

ACETYLCHOLINE AND SEROTONIN IN
THE SPINAL FLUID*

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THIS paper reports quantitative analyses of acetylcholine and serotonin in the spinal fluid of patients with head injuries, brain tumors, and other neurological disorders. It also reports the related findings in dogs subjected to experimental concussion of the brain.

Acetylcholine occurs in the spinal fluid of animals after experimental head injury. This, Bornstein² postulated, is released from the mechanically damaged brain cells, a supposition in keeping with Lorente de Nó's⁸ work showing that acetylcholine is released from peripheral autonomic ganglia by mechanical damage only. Either because of excessive production of acetylcholine or impaired destruction, the acetylcholine, which never normally appears in the spinal fluid, occurs in significant amounts after trauma.

In 1950 Ward¹¹ proposed the use of huge doses of atropine in the treatment of closed head injuries to combat this release of acetylcholine. Since 1951 we have been using Ward's regimen in the treatment of selected severe head injuries. We feel that it may have helped some patients recover who otherwise might not have done so. A control series of patients treated without atropine is being run. We hope with a larger number of cases to ascertain whether there is a significant difference in the results. Recently Jenkner and Lechner,⁷ reporting on 309 patients with severe head injuries, found that 249 of them, treated with anticholinergic drugs, got well quicker and had fewer complications. They also noted that the electroencephalograms in this group returned to normal faster than those in the group not receiving anticholinergic therapy.

It became evident from the first that a quantitative study of the amount of acetylcholine in the spinal fluid of these patients would be of value in order to determine the effectiveness of anticholinergic therapy. Ruge¹⁰ has studied this in animals. The technique of acetylcholine determination is a tedious one, utilizing one of various methods of biological assay. The most sensitive technique makes use of the heart of *Venus mercenaria*, the common clam. This method was introduced by Welsh¹² and is very specific, but any significant amount of blood in the spinal fluid will immediately destroy the

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acetylcholine because of the cholinesterase in the red cells. Therefore, in studying patients with head injuries the traumatic subarachnoid hemorrhage so frequently present invalidates determinations of spinal fluid acetylcholine, but not of serotonin. Likewise, because of the notorious dangers of doing lumbar puncture in the presence of increased intracranial pressure, in many cases we did not take spinal fluid specimens from patients in the acute phase following injury.

Much interest has been manifested recently in the neurohormone, serotonin, chemically identified as 5-hydroxytryptamine.⁹ This substance is identical with enteramine, isolated from the intestinal mucosa.⁵ Hematologists have been interested in serotonin because of its rich occurrence in platelets and its vasoconstrictor effect. However, the discovery of serotonin in the brain¹ is an important landmark in neurology and psychiatry. The

TABLE 1
Neurochemical effects on the regularly contracting heart of the clam

Substance	With Sea Water	With LSD	With Mytolon
Acetylcholine	↓ Contraction	↓ Contraction	Normal contraction
	↓ Rate	↓ Rate	Normal rate
Serotonin	↑ Contraction	Normal contraction	↑ Contraction
	↑ Rate	Normal rate	↑ Rate
"X" Substance	↓ Contraction	↓ Contraction	↓ Contraction
	↑ Rate	↑ Rate	↑ Rate

hypothesis that serotonin has a role in brain function is supported by the fact that lysergic acid diethylamide (known as LSD), a powerful psychogenic and hallucinogenic drug, is the most powerful imitator of serotonin *in vitro*. In addition, reserpine, so widely used in psychiatric and other patients nowadays, has been shown to mobilize the body stores of serotonin and increase its excretion. The following observations, therefore, on serotonin in the spinal fluid of patients with various neurological conditions are of considerable interest.

Quantitative analysis of the spinal fluid of 70 neurological patients has been carried out. The group included 28 patients with head injuries and 16 with brain tumors. Other disease states sampled were epilepsy, electric shock, meningitis, multiple sclerosis, ruptured cerebral aneurysm, gunshot wounds of the brain, and a scattering of other conditions. Both acetylcholine and serotonin can be assayed biologically, using the clam heart technique, because of their opposite inotropic and chronotropic effects. In addition, the specific blocking action of LSD and benzoquinone (commercially known as Mytolon) was used to identify acetylcholine and serotonin (Table 1).

FINDINGS

Acetylcholine. Acetylcholine was encountered in significant amounts in 16 of the 28 cases of head injury studied, at varying times following the injury (Fig. 1). It was never present in more than 1 or 2 $\mu\text{g.}$ per cent; however, the sensitivity of the method was sufficient to make the results definite. The acetylcholine was present during the first few days following severe head injury and disappeared as the patient recovered or reached a stable neurological level, whether this represented complete neurological recovery or not. Massive doses of atropine (2 to 6 mg.) were used in a number of patients; in a few of these, subjected to serial lumbar punctures before and after atropine, distinct reduction of the acetylcholine could be demonstrated.

