the same preparation of HNB-protein which was inactive in the guinea pig in a dose of 100 μg retained full activity in the rabbit, and we have confirmed their results in our laboratory with basic proteins that have been modified with HNB bromide or performic acid. The HNB derivative was fully active in the rabbit but the oxidized protein was reduced in activity by 50 percent. The treatment with performic acid causes oxidation of both tryptophan and tyrosine, in that order. Since the sequence of the active site around tyrosine 69 is identical in the guinea pig and rabbit (*3*), this species difference in encephalitogenic activity suggests that the guinea pig is unable to recognize this site.

Two antigenic sites in myelin basic protein produce EAE in animals. These two sites show striking sequence homology. Guinea pigs are sensitive primarily to the tryptophan-containing site, while rabbits and monkeys are sensitive to the tyrosine-containing site. Modification of both of these sites would be desirable in any preparation used to elicit tolerance to the basic protein in rabbits and primates.

Proteins of known sequence could be surveyed in order to ascertain whether others contain sequences homologous to the above. Such sequences are rare mainly because Gln-Lys or Gln-Arg are found together in very few proteins. Two sequences are found in the human hemoglobin beta chain:

```
 31                                  40
Leu-Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg

121                                 132
Glu-Phe-Thr-Pro-Pro-Val-Gln-Ala-Ala-Tyr-Gln-Lys
```

If one of these tryptic peptides were found to be encephalitogenic, demyelinating diseases, such as multiple sclerosis, might be initiated by proteins that are located elsewhere in the body or in the environment.

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16 April 1971; revised 24 May 1971

Origin of 5-Hydroxyindoleacetic Acid in the Spinal Fluid

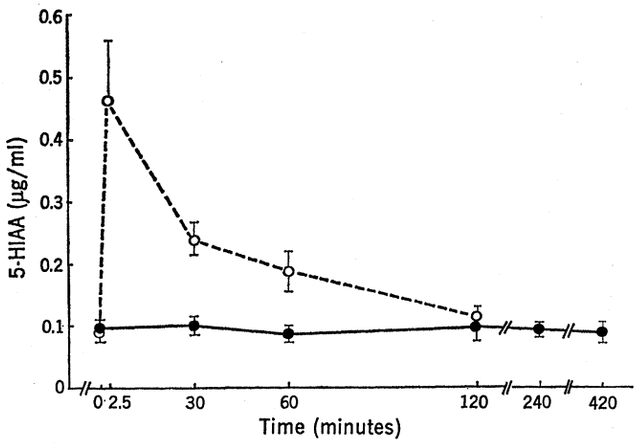
Abstract. *5-Hydroxyindoleacetic acid applied intracisternally in cats does not appear in spinal fluid. Changes of 5-hydroxyindoleacetic acid concentration in the spinal cord are clearly reflected in the perfusate of the spinal subarachnoid space. Thus, 5-hydroxyindoleacetic acid in the spinal fluid originates from the spinal cord and reflects metabolic changes of 5-hydroxytryptamine in the spinal tissue, but not those in the brain.*

Deficiency or altered metabolism of 5-hydroxytryptamine (5-HT, serotonin) in the brain may be the cause of mental diseases (*1*). Investigation of brain 5-HT in man is a difficult task for researchers because experiments on brain in vivo are not possible for ethical reasons. Analysis of brain tissue post mortem, however, can hardly give a reliable insight into metabolism of 5-HT in the brain during life. On the other hand, the concentration of 5-HT and its main metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the urine or blood reflects the metabolism of 5-HT of the whole body rather than that of brain because the latter constitutes only a small percentage of the total 5-HT found in the body.

