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The Use of the Mouse in Studies on Streptomycin.

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The therapeutic value of a drug is determined by 2 general aspects; namely, (a) its physiologic distribution in the host and (b) its specific biologic activity. The physiologic distribution may vary greatly with the host as has been pointed out by Marshall[1] with respect to the sulfonamides. The situation with regard to streptomycin is not clear because of widely divergent results which have been published by various investigators.

The mouse, being extensively used in chemotherapeutic studies, is of particular importance in the study of antibiotic substances where the quantities of drug available for research may be sharply limited. Consequently, knowledge of the comparative behavior of streptomycin in mouse and man is of considerable value. The wide range of results which have been reported in the numerous absorption-excretion studies performed in man is summarized in Table I. Aside from an expected variation in individuals, and probably in the assay method and technics, the explanation for the divergence is not clear.

Absorption of streptomycin in mice, as indicated by blood level studies, has been reported[2,9] but no information has been made available on excretion rates. Such data are essential if one is to examine whether streptomycin is destroyed in the mouse to a greater extent than in man.[2] During the past year and a half we have carried out a number of absorption-excretion studies of streptomycin in the mouse as well as in humans (adults and children). Since our findings have bearing on the question of the relative rates of excretion of this antibiotic in these 2 species it was thought to be of interest to present some of these results.

ABSORPTION OF STREPTOMYCIN IN MICE

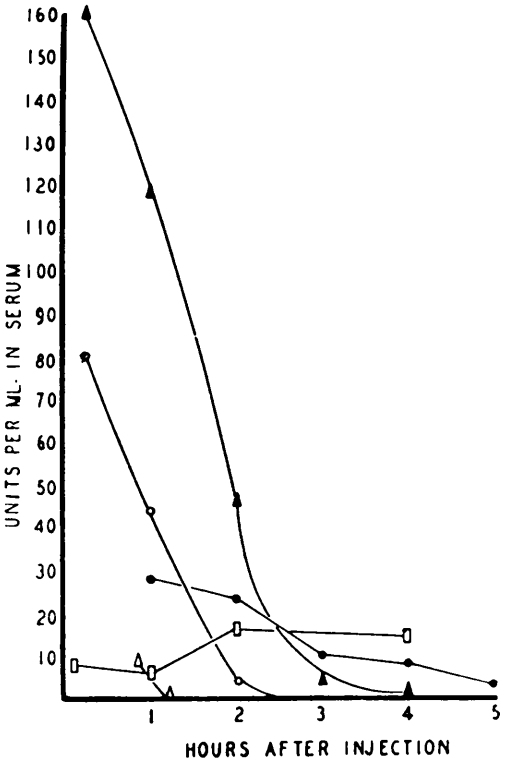

Fig. 1.

▲ 250,000 units per kg. subcutaneously.
○ 104,000 " " " "
△ 23,000 " " ", intramuscularly.
● 1,140 " " ", " in children.
□ 500,000 units, intramuscularly in man, cf Buggs, *et al.*[8]

[1] Marshall, E. K., Jr., *University of Pennsylvania Bicentennial Conference, Chemotherapy*, University of Pennsylvania Press, 1941.

[2] Kornegay, G. B., Forgacs, J., and Henley, T. F., *J. Lab. and Clin. Med.*, 1946, **31**. 523.

[3] Elias, W. F., and Durso, J., *Science*, 1945, **101**, 589.

[4] Heilman, D. H., Heilman, F. R., Hinshaw, H. C., Nichols, D. R., and Herrell, W. E., *Am. J. Med. Sci.*, 1945, **210**, 576.

[5] Rutstein, D. D., Stebbins, R. B., Cathcart, R. T., and Harvey, R. M., *J. Clin. Invest.*, 1945, **24**, 898.

[6] Anderson, D. G., and Jewell, M., *New England J. Med.*, 1946, **233**, 485.

[7] Zintel, H. A., Flippin, H. F., Nichols, A. C., Whiley, M. M., and Rhoads, J. E., *Am. J. Med. Sci.*, 1945, **210**, 421.

[8] Buggs, C. W., Pilling, M. A., Bronstein, B., and Hirshfeld, J. W., *J. Clin. Invest.*, 1946, **25**, 94.

[9] Stebbins, R. B., Graessle, O. E., and Robinson, H. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 68.

TABLE I.
Composite Table of Excretion of Streptomycin in Man, Dog, and Monkey as Reported in the Literature.

