dissolved in chloroform and passed through a short column of silica gel to remove colored impurities. The rotation of a weighed sample (80 mg) in chloroform (2 ml) was then measured (Perkin-Elmer model 141 polarimeter, 1-ml, 10-cm cuvettes) and the specific rotation $[\alpha]_D$ was calculated. For enantiomerically pure **2**, $[\alpha]_D = 167°$ (*2*).

6. R. E. Pincock, R. R. Perkins, A. S. Ma, K. R. Wilson, *Science* **174**, 1018 (1971).
7. Solutions containing 180 mg of **1** and 12 mg of **2** (having $[\alpha]_D = 103°$) in 6 ml of ethyl acetate were evaporated to dryness overnight. The samples were then powdered and exposed to bromine for 1 week, leading to about 55 percent reaction. The rotations of weighed samples (about 80 mg) were corrected for the rotation of the dibromide added initially. In a separate experiment, six methylene chloride solutions, each containing 90 mg of **1** and 8 mg of **2** ($[\alpha]_D = 137°$), were evaporated and then brominated (70 to 90 percent reaction). All gave levorotary material, mean $[\alpha]_D = 25.5°$.
8. The solutions contained 210 mg of **1** and 14 mg of **2** ($[\alpha]_D = -28.4°$) and were evaporated over a 1-day period. Bromination for 1 week led to 40 to 60 percent reaction. The rotations were corrected for the presence of (—)-**2** initially present. This experiment was repeated on ten samples, each containing 260 mg of **1** and 22 mg of **2** ($[\alpha]_D = -135°$) in 6 ml of ethyl acetate. All afforded, after treatment as above, dextrorotary dibromide, mean $[\alpha]_D = 26.2°$.
9. R. M. Secor, *Chem. Rev.* **63**, 297 (1963).
10. The scheme in Fig. 2 is an illustration of the stereospecific autocatalytic production of enantiomer excess as formulated by Calvin [M. Calvin, *Chemical Evolution* (Oxford Univ. Press, London, 1969), pp. 149–152]. See also: A. Dauvillier. The *Photochemical Origin of Life* (Academic Press, New York, 1965), pp. 107–115; F. F. Seelig, *J. Theor. Biol.* **34**, 197 (1972), and references therein; L. G. Harrison, *ibid.* **39**, 333 (1973).
11. See J. H. Stout and L. H. Jensen, *X-ray Structure Determination* (Macmillan, London, 1968), pp. 134–135.

4 February 1974

5-Hydroxyindoleacetic Acid in the Lumbar Fluid: A Specific Indicator of Spinal Cord Injury

Abstract. *In cats, 19 days after the lower thoracic cord was injured, the concentrations of 5-hydroxytryptamine and its metabolite 5-hydroxyindoleacetic acid in the lumbosacral cord and that of 5-hydroxyindoleacetic acid in the lumbar fluid decreased. At the same time the concentrations of these substances in the cord above the lesion and that of 5-hydroxyindoleacetic acid in the cisternal fluid was not significantly altered. Since high concentrations of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid are present in the human lumbosacral cord, it appears that the concentration of 5-hydroxyindoleacetic acid in the lumbar fluid of animals and man reflects the biochemical changes of 5-hydroxytryptamine in the normal and injured spinal cord.*

The number of injuries of the spinal cord in man is increasing. Efforts are being made to advance the care and study of injuries to the spinal cord in humans (*1*).

In an effort to develop a method for studying the biochemical changes in the injured spinal cord in animals and potentially in man in vivo, we considered two relevant findings on animals. (i) After transection of the spinal cord, the degeneration of the descending serotonergic nerve fibers takes place in the cord below the lesion with simultaneous decrease of 5-hydroxytryptamine (5-HT, serotonin) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) (*2*). (ii) The concentration of 5-HIAA in the spinal fluid of lumbosacral region (lumbar fluid) reflects the metabolism of 5-HT in the spinal cord (*3, 4*). Thus, after injury of the serotonergic fibers, a decrease of 5-HT and 5-HIAA in the spinal cord below the lesion followed by a similar decrease of 5-HIAA in the lumbar fluid might be expected. If so, this would open the possibility of studying the metabolism of 5-HT in the injured spinal cord by analysis of the lumbar fluid, which can be obtained by lumbar puncture in man in vivo. We now report that the concentration of 5-HIAA in the lumbar fluid reflects the changes of 5-HT metabolism in the injured spinal cord of cats.