The finding that 5-HIAA in the cerebral fluid (*2*) of animals reflects the metabolism of 5-HT in the brain (*3*) has opened a new and promising avenue in clinical research. Numerous studies have been undertaken in patients in which concentration of 5-HIAA in the spinal fluid, obtained by lumbar puncture, was used presumably as an index of brain 5-HT metabolism in various mental disturbances (*4*). However, this presumption is of doubtful value, because the origin of 5-HIAA in the spinal fluid is obscure. We now report that 5-HIAA in the spinal fluid derives from the spinal cord and reflects metabolic changes of 5-HT in the spinal tissue.

The 5-HIAA in the spinal fluid could originate from three sources: brain (via cisternal fluid), spinal cord, and blood. We examined these three possibilities in cats. Experiments were performed on adult animals lightly anesthetized with thiopental sodium. After thoracolumbar laminectomy 0.10 ml of cisternal fluid was withdrawn by percutaneous puncture of the cisterna magna, and 0.5 μg of 5-HIAA in 0.10 ml of artificial spinal fluid (*5*) was given over 30 seconds. At a fixed time interval after the injection (Fig. 1) an extradural ligature of the spinal cord at the T_{11} segment was positioned, and samples of spinal fluid below the ligature as well as cisternal fluid were taken (*2*). Only one sample each of cisternal (about 0.8 ml) and spinal (about 0.5 ml) fluid was obtained from the same cat.

In the other group of experiments the spinal subarachnoid space was perfused with warm (37°C) artificial spinal fluid in saline (control) and reserpine-treated animals (Fig. 2). Thus, perfusion was performed by introducing two fine polyethylene tubes subarachnoidally, one at the T_{11} segment (inflow) and the other one at the cauda equina (outflow). A closed spinal subarachnoid space, separated from the rest of the spinal fluid, was formed by a thread ligature placed extradurally at the T_{11} segment. The inflow tube was connected with a Harvard pump which delivered artificial spinal fluid through the spinal subarachnoid space at a rate of 109 μl/min. The end of the outflow tube was lifted 7 cm above the horizontal level of the spinal cord



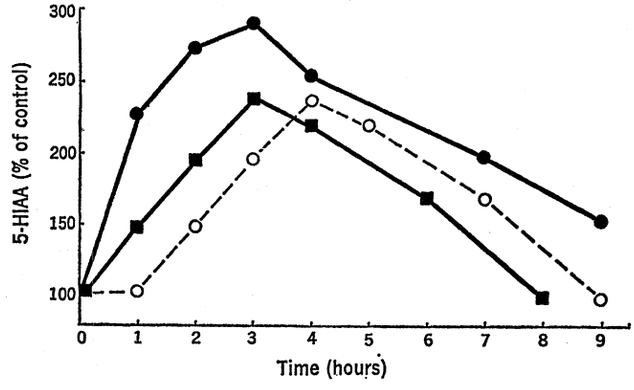


Fig. 1 (left). Concentration of 5-HIAA in cisternal (open circles) and spinal (closed circles) fluid (μg/ml). 5-HIAA (0.5 μg) was injected intracisternally over the first 30 seconds and samples of cisternal and spinal fluid were taken at one of the time intervals after the injection. Each point represents the mean of four experimental values ± S.E.M. (vertical bars). Fig. 2 (right). 5-HIAA (percent of control) in spinal cord (closed circles) and perfusate of spinal subarachnoid space (open circles) at different intervals after reserpine application. Curve with quadrangles is the same as that with open circles but shifted to the left by an interval of 1 hour. Reserpine (2 mg/kg) was applied intravenously over the first 15 minutes. Cats injected intravenously with saline were used as controls (100 percent). Control concentrations of 5-HT (0.504 μg/g) and 5-HIAA (0.147 μg/g) in spinal cord, and 5-HIAA in perfusate (see text) were not changed significantly during perfusion. Each point on the curves represents the mean of approximately five experimental values. The S.E.M. (not shown in the figure) was less than 18 percent (spinal cord) or 14 percent (perfusate).

because we found that normal pressure of the spinal fluid in cats was about 7 cm of spinal fluid.