Animal	Streptomycin administered		% recovered in urine in 24 hr or longer	Reference
	Route	Dose × 1000 (units)		
Man	I.M.	25	6.1-6.5	(8)
	"	50	19.5	(8)
	"	100	59.1-68.1	(4)
	"	200	42-78.2	(8)
	"	200	63 (4 hr)	(6)
	"	300	72	(6)
	"	400	16.7-22.2	(4)
	"	400	41-53.8	(8)
	"	500	56-71	(8)
	"	600	45.5-75.5	(5)
	"	600	87 (12 hr)	(6)
	"	20,000 in 6 days	44	(3)
	"	varied, over 29 days	30†	(2)*
	"	varied, over 25 days	103‡	(2)*
	I.V.	50	50-85.6	(8)
	"	100	52.3-80.0	(4)
	"	200	64-91.7	(8)
	"	400	62.9	(4)
	"	400	42.7-48	(8)
	"	500	79-83.5	(8)
	"	600	29-89§	(7)
	"	28,000 in 7 days	70	(3)
Monkey	I.M.	10 per kg/day	60-66	(9)
	"	50 per kg/day	38-44	(9)
Dog	I.M.	Not indicated	*ca.* 50-80‖	(9)

* Calculated from the published data.
† Total recovery over 29-day period of treatment.
‡ Total recovery over 25-day period of treatment.
§ Average recovery of streptomycin in the urine in 10 cases was 66%.
‖ Recoveries published as approximate figures.

Absorption and Excretion of Streptomycin in Mice. Procedure. Large groups (usually 100) of female Swiss mice weighing between 19 and 21 g were given a single dose of streptomycin subcutaneously or intramuscularly. At given intervals, beginning 15 to 30 minutes after injection, 10 mice were sacrificed by bleeding from the heart, the blood pooled, and assays run on the serum. Immediately prior to each bleeding period, urine samples were taken from all surviving mice (by applying slight pressure over the bladder region) and the urine pooled, measured and assayed by the broth dilution method.[10]*

Results. In Fig. 1 are shown the serum levels reached in mice following a single injection of streptomycin at 3 dose levels. Also included, for purposes of comparison, are a composite curve of similar data we have obtained in infants as well as a curve showing data taken from the work of Buggs, *et al.*[8] In the latter case, the body weight of the patient was not indicated and hence the dosage cannot be given in terms of units per kg. It will be noted that serum levels of streptomycin in mice drop considerably faster than in humans following a single dose of the antibiotic.

The excretion rates as shown by the appearance of antibiotic in the urine of mice as compared to data in humans reported by

[10] Donovick, R., Hamre, D., Kavanagh, F., and Rake, G., *J. Bact.*, 1945, **50**, 623.

* The various sera studied caused no apparent inhibition of the test organisms. However, assays of sera to which small amounts of streptomycin were added gave results ranging from 34-40% higher than aqueous controls. The streptomycin concentrations were such in these assays that they were carried out on undiluted or low dilutions of serum. Similar studies with low dilutions of urine gave results of 9-23% higher than controls. However, in the absorption-excretion studies in mice the streptomycin concentrations in most of the serum and urine samples were great enough to require considerable dilution of the sample for assay, thereby minimizing this effect to an extent that it may be neglected in the calculations.

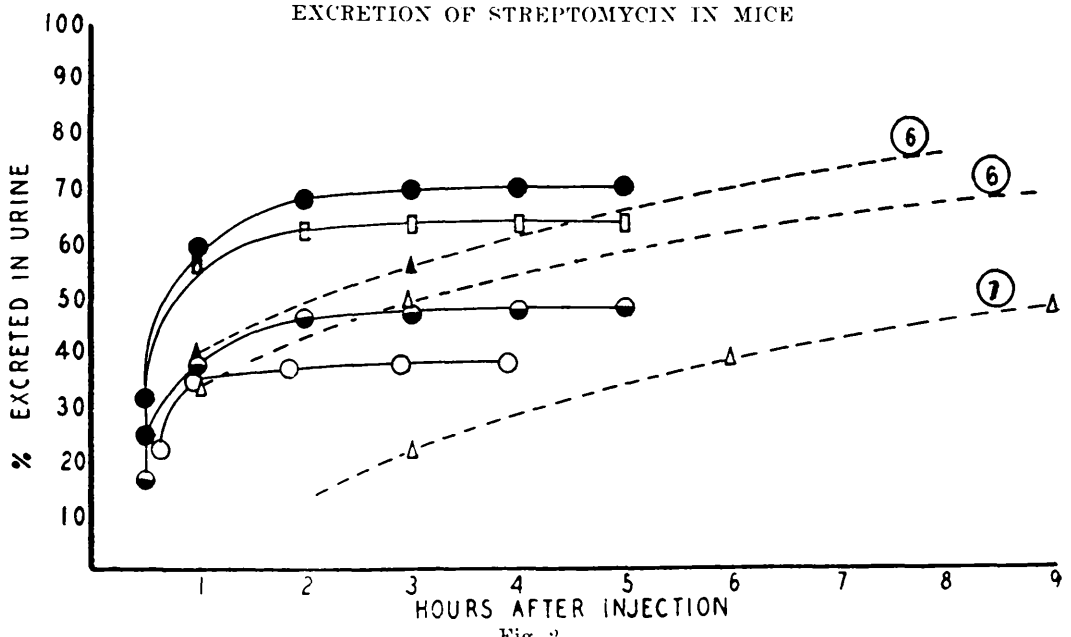

Fig. 2.

