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Concentration of Streptomycin in Brain and Other Tissues of Cats After Acute and Chronic Intoxication.* (18334)

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The disturbance of equilibrium which can be produced in the cat by the parenteral administration of suitably large doses of streptomycin develops only after several days of treatment(1). The time of onset of the characteristic ataxia, accompanied by loss of nystagmus in response to rotation, varies inversely with the size of the dose employed (Fig. 1). The manner in which this cumulative toxic effect is produced is still unknown. The experiments to be described were made to test one possible explanation, namely that chronic streptomycin intoxication is associated with an accumulation of the drug in the cerebrospinal fluid and in the central nervous system. The negative results, demonstrating that such an accumulation does not occur, are in accord with histological(2,3) and electrophysiological(4,5) studies which have been published since this work was done, indicating that the primary site of the toxic action of streptomycin is not in the brain stem but rather in the vestibular and cochlear end-organs. On the other hand the present experiments are of interest because they show that, unlike the brain, other organs such as the lungs and liver contain significant concentrations of the drug even 72 hours after a single dose.

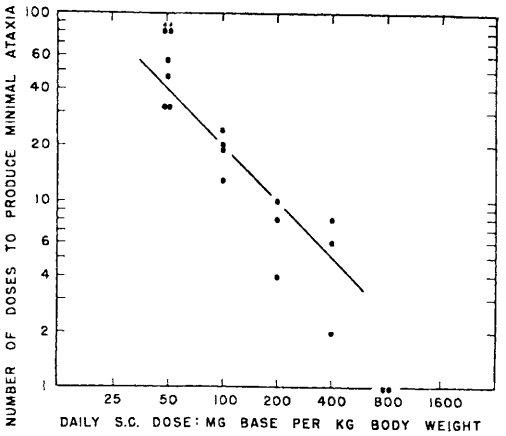

FIG. 1.

No. of doses of streptomycin required to produce minimal ataxia in cats, plotted as a function of the size of the daily dose. The straight line represents a *constant total dose* of 2 g per kg, *e.g.*, 200 mg/kg/day for 10 days. The data may be interpreted to mean that within the range 50 to 400 mg/kg an approximately constant total quantity of streptomycin is required to produce signs of vestibular disturbance. A single dose of 800 mg/kg is lethal. Streptomycin hydrochloride Merck, Lot No. 731. (From data of Hawkins and O'Shanny presented at the meeting of the Fed. Am. Soc. Exp. Biol., Atlantic City, March 1948).

Method. The fluorometric method for the determination of streptomycin in body fluids(6) and in tissues(7) was employed.

* Presented at the meeting of the Federation of American Societies for Experimental Biology, Detroit, April 1949.

1. Hawkins, J. E., Jr., *Fed. Proc.*, 1947, v6, 125.

2. Caussé, R., Gondet, I., and Vallancien, B., *Compt. rend. Soc. Biol.*, 1949, v143, 619.

3. Berg, K., *Ann. Otol.*, 1949, v58, 448.

4. Vanderhaeghe, H., *Arch. Int. Pharm.*, 1949, v79, 269.

5. Hawkins, J. E., Jr., *J. Pharmacol.*, 1950, v100, 38.

6. Boxer, G. E., and Jelinek, V. C., *J. Biol. Chem.*, 1947, v170, 491.

TABLE I. Acute Intoxication. Streptomycin concentrations in body fluids and tissues of cats after single dose of 400 mg per kg S.C.

Cat	Hr after dose	Serum	CSF	Hemispheres	Cerebellum	Brain stem	Lung	Liver
1	2	796	5					
	5	680						
	24	116	35	14	18.5	14	86	84
2	2	517	5					
	5	313						
	26	21	1.5					
	48	1.5	1	2.5	3.5	4	17	20
3	2	874						
	5.5	464						
	26.5	31						
	48.5	4						
	72	1	2	2	4	3.5	23	38

SM conc. in body fluids, μg per ml; in tissues, μg per ml (wet wt).

The lower limit of sensitivity of the method for body fluids is 1 μg of streptomycin in 1 ml of fluid, and for tissues 2 μg per g of tissue.

Acute Intoxication. In order to determine whether streptomycin remains in the brain after a single large dose, the drug in the form of the calcium chloride complex was given to 3 cats by subcutaneous injection of a dose equivalent to 400 mg streptomycin base per kg of body weight (corresponding to 24 g for a 60 kg man). Typical signs of acute intoxication appeared: vomiting, hyperpnea, prostration and finally stupor lasting for several hours. Two hours after the injection the concentration of the drug in the serum ranged from 500 to 900 μg per ml. No tissues were taken for analysis at this time, however, since the actual concentration in the tissues cannot be determined in the presence of high levels in the blood. Instead the method chosen was to measure the concentration remaining in the tissues at a time when it was expected that removal of the drug from the blood by renal excretion would be almost complete. The 3 animals were sacrificed and tissues were taken for analysis only after 24, 48 and 72 hours respectively. Data from this experiment are presented in Table I. It was concluded that streptomycin does enter the brain after a very large dose and can persist there for as long as 3 days. The amount in the brain, however, is small as compared with that in other tissues.

