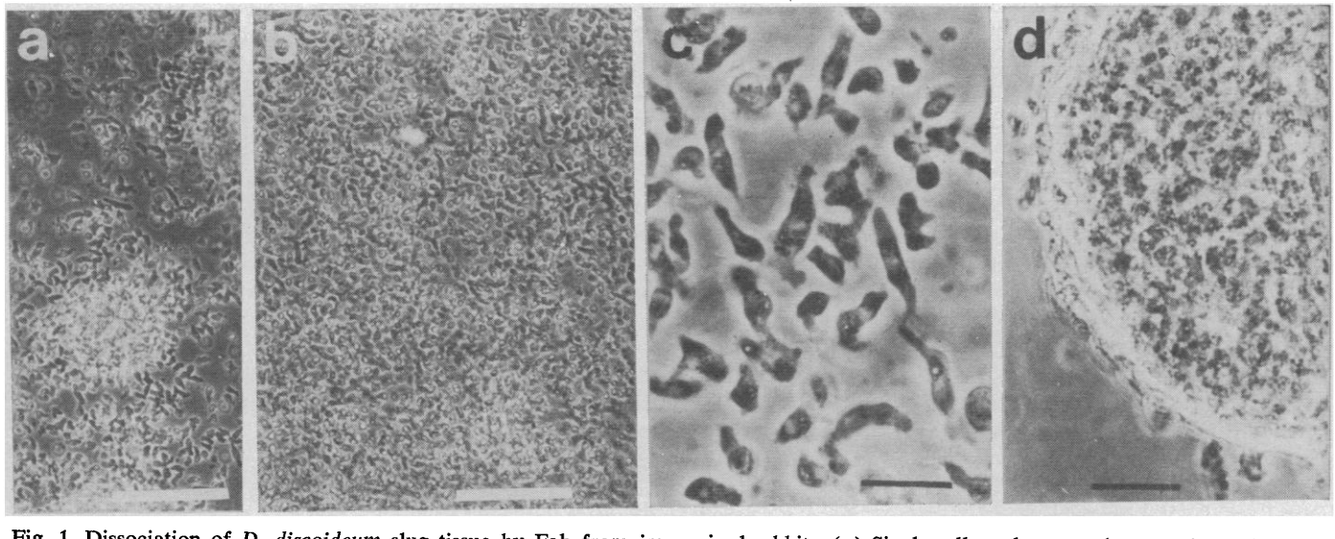

Fig. 1. Dissociation of *D. discoideum* slug tissue by Fab from immunized rabbits. (a) Single cells and groups that remained after incubation for 3 hours; (b and c) completely dissociated slug fragment after an additional 3-hour incubation period; (d) control: undissociated slug fragment incubated for 6 hours with Fab from nonimmunized rabbits. White scales, 50 μm; black scales 10 μm.