Adult cats were anesthetized with thiopental sodium, and a partial laminectomy at T_{11} vertebrae was performed. The exposed spinal cord of each cat was squeezed epidurally with a fine dissecting forceps for 10 minutes. Control animals underwent laminectomy, but their spinal cords were left intact. The wound in each animal was sprinkled with xanthocillin and sewed up. The animals with injury of the cord developed clinical evidence of complete loss of function below the lesion (paraplegia, urine retention, and loss of sensitivity). After the operation the cats were treated daily with procaine penicillin (200,000 international units), and urine retention was relieved by manual expression. There was no evidence of blockage of communication between spinal fluid above and below the site of injury (*5*).

Nineteen days after the operation, the spinal cord (about 2 g) below the lesion (lumbosacral cord), part of thoracic cord (about 1 g) above the lesion, and samples of cisternal (about 1 ml) and lumbar (about 0.3 ml) fluid were taken for analysis in animals under thiopental anesthesia (*3, 4*); the samples were then frozen (— 20°C). Determinations of 5-HT (*6*), 5-HIAA (*6*), and norepinephrine (*7*) in the spinal cord and of 5-HIAA in the cerebrospinal fluid (*8*) were performed the next day. The concentration of 5-HT and 5-HIAA in the lumbosacral cord in humans was also measured.

Nineteen days after injury to the cat's spinal cord, there was a 75 percent decrease of 5-HT in the cord below the lesion (lumbosacral cord), while the concentration of 5-HT in the thoracic cord above the lesion was not changed. The concentration of 5-HIAA in the lumbosacral cord and in the lumbar fluid was lowered (by 80 and 68 percent, respectively; $P < .001$), while the concentration in the thoracic cord as well as in the cisternal fluid was not significantly altered ($P > .05$) (Fig. 1). Since the decrease of 5-HT and 5-HIAA in the injured lumbosacral cord was followed by a corresponding decrease of 5-HIAA in the lumbar fluid (Fig. 1), it appears that by measuring the concentration of 5-HIAA in the lumbar fluid we can obtain a better understanding of the metabolism of 5-HT in the injured spinal cord. Further, after blockage of the active transport of 5-HIAA from the spinal cord and spinal fluid by probenecid (*4, 9*), we found a greater increase of 5-HIAA in the lumbosacral cord and lumbar fluid in control as compared to injured animals. This indicates that the

Table 1. Concentration of 5-HT and 5-HIAA in the human lumbosacral cord.

Subject	Age	Sex	Cause of death	Time from death to autopsy (hours)	5-HT (ng/g)	5-HIAA (ng/g)
S.M.	78	Female	Auto accident	23	454	371
T.Z.	35	Female	Cerebral edema	17	557	491
P.L.	77	Male	Pulmonary embolism	10	454	673
D.Z.	27	Female	Auto accident	3	650	617
S.M.	84	Male	Pneumonia	9	563	799
				Mean ± S.E.M.	536 ± 37	590 ± 74

turnover of 5-HT in the injured cord is reduced.

After injury to the cord the concentration of 5-HIAA in the cisternal fluid was three times higher than that in the lumbar fluid (Fig. 1). Thus, it appears that not only after a single intracisternal injection of 5-HIAA (*3*) but also in these long-term experiments, the concentrations of 5-HIAA in the cisternal and lumbar fluid do not show a tendency of equilibration. This supports our previous conclusion that 5-HIAA in the lumbar fluid reflects the metabolism of 5-HT in the spinal cord but not that in the brain (*3, 4*). Furthermore, after injury to the spinal cord, the concentration of 5-HIAA in the lumbar fluid follows that in the adjacent lumbosacral cord, but not its concentration in the cranially located thoracic cord (Fig. 1). This indicates that 5-HIAA in the lumbar fluid derives primarily from adjacent spinal tissue and that little, if any, derives from the remote parts of the spinal cord.

