Effects of Reserpine and Chlorpromazine in Prevention of Cerebral Edema and Reversible Cell Damage.* (23010)

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In an attempt to elucidate the mechanism which may cause or contribute to edema formation, the action of serotonin (5-hydroxytryptamine) on the brain was investigated. It is known that this substance is liberated or activated whenever thrombocytes disintegrate during clotting(1) and that it induces local edema upon subcutaneous injection(2). As a result of *in vivo* experiments it was found to act on the synaptic transmission(3). This last finding suggested that serotonin may have a neurotropic property through which it could theoretically contribute to the production of reversible hemiplegic symptoms. A series of tests was, therefore, carried out to establish the possible existence of such a relationship.

Should serotonin prove to be capable of causing cerebral edema as well as hemiplegic symptoms, it would then be logical to search for compounds counteracting these effects. Among drugs known to be inhibitory to certain actions of serotonin, reserpine and chlorpromazine were chosen. These two agents seemed particularly promising as reserpine was reported to prevent subcutaneous edema resulting from a challenging dose of egg-albumin in a heterogeneous animal(4) and chlorpromazine inhibited serotonin-induced subcutaneous edema(2).

Methods. 1) The edematous response of brain-tissue to serotonin was tested in albino mice. Groups of 40 mice, weighing approximately 25 g were used. Each experimental animal received 0.2 ml of a 0.4% solution of T-1824 (Evans' blue) in isotonic saline, intravenously. (This dye, which is bound to the plasma protein, stays within the vascular bed. Leakage of plasma into the tissue can thus be determined from the intense blue coloring.) Immediately upon each dye administration, one cerebral hemisphere of each experimental animal was injected with 0.05 ml of a 0.1% solution of serotonin in isotonic saline. Incidence of hemorrhage due to cerebral serotonin injection was not greater than that due to isotonic saline injection. Serotonin, however, induced edema, whereas saline did not. This indicates that under the experimental conditions serotonin did not increase the fragility of cerebral vessels. Twenty-four hours after injection, the test animals were killed by ether vapor inhalation and the intracranial contents carefully removed, frozen, and sliced by microtome technic. 2) Control studies were carried out with the same number and strain of mice, and animals treated in an identical manner, except that instead of serotonin-isotonic saline, Tyrode or Locke solution were injected intracerebrally. 3) The method for testing efficacy of reserpine and chlorpromazine in the prophylactic treatment of cerebral edema included intraperitoneal administration of varying amounts of the 2 drugs in isotonic saline solution to albino mice. Forty-five to 75 minutes later, each experimental animal received Evans' blue intravenously, immediately followed by intracerebral administration of serotonin. 4) The investigation of the role of serotonin in production of reversible hemiplegic symptoms was tested in 2 series of experiments: a) Four dogs received a unilateral internal-carotid injection of 3 mg/kg body weight of serotonin creatinine sulfate (of a 0.3% solution in isotonic saline). b) Another 4 dogs received unilateral internal-carotid injection of 0.003 μg/kg serotonin (a 0.003% solution in isotonic saline). 5) Alterations in the neurological serotonin response of the brain due to premedication with chlorpromazine and reserpine were tested in 2 series of experiments: a) Two dogs were

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premedicated with 0.25 mg and 2 dogs with 1 mg/kg body weight chlorpromazine, intravenously, 2 hours prior to serotonin injection (route of administration and dosage identical to those in 4a). b) Another 2 dogs were premedicated with 0.0001 mg and 2 dogs with 1 mg kg reserpine, intravenously, 3 hours prior to serotonin injection (route of administration and dosage identical as in 4b). 6) In order to establish whether the hemiplegic symptoms resulting from serotonin administration are due to cerebral edema or possibly to a neurotropic effect of that agent, experiments 4a), 5a) and 5b) were repeated with the only modification that each animal, immediately prior to serotonin administration, received an intravenous injection of 2 ml kg of 0.4% solution of Evans' blue.† After approximately 1 hour, the dogs were killed by sodium pentobarbital injection, the brains removed and examined for dye diffusion or increase of moisture content in serotonin-injected hemispheres (by comparison of weight of the 2 hemispheres). 7.) In order to establish whether the hemiplegia-like symptoms are due to the neurotropic or the musculotropic property of serotonin, determinations of internal jugular blood-flow were carried out (bubble-flow meter).

