THE EFFECT OF IRRITATION ON THE PERMEABILITY OF THE MENINGES FOR SALVARSAN.

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One of the explanations offered for the beneficial effects of intraspinal injections in the treatment of syphilis of the central nervous system is that there may thus be induced an increased permeability of the meninges following their irritation by the injected substance. The purpose of this investigation was to determine whether the intraspinal, or rather subdural, injection of various substances used in intraspinal treatment would increase the amount of arsenic in the cord and brain of animals which received intravenous injections of salvarsan at the same time.

Sicard and Reilly (1) described a method for increasing the permeability of the brain substance for drugs by trephining the skull and injecting 5 cc. of a 0.5 per cent solution of sodium chloride under the dura with a fine hypodermic needle. They showed that in the cadaver a similar amount of India ink injected subdurally was distributed over an area 8 or 10 cm. in diameter, and suggested from this experimental evidence that two subdural injections of saline, *i. e.*, one in each temporofrontal region, would be sufficient to increase the permeability of a large part of the cerebrum for salvarsan introduced intravenously. They also state that they have injected 0.1 mg. of cyanide of mercury subdurally in the frontal region without harmful effect, and on the following day given salvarsan or neosalvarsan intravenously to the same patient. The cyanide of mercury was used simply to increase the permeability of the tissues. Viton (2) states that after a slight chemical irritation the meninges are rendered more permeable, and he used for this purpose a preparation suggested by Sicard (3) which consists of cyanide of mercury 0.1 mg., and novocain 0.015 gm. in 2 cc. of a 0.5 per cent saline solution. The intraspinal injections were made at intervals of 1 month, mercury or salvarsan being given in the usual way. He states that with this method of treatment the irritative symptoms of tabes are much relieved. Tinel and Leroide (4) injected several drops of a 1 per cent solution of sodium nucleinate into the fourth ventricles of rabbits and immediately afterwards gave the animals 10 cg. of neosalvarsan intravenously. One hour later they again punctured the fourth ventricles and obtained 4 cc. of fibrinous fluid,

which contained 0.008 mg. of arsenic. Fluid from the fourth ventricles of control animals contained no arsenic. The fibrinous fluid was evidence of a much more intense irritation than is usually produced by subdural injections in patients, so the increase in permeability which these authors demonstrated cannot be applied to bedside treatment.

The intraspinal injection of normal salt solution, or serum, does produce, however, a temporary irritation of the meninges, as is shown both clinically by pains, and by examination of the cerebrospinal fluid a few hours after such an injection, when several hundred cells per cmm. may be found. It is reasonable to suppose that this irritation might increase the permeability of the tissues, and that salvarsan circulating in the blood would be deposited in larger amounts in the tissues contiguous to the irritated meninges, than when the meninges are in their normal impermeable condition.

Methods.

To determine whether a demonstrable increased deposition of arsenic actually does occur, the following experiments were performed. Cats were selected as the experimental animals, for the following reasons: Intravenous injections can be easily given into the marginal vein of the ear, and there is a fairly large subdural cistern surrounding the cauda equina, so that subdural injections in the cat are similar in nature to those in man in that the injecting needle is not brought into direct contact with the cord. In rabbits the cord extends so low that it is impossible to introduce a needle under the dura and inject a solution without injuring the cord. In the experiments under discussion it was necessary that the nervous tissue be injured in no other way than that possibly resulting from the presence of the substance injected. The intraspinal injections in the cats were made in the following manner: The animals were etherized, and the dura was exposed by laminectomy of the two lower lumbar vertebræ. A fine curved hollow needle attached to a syringe was introduced through the dura, and cerebrospinal fluid was aspirated into the syringe, which was then detached, and another syringe containing the solution to be injected was attached to the needle, and the solution slowly injected. The injections were always preceded by aspiration of cerebrospinal fluid, to be sure that the needle was properly placed. All the animals were

injected intravenously with salvarsan in alkaline solution in the proportion of 0.05 of a gram per kilo of body weight. Some of the animals received the intravenous injections one hour before the intraspinal treatment, and in others the order was reversed, so that at the time of intravenous injection the meninges would be already in a state of irritation. On the following day the animals were exsanguinated by opening the jugular veins and carotid arteries, in order to remove as much arsenic-containing blood from the tissues as possible. The cord and brain were immediately removed from the body and the brain was divided into three portions: cerebrum, cerebellum, and midbrain, pons, and medulla; the cord was separated from the medulla, and all the specimens were dried separately. The dried tissue was powdered, thoroughly mixed, and weighed, and duplicates of each specimen were analyzed quantitatively for arsenic.

The analyses were made by a special method devised for the purpose by Vinograd (5). This method depends for its accuracy upon the principle of oxidizing the tissues with small amounts of arsenic-free nitric acid, at 260° C. in a sealed glass bomb. The bomb is then opened, sulphuric acid added, the nitric acid driven off by heating, and the sulphuric acid-arsenic mixture quantitated for arsenic by Sanger and Black's modification of Gutzeit's method. All reagents and utensils were carefully tested for arsenic before using. The standard scales for comparison were made with both arsenious acid and salvarsan. In the results here presented the figures are given in fractions of mg. of salvarsan per gram of dried tissue.

RESULTS.

