TABLE I. Effect of Cortisone on Content of Free Hydroxyproline in the Tissues of 18-Day Chick Embryos.

Series	Treatment	Hydroxyproline content; γ per 100 mg of fresh tissue		
		Brain	Liver	Heart
1	Control	12.0	—	—
	Cortisone	68.0	—	—
2	Control	4.0	3.8	5.6
	Cortisone	15.9	9.8	11.3
3	Control	12.0	10.0	12.4
	Inactive steroid*	10.4	6.4	8.4
	Cortisone	30.2	26.4	32.8

* Δ^1-17 α-hydroxy progesterone.

proline may only indirectly reflect the inhibition of the synthesis of collagen or collagen-precursors.

Summary. A chromatographic examination was made of the free amino acids of tissues of normal and cortisone-injected chick embryos. The content of free hydroxyproline was increased markedly in the tissues of the cortisone-treated embryos. The results were confirmed by a colorimetric procedure. With the exception of glycine, in which increases were noted in some instances, the other amino acids showed no consistent changes as a result of the injection of cortisone. The injection of Δ^1-17 α-hydroxy progesterone produced slight decreases in free hydroxyproline content.

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Absorption and Excretion of Viomycin in Humans.* (18467)

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(Introduced by David P. Barr)

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Viomycin, a derivative of a species of *Streptomyces,* is a compound which has been found to have antimicrobial activity for a number of species of bacteria *in vitro* and to protect mice against experimental infections (1,2). It has been of particular interest because of an inhibitory effect upon mycobacteria, including *M. tuberculosis,* and because of its ability to suppress tuberculosis in animals(3). Preliminary clinical studies with viomycin in tuberculous patients have been carried out(4). The present study concerns the absorption and excretion of viomycin in human subjects. In addition, serum concentrations in patients receiving maintenance therapy and concentrations in the cerebrospinal fluid have been determined.

Materials and methods. Preparation of drug. Viomycin‡ was supplied as the highly purified sulfate salt in 1.0 g vials and was dissolved in sterile distilled water in a concentration of 250 to 500 mg per ml for intramuscular injection.

Collection of specimens. In the studies of absorption and excretion after a single intramuscular dose, specimens of blood were drawn under sterile precautions at prescribed inter-

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‡ Supplied by Charles Pfizer and Co., Brooklyn, N. Y.

vals, and the total 24-hour output of urine following the administration of drug was collected in fractional portions. The blood was allowed to clot in the refrigerator at 4°C, and the serum was removed following centrifugation. Aliquots of the urine fractions were Seitz-filtered immediately after collection had been completed. Specimens of blood for serum assay were withdrawn at random from patients receiving daily maintenance doses of viomycin parenterally, and simultaneous withdrawals of blood and cerebrospinal fluid for assay were made in certain subjects. All specimens of serum, urine, and cerebrospinal fluid were kept frozen until time of assay.

Assay technic. Microbiologic assay of the sera was carried out by means of a tube-dilution method previously described(5). With this technic a preliminary 1:4 dilution was made of the unknown serum in broth. Following this dilution, subsequent dilutions were made in 25% pooled human serum broth. When the broth inoculum of culture was added, the final concentration of serum in each tube became 20%. The final dilutions of the unknown were 1:5, 1:6.6, 1:8, 1:10, etc. This method obviated the effect which varying concentrations of serum proteins may exert in the assay procedure. In assaying the urine and cerebrospinal fluid specimens a conventional technic of 2-fold serial dilutions in broth was utilized. In addition, because of expected low concentrations of drug in the specimens of cerebrospinal fluid, further dilutions of 8:10, 7:10, and 6:10 were made of these specimens in broth. The test-organism employed was a strain of *Klebsiella pneumoniae,* type A-D, and the final concentration was a $1:10^6$ dilution of 18 hour growth at 37°C in infusion broth. All assays were read after 18 to 24 hours incubation at 37°C. A standard solution of viomycin in serum or in broth was diluted, inoculated, and incubated concurrently with each series of unknown specimens. The end-point was taken to be the first tube of each series in which macroscopically visible growth could not be detected. All concen-

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TABLE I. Serum Concentrations and Data for Urinary Excretion of Viomycin in 5 Subjects Following a Single Intramuscular Dose.