Chronic Intoxication. For repeated subcutaneous administration a dose of 100 mg streptomycin base per kg per day was chosen. Four cats were so treated for 7, 14, 21 and 28 days respectively. The serum concentrations of streptomycin obtained after the first dose, which was given intramuscularly, are shown in Fig. 2. One representative case is illustrated, since the curves for the 4 cats are practically superimposable. Blood samples taken on various days during the experiment, in each case 24 hours after the previous injection, showed plasma concentrations of 1 to 4 μg per ml. There was no evidence of accumulation of streptomycin in the blood or CSF with continued treatment, a finding which is in agreement with the experiments on the effect of multiple doses on blood concentrations reported by Boxer *et al.*(8). Data obtained at the time of sacrifice of these four animals are presented in Table II.

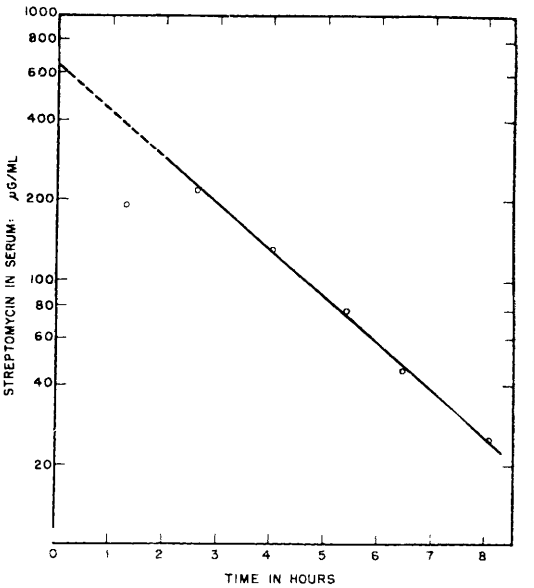

FIG. 2.

Streptomycin concentrations in the serum of cat 6 after a single intramuscular inj. of 100 mg base/kg.

7. Jelinek, V. C., and Boxer, G. E., *J. Biol. Chem.*, 1948, v175, 367.

8. Boxer, G. E., Jelinek, V. C., Tompsett, R., Dubois, R., and Edison, A. O., *J. Pharmacol.*, 1948, v92, 226.

TABLE II. Chronic Intoxication. Streptomycin concentrations in body fluids and tissues of cats after daily doses of 100 mg per kg SC.

Cat	No. of doses	Hr after last dose	Ataxia	Serum	CSF	Hemispheres	Cerebellum	Brain-stem	Spinal cord
4	7	48	—	1	<1				
5	14	24	1+	2	1	1	1	1	—
6	21	24	4+	3	2	2	2		1
7	28	24	4+	2.5	3	1	1	1	1

Cat	Lung	Liver	Spleen	Parotid	Kidney	Urine
4	7	16.5	12	7	119	21
5	16	23	24	22	234	—
6	32	41	21	18	176	360
7	20	10	22	12.5	148	90

SM conc. in body fluids, μg per ml; in tissues, μg per ml (wet wt).

Discussion. The present experiments show that although a small quantity of streptomycin can be detected in brain tissue after a single large subcutaneous dose, there is no accumulation of the drug in the brain as a whole or in the brain stem during the development of a severe chronic intoxication. If there had been a striking accumulation in particular medullary nuclei, this should have been reflected in the concentration measured in the brain stem. On the contrary, there was no difference between the concentration in the stem and that in other portions of the brain. This finding is in agreement with the results of the investigations already cited(2-5), which demonstrate that the labyrinthine and cochlear end-organs are the primary site of the neurotoxic action. Unfortunately no data on streptomycin concentrations in the endolymph and perilymph have been obtained because of obvious technical difficulties. Nevertheless it is reasonable to assume that the concentration in the perilymph corresponds to that in the CSF, and that the drug reaches the end-organs by this route. The same considerations presumably apply in the case of the less neurotoxic dihydrostreptomycin(9,5).

Concentrations determined in the lungs, liver and other organs must represent streptomycin actually contained in the cells of these tissues and not merely in the extracellular fluid, where the concentration should correspond approximately to that in the serum. No conclusion can be drawn concerning possible binding to the tissue proteins, since the deproteinization technique employed in the analytical procedure liberates any protein-bound streptomycin.

The data in Table I indicate that streptomycin once present in the tissues is removed only slowly. When the values at 24, 48 and 72 hours after injection are compared, it is apparent that the fraction of the drug present in the blood and extracellular fluid is removed rather rapidly by excretion through the kidney, while the tissue concentration is decreasing at a much slower rate. A strikingly high tissue concentration was found in the kidney itself (Table II). This observation is in accord with that of Adcock and Hettig(10), who reported streptomycin in the kidneys of 2 patients who had died during treatment for tuberculous meningitis.

Summary. Streptomycin was found in the brain, lungs and liver of cats for as long as 3 days after a single injection of 400 mg per kg. At this time the drug had virtually disappeared from the blood and CSF. The concentration in the brain was small compared with that in the other organs. In cats showing typical chronic intoxication during treatment with streptomycin in daily doses of 100 mg per kg, no accumulation of the drug was found in the serum, CSF or brain 24 hours after last dose. Significant concentrations of the drug were present in other tissues, especially in the kidney. It is concluded that the chronic neurotoxic action of streptomycin cannot be attributed to accumulation of the drug in the brain as a whole or in the brain stem.

9. Edison, A. O., Frost, B. M., Graessle, O. E., Hawkins, J. E., Jr., Kuna, S., Mushett, C. W., Silber, R. H., and Solotorovsky, M., *Am. Rev. Tuberc.*, 1948, v58, 487.

10. Adcock, J. D., and Hettig, R. A., *Arch. Int. Med.*, 1946, v77, 179.

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