Cisternal fluid, spinal fluid, perfusate, and the superfused portion of spinal cord were analyzed for 5-HIAA or 5-HT. We determined 5-HIAA by a modified and improved (*6*) method of Ashcroft and Sharman (*7*). We estimated 5-HT according to the method of Bogdanski *et al.* (*8*). The following data indicate that the superfused portion of spinal cord was in good physiological condition during superfusion: (i) The concentrations of 5-HT and 5-HIAA in the spinal cord and 5-HIAA in perfusate were at constant levels. (ii) Permeability of blood vessels in the spinal cord, evaluated by intravenous application of Evans blue or trypan blue, was not altered. (iii) In preliminary experiments we found that segmental reflex activity through the superfused portion of spinal cord, tested by stimulation of and recording from hind limb nerves, did not change appreciably.

If 5-HIAA in the spinal fluid derives from the brain, then an increase of 5-HIAA in the cisternal fluid should be followed by augmentation of 5-HIAA in the spinal fluid. After intracisternal application of 5-HIAA (Fig. 1), a prominent increase of 5-HIAA in the cisternal fluid, which lasts for about 2 hours, is observed. However, there is no significant increase of 5-HIAA in the spinal fluid (Fig. 1). In explaining these results one should bear in mind that substances move slowly from cisternal into spinal fluid (*9*). In addition, we have found (*6*) that 5-HIAA is constantly removed from spinal fluid to the blood and that this process can be suppressed by probenecid, a substance known to block active transport of 5-HIAA from ventricular fluid into the blood (*10*). Therefore, removal of 5-HIAA from spinal fluid seems to be, at least partly, an active mechanism. This supports a recent view (*11*) that not only choroid plexus or ependyma in brain ventricles but also nonependymal tissue in spinal subarachnoid space are capable of actively transporting some substances from spinal fluid to blood.

Does 5-HIAA in the spinal fluid derive from the blood? Concentration of 5-HIAA in the blood is low or unmeasurable (*12*). We have found (*6*) that an increase of 5-HIAA in the spinal fluid can be observed only when the concentration of 5-HIAA is extremely elevated in the blood. Further, an active mechanism removes 5-HIAA from the spinal fluid to blood (see above). All this evidence suggests that 5-HIAA in the spinal fluid does not originate normally from the blood. A similar conclusion had been reached about potential origin from blood of 5-HIAA in cerebral fluid (*10*).

5-Hydroxytryptamine (5-HT) in the spinal cord (*13*) might be the source of 5-HIAA in the spinal fluid. If so, 5-HIAA ought to enter the perfusate of the spinal subarachnoid space. We have found that 5-HIAA appears in the perfusate at a rate (37 ng per gram of superfused spinal cord per hour) that does not vary significantly in the course of perfusion. If this 5-HIAA is derived from the spinal cord, the increase or decrease of 5-HIAA in that tissue should be followed by corresponding changes of 5-HIAA in the perfusate. Reserpine seems to be the drug of choice for such an investigation because it is known that after reserpine application the cerebral 5-HIAA increases for several hours and then decreases toward control values (*14*). In Fig. 2 it is seen that after application of reserpine, the concentration of 5-HIAA increases in the spinal cord for 3 hours and thereafter declines, approaching control values at 9 hours. However, 5-HIAA in the perfusate increases for 2 to 4 hours, returning to control levels at 9 hours. Thus, there is a time delay in the increase of 5-HIAA in the perfusate, as compared to the increase in the spinal cord.

If the curve for 5-HIAA in the perfusate is shifted to the left by an interval of 1 hour, the changes in 5-HIAA concentration in the spinal cord are clearly followed by similar fluctuations in the perfusate (Fig. 2). This indicates that 5-HIAA in the perfusate originates from the spinal cord. The delayed appearance of an increase in the 5-HIAA in the perfusate (Fig. 2) can be explained simply by taking into account the facts that 5-HT (*15*) and monoamine oxidase (*16*) are located mainly

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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20 May 1971

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