Results. 1) Each of the cerebral hemispheres injected with serotonin contained one blue globule of 2 mm diameter, indicative of edema. 2) None of the hemispheres injected with physiological salt solutions showed edema formation, with the exception of those which had trauma-induced hemorrhages. In other words, it could be established that exogenous serotonin produces cerebral edema, whereas physiological salt solutions do not. 3) Examination of the brains of mice, pretreated with reserpine or chlorpromazine, showed that reserpine in concentration of $1x10^{-5}$ μg/kg body weight in 0.1 ml saline inhibited the serotonin-induced edematous response of brain tissue in all test animals. With the use of $1x10^{-7}$ μg/kg in 0.1 ml of isotonic saline, 50% of the experimental animals were still protected against the edema-producing property of serotonin (Table I). In the case of chlorpromazine, 0.1 μg/kg body weight was needed to protect all animals, and 0.01 μg/kg to prevent edema formation in 50% (Table II). 4a) Serotonin in very large dosage (3 mg/kg) induced in all 4 animals the following immediate reactions: ipsilateral ptosis, mydriasis and slight facial paralysis, together with contralateral flaccid paralysis of all musculature innervated by spinal nerves. These hemiplegia-like signs were of a very transient nature, lasting less than 5 minutes, during which time the dogs defecated and urinated. 4b) Upon serotonin administration in very small dosage (0.003 μg kg), all 4 animals showed ipsilateral miosis and spastic facialis paralysis along with contralateral spastic paralyses. These hemiplegia-like signs were evident for less than 5 minutes. (To exclude errors from individual variations, the same dogs, after full neuro-

† All surgery was performed on animals under ethyl chloride anesthesia.

TABLE I. Effects of Reserpine on Serotonin-Induced Edema in Mice.

Reserpine — Intraper. admin. Dosages shown in tabulation.

Evans' blue— Intrav. admin. (tail vein, 0.2 ml of 0.4% in isotonic saline) 2 hr following reserpine.

Serotonin — Immediately following Evans' blue, 50 μg total intracer. admin. in 0.05 ml of isotonic saline.

No. of animals surviving*	Reserpine dosage (μg/kg)	With hemorrhage		No hemorrhage	
		No diffusion	Diffusion	No diffusion	Diffusion
10	1000	2	1	7	0
10	100	2	2	6	0
10	10	3	1	6	0
10	1	2	2	6	0
15	.1	0	4	11	0
12	.01	0	4	8	0
12	.001	0	5	7	0
16	.0001	0	7	9	0
13	.00001	0	4	9	0
12	.000001 1:million	0	3	8	1
13	.0000001 1:10 mil.	0	3	6	4
13	.00000001 1:100 mil.	0	4	3	6
12	.000000001 1:1000 mil.	0	2	2	8

* 40 mice used at each dose level.

TABLE II. Effect of chlorpromazine on Serotonin-Induced Edema in Mice.

Chlorpromazine— Intraper. admin. Dosages shown in tabulation.
Evans' blue — Intrav. admin. (tail vein, 0.2 ml of 0.4% in isotonic saline) 2 hr following chlorpromazine.
Serotonin — Immediately following Evans' blue, 50 μg total intracer. admin. in 0.05 ml of isotonic saline.

No. of animals surviving*	Chlorpromazine dosage (μg/kg)	With hemorrhage		No hemorrhage	
		No diffusion	Diffusion	No diffusion	Diffusion
13	10,000	0	3	10	0
21	1,000	2	5	14	0
16	100	1	3	12	0
18	10	2	5	11	0
15	1	3	2	10	0
22	.1	4	7	11	0
23	.01	3	10	5	5
24	.001	7	4	4	9
28	.0001	2	9	1	16
19	.00001	0	8	0	11

* 40 mice used at each dose level.

Approximately 30% of the animals, which received intracer. inj. (Tables I and II) died within 20 min. due to extensive hemorrhages. Approximately 30% of the surviving animals showed minute hemorrhages in the area of needle insertion, in histological examinations of serial sections. In the "no-hemorrhage" category of animals, the absence of hemorrhage was determined by histological examination of serial sections.

logical recovery, were subjected to a second serotonin injection into the same artery with identical amounts and concentrations as used in 4a. The resultant neurological findings were exactly the same as observed in 4a. In other words, the spastic paralysis from low dosage was converted into a flaccid paralysis from high dosage.) 5a) The response of all chlorpromazine premedicated animals to serotonin administration consisted in a marked amplification of the signs and their duration observed in control animals in 4a. In addition, the ipsilateral musculature of the face in some instances showed fasciculation. All these symptoms were evident for more than one hour. In analogous experiments, chlorpromazine did not noticeably alter the stimulating effect of serotonin administered in small doses. 5b) The result of reserpine premedication in all 4 animals was a very slight ipsilateral pupillary dilatation (during the large dose injection period only, altogether lasting less than 3 seconds). The dogs did not show any signs of ipsilateral spastic or flaccid facialis paralyses, nor did they have contralateral spastic, respectively-flaccid paralyses. 6) Dye administration did not alter the serotonin-induced hemiplegic symptoms, nor could gross edema formation and increase of weight be detected in the serotonin-injected hemispheres of any animal. 7) Internal jugular blood-flow was not altered due to injection of serotonin into the internal-carotid artery, thereby suggesting that the neurotropic effect of serotonin is responsible for the development of hemiplegic symptoms.