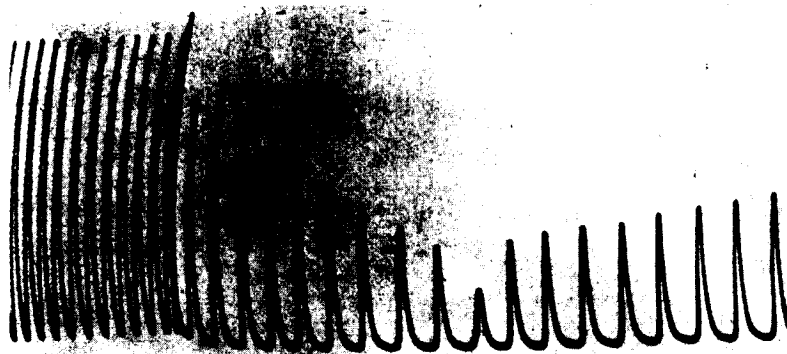


FIG. 1. Cerebrospinal fluid from patient with head injury, showing presence of *acetylcholine*: slowing heart and decreasing contractions.

As a rule this seemed to coincide with clinical improvement of the patient, but this was not always the case. In brief, this quantitative study tends to support the empirical assumption of Ward that release of acetylcholine, medication with atropine, and the patient's clinical course may be related.

Serotonin. In 6 of the 28 cases of head injuries, 5 of the 16 cases of brain tumors, and a single case of traumatic paraplegia, serotonin was found in the spinal fluid (Fig. 2). In 4 of the 6 cases of head injuries in which serotonin was recovered from the spinal fluid, it was associated with acetylcholine in the same specimens. These were separated by virtue of the selective blocking action of Mytolon and LSD. The serotonin in the spinal fluid was not associated with sufficient amounts of blood to account for its presence.

In 5 of the 16 cases of brain tumor, serotonin was demonstrated in the spinal fluid; in 2 cases it was associated with acetylcholine. In all of the cases of brain tumor, the assays were done postoperatively and the findings may simply represent another form of head injury, namely, the trauma of surgery. Serotonin was also found in the 1 case of traumatic paraplegia, and in 1 case in which no precise neurological diagnosis could be made.

In 8 cases, namely, 5 brain injuries, 2 brain tumors and 1 meningitis,



FIG. 2. Cerebrospinal fluid from patient with head injury, showing presence of *serotonin*: speeding heart and increasing contractions.

an unidentified substance was encountered in the spinal fluid. Like serotonin, it caused augmentation of the heart rate of the clam; however, unlike serotonin, it could not be blocked by LSD, and caused diminution of the contracting heart, in which respect it resembled acetylcholine. This substance has not yet been identified, but we hope to do so in the next phase of our study (Fig. 3).

Animal Experiments. Fourteen dogs and many rats have been submitted to varying degrees of quantitatively controlled concussion. Analysis of the cisternal spinal fluid of the dogs has been done by the above method. Morphine analgesia has been used because of the preservation of consciousness, as suggested by Gurdjian and Webster.⁶ An air concussion gun has



FIG. 3. Cerebrospinal fluid from patient after operation for brain tumor, showing presence of "X" substance: speeding heart and decreasing contractions.

been utilized for these experiments. Blows of variable known energies and velocities can be obtained with this instrument. The blood pressure, pulse and respiratory rates, corneal reflex, and level of consciousness have been closely followed and correlated with determinations of spinal fluid acetylcholine. Blows varying from 280- to 460-inch pounds of energy have been used so far. At the lower energies, minimum concussion was observed and no acetylcholine was found in the spinal fluid. At the higher levels of energy, severe head injuries were incurred and the majority of the animals died. In this group acetylcholine appeared in the spinal fluid as early as 10 minutes after trauma and lasted for as long as 10 days if the animal survived. Serotonin was found in 1 animal 3 weeks after trauma. The unidentified substance has not been found in any of the animals' spinal fluids analyzed so far.

DISCUSSION

The work of Bornstein and of Ward has been corroborated by quantitative acetylcholine determinations on the cerebrospinal fluid of patients with head injuries and brain tumors as well as dogs subjected to experimental concussion. The new finding of serotonin in the spinal fluid of these patients poses more questions than it answers. Brown and his co-workers⁴ have shown that the cerebrovascular and cardiovascular effects of concussion and electroshock therapy are almost identical. The fact that LSD inhibits serotonin and produces psychoses suggests that serotonin may play a rôle in mental function.³ One might postulate that since shock therapy helps some patients recover from psychoses, and since concussion and shock therapy are so similar, perhaps concussion has an anti-LSD-like effect on serotonin metabolism. This hypothesis is being tested in the laboratory by direct tissue analysis of the brain for acetylcholine and serotonin in biopsies taken from concussed rats and dogs.

SUMMARY

1. Spinal fluid acetylcholine and serotonin determinations have been done on a series of 70* patients suffering from head injuries and brain tumors and a variety of other neurologic disorders.

2. Both acetylcholine and serotonin, as well as another unidentified substance, have been found in a large percentage of these cases. Serotonin has not been reported previously in the spinal fluid of man.

3. Comparable studies on experimentally concussed animals have also been reported.

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* Since presentation of this paper, the series of patients has been enlarged to 140.

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