● 105,000 units per kg (with alum), subcutaneously.
□ 250,000 " " ", subcutaneously.
○ (half solid) 104,000 units per kg, subcutaneously.
○ 23,000 units per kg, intramuscularly.
▲ 600,000 units, I.M., (total dose) in man, cf Anderson, *et al.*[6]
△ 300,000 " " (total dose) in man, cf Anderson, *et al.*[6]
△ 600,000 " , I.V., (total dose) in man, cf Zintel, *et al.*[7]

Anderson, *et al.*[6] and Zintel, *et al.*[7] are shown in Fig. 2. Here again, unfortunately, the dosage in humans was not reported with regard to body weight. Nevertheless, it appears evident that the excretion of streptomycin into the urine is more rapid in mice than in man. These data parallel the findings on the rate of decrease in serum levels in these 2 species.

The percentage of streptomycin recovered in the urine of mice by the procedure used must be considered minimal since some urine might have been lost between samples. However, the total recovery was similar to that found in children (see below) and that reported in adults (Table I). Thus, the recovery of streptomycin in the urine ranged from 37.5 to 69.5% of the dose in mice receiving from 460 to 5,000 units intramuscularly or subcutaneously (23,000 to 250,000 units per kg). Except for a more rapid rate of excretion, the mouse does not appear to handle streptomycin differently than does man.

Judging from the data published by Stebbins, Graessle and Robinson[9] the rate at which the blood levels drop in dogs, following a single intramuscular injection, is not greatly different from that in mice. In monkeys it appears to be somewhat slower.

In Fig. 2 it will be noted that one excretion study in mice was carried out with a streptomycin-alum mixture. Carter, *et al.*[11] reported that streptomycin was adsorbed on alkaline alumina. It was therefore of interest to know whether streptomycin injected in mixture with alum was excreted at a slower rate than was streptomycin alone. For this purpose a solution containing 9200 units of streptomycin-sulfate per cc was mixed with an equal volume of 4% aluminum-potassium sulfate and sufficient N/1 NaOH was added to bring to pH 7.6. Female mice were injected subcutaneously with 0.5 cc of the mixture and the urine collected and pooled at given intervals as in the other experiments. In this instance blood levels were not studied.

11 Carter, H. E., Clark, R. K., Jr., Dickman, S. R., Loo, Y. H., Skell, P. S., and Strong, W. A., *J. Biol. Chem.*, 1945, **160**, 337.

It was found that the excretion rate of the streptomycin administered together with alum was similar to that of streptomycin alone. The calculated recovery of the antibiotic in the urine was somewhat higher but not significantly so. Apparently the alum did not retard excretion. However, in all previous excretion studies in mice, the urine collected during the first hour or 2 following injection was a deep, reddish brown in color, very similar to that of the concentrated streptomycin solutions used. In the alum study, on the other hand, the urine was normal in color throughout the experiment, indicating that some of the pigmented impurities of the streptomycin preparation had been retained by the alum while the streptomycin was not.

Excretion of Streptomycin in Humans. Considerable data on the absorption and excretion of streptomycin in humans has already been reported (Table I). Consequently no special attempt was made to collect extensive data on this species. Limited data on streptomycin excretion have been collected on one adult and 3 infants to compare recovery rates in the urine with those found in mice, using the same bio-assay procedure.[10]

An adult patient, weighing 160 lb, was given 1,850,000 units of streptomycin subcutaneously in divided doses over a 6-day period. The average daily dose was 4,200 units per kg. The overall recovery of antibiotic in the urine was 75% of the streptomycin administered, but the ratio of units administered to units recovered in the urine varied somewhat during various stages of the study.

In 3 small children receiving 20,000 units of streptomycin per lb (ca 9100 units per kg) per day, intramuscularly, in interrupted doses or by continuous drip, the streptomycin administered which could be accounted for in the urine at any given period during the course of treatment again varied considerably with the individual. The overall recoveries of antibiotic in the urine were 31.5, 45.0 and 45.5% respectively. Although these are lower recoveries than some reported in adults, they fall within the general range found in man.

Conclusions. Whereas streptomycin is absorbed and excreted considerably faster in mice than in man, the percentage recovery of streptomycin in the urine of these 2 species is very similar. Hence, there is no reason to believe that this antibiotic is destroyed in mouse to any greater extent than in man.

Despite the fact that streptomycin is adsorbed on alkaline alumina, streptomycin administered in mixture with alkaline potassium aluminum sulfate is excreted as rapidly in mice as is streptomycin given alone.

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Proteins with Growth-Promoting Action on Tissue Cells *in vitro*.

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Among the many substances which contribute to the effectiveness of embryonic tissue extracts as growth-promoting media for cells *in vitro*, some have been found to be associated with the proteins of the extracts. The ease of inactivation of the extracts by heat and by proteolytic enzymes were early indications that proteins played some role in the effects which result in increasing the area of explanted tissue relative to the area of sister control-cultures.

The lability of the high molecular substances in the embryonic extracts makes it necessary to use only very mild methods in attempts to extract or purify active fractions. It has been found by Fischer and Astrup[1] that a rather high activity was associated with a fraction separated by the method used by Hammarsten[2] for preparation of nucleoproteins. Experiments have now confirmed

[1] Fischer, A., and Astrup, T., *Pflüg. Arch. ges. Physiol.*, 1943, **247**, 34.

[2] Hammarsten, E., *Hoppe-Seyl Z.*, 1920, **109**, 141.