However, whether 5-HT and 5-HIAA are present in the spinal cord of man, and at what concentration, remains unknown. Since we did not find any data concerning the presence of 5-HT and 5-HIAA in the human spinal cord, we took a sample of the spinal tissue (about 1.5 g) from the end of the lumbosacral cord at autopsy and froze it (− 20°C) immediately. Determinations of 5-HT and 5-HIAA in the cord were made within 24 hours after autopsy. Human lumbosacral cord contains a relatively high concentration of both 5-HT and 5-HIAA (Table 1). The concentration of 5-HIAA is about three times higher in lumbosacral cord of man than in that of the cat (compare Fig. 1 and Table 1). Despite the fact that the cord samples were taken from subjects that differed with respect to cause of death, the interval between death and autopsy, age, and sex, the lumbosacral cord samples contained in each case relatively high concentrations of 5-HT and 5-HIAA (Table 1), suggesting that in man the concentration of these substances in vivo is also great. The high concentration of 5-HIAA in the lumbosacral cord suggests that lumbar fluid in man is also supplied with 5-HIAA from this nearby potent source.

It seems paradoxical that the concentration of 5-HIAA in the human lumbar fluid is about three to four times lower (*10*) than that in the lumbar fluid of the cat (Fig. 1), although

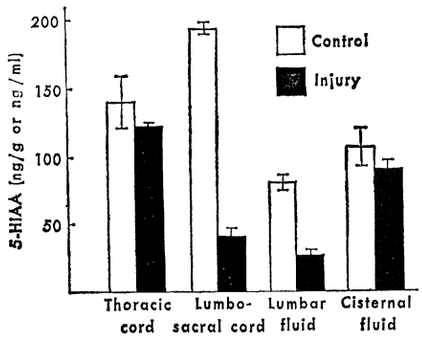

Fig. 1. Concentration of 5-HIAA in different parts of the spinal cord (nanograms per gram) and cerebrospinal fluid (nanograms per milliliter) in control cats and in cats with injury to the spinal cord. Four to six animals were used to obtain the means ± S.E.M. (vertical bars).

the concentration of 5-HIAA in the human lumbosacral cord is about three times higher than that in the cat (compare Fig. 1 and Table 1). The anatomical relationships of the lumbar fluid in man and in cat differ in that lumbar fluid taken for analysis in man is located predominantly in the voluminous lumbar sac positioned caudally to the lumbosacral cord. But in cats there is no lumbar sac so that their lumbosacral cord is in a direct contact with lumbar fluid located in a thin subarachnoid space. Thus, 5-HIAA that diffuses from the human lumbosacral cord down into the lumbar sac is diluted by the large volume of the fluid. In addition, 5-HIAA is continuously removed by an effective transport mechanism from the spinal subarachnoid space into the blood (*9, 11*). These mechanisms probably keep the concentration of 5-HIAA in the lumbar sac extremely low, at the limit of sensitivity of most methods used for 5-HIAA measurements, so that 5-HIAA in the fluid of the lumbar sac cannot sometimes be identified at all (*12*). If so, then the changes of 5-HT metabolism in the human spinal cord might hardly be detected by analysis of 5-HIAA in the fluid of the lumbar sac (*13*).

The concentration of 5-HIAA in the lumbar fluid which is adjacent to the human cord is probably much higher than that in the lumbar sac because, as shown above, 5-HIAA in the lumbar fluid reflects primarily the concentration of 5-HIAA in the adjacent spinal tissue, but not that from the remote parts of the cord.

Our experiments with cats indicate that the concentration of 5-HIAA in the lumbar fluid is an indicator of injury of serotonergic nerve fibers and that it may serve as an index of injury, degeneration, or eventual regeneration of these fibers in the spinal cord. To make a similar study in man, a method has to be devised for sampling the lumbar fluid not from the lumbar sac but from the spinal subarachnoid space adjacent to the lumbosacral cord. Not only 5-HIAA but also probably some other substances in the lumbar fluid might serve as indicators of injury of nerve pathways in the spinal cord. For instance, we and others (*2*) have found that the concentration of norepinephrine as well as that of 5-HT decreases in the cord below the lesion. Possibly the concentration of 3-methoxy-4-hydroxyphenyl glycol, a metabolite of norepinephrine, in the lumbar fluid (*14*) could reflect the injury of adrenergic descending nerve pathways in the spinal cord.

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