The operative procedures, substances injected, time relations, and results of the analyses are given in Table I. Four control animals were first treated. Two of them had only intravenous injections of salvarsan. One of these killed one and one half hours later showed no more arsenic in its cerebrospinal axis than one killed after eighteen hours. The comparatively slight neurotropic action of salvarsan demonstrated by Ullmann (6) and by Stühmer (7) probably explains this similarity in arsenic content at such different intervals after treatment. In the other two controls the operative procedure, laminectomy and withdrawal of cerebrospinal fluid, was shown to

have no effect. The effect of irritation with the following substances was studied: isotonic salt solution; 50 per cent cat serum diluted with isotonic salt solution; pure cat serum; salvarsanized cat serum obtained by treating cats with 0.05 of a gram of salvarsan per kilo of body weight, bleeding one hour later, separating the serum, and heating to 56° C. This serum contained between 0.015 and 0.025 mg. of salvarsan per cc. of serum. It was injected in 50 per cent dilution and also undiluted. A mixture of cyanide of mercury, novocain, and 0.5 per cent saline, similar to that used by Sicard and by Viton, was also injected.

As a rule, the cords of the ten animals which received intraspinal injections contained no more arsenic than the four controls. The two exceptions are Animals H 104 and H 106, which received intraspinal injections of normal cat serum in a 50 per cent dilution. There are two possible explanations for this variation. First, the 50 per cent serum may have been more irritating than the other substances injected; or, second, the animals were not so completely exsanguinated. We are inclined to attribute the increased amount of arsenic in the cords of these animals to incomplete exsanguination, for in both animals the cerebrum and cerebellum contained considerably more arsenic than the average. Furthermore, if the 50 per cent serum were the important factor, one would expect that the cords of Cats H 110 and H 112, which received intraspinal injections of 50 per cent dilution of salvarsanized serum, would show a similar increase in arsenic, but in these animals both the cords and brains showed the average arsenic content. Upon first thought, one would expect that the cerebrospinal axis of the animals that received subdural injections of salvarsanized serum would contain more arsenic than the animals injected with normal serum or mercury. The rapid diffusion of the small amount of arsenic in the serum through the cerebrospinal fluid, and the rapid excretion of substances from the cerebrospinal fluid into the blood stream, probably explain the fact that the cords and brains of animals which received salvarsanized serum intraspinally contained no more arsenic than the average. Both of these factors have been conclusively demonstrated by Dandy and Blackfan (8). Hall (9) has also shown that the cerebrospinal fluid of patients who received

TABLE I.

Animal No.	Weight.	Operative procedure.	Substance injected intraspinally.	Time relation of intraspinal injection (I.S.) to intravenous injection (I.V.).	Killed after intravenous injection.	Salvarsan per gm. of dried tissue.				Remarks.
						Cerebrum.	Cerebellum.	Midbrain, pons, medulla.	Spinal cord.	
	gm.				*hrs.*	*mg.*	*mg.*	*mg.*	*mg.*	
H 115	3,000	None	None		1½	0.025	0.025	0.025	0.017	
H 113	2,560	"	"		18	0.017 (N)*	Traces	0.025	0.025 (N)*	
H 105	3,000	Laminectomy	"		19	0.037	0.037	0.025	0.025	
H 107	1,080	Laminectomy and 1 cc. cerebrospinal fluid withdrawn	"	1 hr. before I.V.	18	0.025	0.025	0.017	0.017	
H 116	3,100	Laminectomy and subdural injection	1 cc. 0.9% sodium chloride solution	I.S. 1 hr. after I.V.	17	0.017	Traces	0.025	Traces	
H 104	2,800	" "	1 cc. 50% normal cat serum	" " before "	13	0.037	0.037	0.025	0.075	
H 106	1,900	" "	" "	" " after "	17	0.075	0.075	0.025	0.037 (N)*	
H 109	1,550	" "	1 cc. 100 % normal cat serum	" " before "	16	0.025	0.017	0.025	0.025	
H 108	2,050	" "	" "	" " after "	16	0.025 0.025	Traces	0.025 0.017	0.025	Weakness in both hind legs day after operation.
H 112	2,650	" "	1 cc. 50% salvarsanized cat serum	" " before "	15	0.017	0.025	0.017	0.025	
H 110	2,250	" "	" "	" " after "	19	0.017	0.017	Traces	0.025	Weakness in right hind leg day after operation.
H 111	1,670	" "	1 cc. 100% salvarsanized cat serum	" " " "	19	0.025	Traces	0.017	0.025	
H 117	2,500	" "	Mercury cyanide solution	" " before "	19	0.025	0.017 (N)*	0.025	0.025	Weakness in both hind legs day after operation.
H 118	2,850	" "	" "	" " after "	16	0.017	Traces	Traces	0.025	

* (N), duplicate lost. All results are in duplicate unless otherwise noted.

intraspinal injections of neosalvarsan often contains no arsenic twenty-four hours after the injection.

If irritation alone were the important factor in determining the deposition of arsenic in the nervous tissue, the cords, which were more exposed to the irritating effect of the substances injected than the brains, should have shown a higher arsenic concentration. The average arsenic content of the cord and of the cerebrum was practically the same; *i. e.*, 0.029 mg. for the former, and 0.028 mg. for the latter. The cords of the three animals, H 108, H 112, and H 113, which showed clinical evidences of cord or cauda equina injury did not contain more arsenic than the cords of animals which showed no evidence of local lesions. Nor did the more irritating mercury solutions increase the arsenic in the cords or brains of the animals which received it. There was no difference between the animals that received intraspinal injections an hour before the intravenous injections of salvarsan, and those that first received the salvarsan intravenously.

CONCLUSIONS.

The subdural injection of normal salt solution, normal serum, serum salvarsanized *in vivo* or weak solutions of cyanide of mercury does not demonstrably increase the permeability of the spinal cord or brain for salvarsan which is circulating in the blood at the time of the subdural injection.

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