Discussion. Our findings raise the question as to whether or not a portion of the post-apoplectic edema may be due to serotonin release from disintegrating thrombocytes. A possible explanation for the mechanism of such edema formation may be sought in the known positive musculotropic effects of serotonin. This agent, entering the interstitial spaces of the brain, may thus be expected to cause local vasoconstriction, which in turn leads to ischemia and an increase of the cell permeability. Concomittant neurological changes in the contralateral musculature of extremities indicate that the facial paralyses were due to brain responses rather than to spilling of injection fluid. Moreover, the internal carotid artery of the dog has no branches supplying facial musculature. Reserpine and chlorpromazine were found to prevent the cerebral edema-inducing effect of serotonin. It appeared, however, that cerebral edema in itself does not produce the reversible hemiplegic symptoms, but that it is the neurotropic action of serotonin which may be held responsible for these symptoms. Edema *per se* may, of course, prolong and thus aggravate the damage to the brain-cells involved. Chlorpromazine premedication potentiates the neurotropic action of large doses of serotonin; in other words, it aggravates flaccid paralyses. It does not influence the spastic paralyses as caused by serotonin in small dosage. Reserpine premedication inhibits the neurotropic action of serotonin, regardless of whether the latter agent was ad-

ministered in stimulating or depressing dosage. It was not possible to test the therapeutic efficacy of reserpine after serotonin administration, as the neurological symptoms induced by intra-arterially injected serotonin were of too short duration.

Summary. 1) Serotonin, administered into brain-tissue via an extravascular route, induces edema, possibly due to its musculotropic property. 2) Serotonin was confirmed to have a neurotropic action which appeared to be responsible for the reversible hemiplegic symptoms. 3) The neurotropic effect of serotonin is biphasic, *i.e.* when administered in low dosage via internal-carotid artery, it causes transient spastic paralyses. In high dosage, it causes flaccid paralyses without inducing cerebral edema. 4) Chlorpromazine and reserpine both block the edema-inducing action of serotonin. Reserpine is, however, effective in a much lower dosage. 5) Chlorpromazine potentiates the neurotropic depressant effect of serotonin, but apparently does not alter its stimulating action. 6) Reserpine inhibits the neurotropic effect of serotonin. This holds true for the spastic as well as for the flaccid symptoms. 7) Therapeutic considerations suggest that reserpine may be specifically indicated in prophylactic treatment of apoplexy.

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Japanese B Encephalitis Virus in Tissue Culture.* (23011)

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The virus of Japanese B encephalitis (JBE) grows readily in a variety of tissue culture systems. Kawakita(1) successfully propagated JBE virus serially in Maitland-type cultures of minced chick embryo brain, and in minced whole chick embryo(2). Scherer and Syverton(3) reported 13 serial passages of JBE virus in HeLa cell cultures. Cytopathogenic effect (CPE) was noted only with the first 4 passages although continued virus proliferation was evidenced by mouse pathogenicity of subsequent tissue culture passages. Mason reported growth of 2 strains of JBE virus in HeLa cell cultures with CPE noted only in 13th and 41st passages of one strain and only in 32nd passage of the other. In general, however, consistent and reproducible CPE has not been observed. The present report is concerned with the propagation of JBE virus in several tissue culture systems, primarily in the cell line currently known as Detroit-6 (originally isolated by Berman, Stulberg and Ruddle(4) from human bone marrow), the only type of cells which has shown consistent CPE in our experience.

Materials and methods. Viruses: *a) Japanese B. encephalitis.* Strain M_1311 was received as the seventh mouse brain passage of a virus isolated from a pool of *Culex tritaeniorrhynchus* mosquitoes. These were trapped near Tokyo, Japan in 1951 by members of the 406th Medical General Laboratory (U.S. Army). Strain M_32204 B was received as the third mouse brain passage of a similar isolation made in 1953. Both were kindly supplied by Major Edward L. Buescher, Walter Reed Army Institute for Research. Both strains were passed one time in suckling Swiss albino mice for stock suspensions, used to initiate tissue culture passages. *b) West Nile.* Strain EV 101 was supplied as third mouse brain passage by Dr. Joseph L.

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