Subject	Dose, mg/kg	Serum conc., μg/ml						Urine conc., μg/ml						% dose recovered					
		½	1	2	4	8	24 hr	0-1	1-2	2-4	4-8	8-23	23-24 hr	1	2	4	8	24 hr
1	52	100	125	125	100	25	<12	100	12500	625	800	100	25	.3	28	49	69	72
2	50	125	100	100	62	16	<12	1240	1240	2480	800	200	100	11	28	55	68	77
3	47	62	62	82	33	12	<12	2480	1240	1240	800	200	200	10	32	56	77	91
4	35	60	120	75	30	<15	<15		3170*	5000	800	200	50		18	42	58	65
5	25	100	100	75	20	<15	<15	3170	5000	3170	800	100	<6	25	47	73	95	105

* 0-2 hr specimen.

trations were expressed in terms of pure viomycin base having a potency of 1000 μg per mg.

Results. Absorption and excretion. A single intramuscular dose of viomycin was administered to each of 5 adult males. Three of the subjects received 3 g, and the other 2 received 2 g and 1 g respectively. On a weight basis these doses represented from 25 to 50 mg per kilo. The data concerning the serum and urine concentrations which were achieved and the amounts of viomycin which were recovered in the urine following the single injections of drug have been presented in Table I. The curves of the serum concentrations of 3 subjects which had received doses of 50 mg per kilo have been shown in Fig. 1 together with the mean values for the cumulative urinary excretion of the drug in all 5 subjects. From Table I and Fig. 1 it may be seen that the highest serum concentrations were attained within one-half to 2 hours after injection and ranged from 82 to 125 μg per ml. Thereafter the values diminished, and no drug could be detected in the sera which were obtained 24 hours after injection. The smallest concentration of viomycin which could be measured in the serum by this method was 7.5 μg per ml. Urinary excretion of the drug commenced within an hour after injection, and concentrations of viomycin up to 12 mg per ml were achieved in the urine. Four of the 5 subjects had measurable concentrations of viomycin in the urine specimen which was collected between 23 and 24 hours after the initial dose. Concentrations of viomycin in the urine of less than 6 μg per ml could not be detected with the assay method. The period of greatest urinary excretion of the drug was between 2 and 4 hours after injection. From 65 to 100% of the administered dose of viomycin was recovered in the urine within 24 hours after the single dose had been given (Table I and Fig. 1).

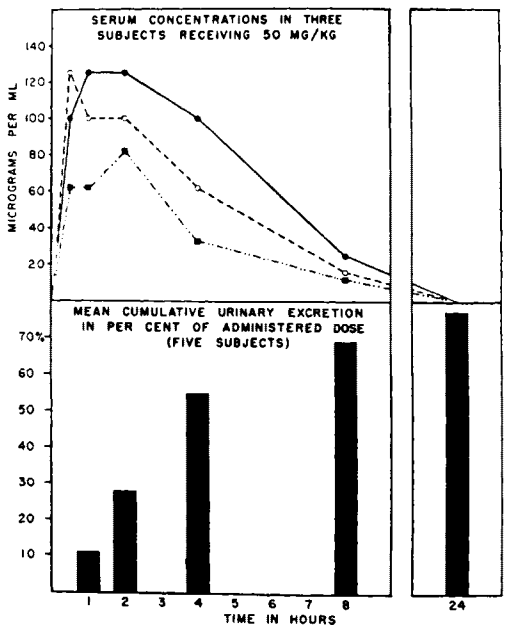

FIG. 1.
Absorption and excretion of viomycin following a single intramuscular dose.

Serum concentrations during maintenance therapy. Determinations of viomycin were made in 22 sera obtained from 6 patients with pulmonary tuberculosis who were receiving daily maintenance doses of 1 g 2 or 3 times daily intramuscularly. These doses represented total amounts of the drug of 50 to 72 mg per kg per day, and the sera were obtained after 14 to 53 days of continuous therapy. In 8 of 14 sera which were obtained 12 hours after the last preceding dose of viomycin no drug was measured, while in the remaining 6 specimens concentrations of 7.5 to 30 μg per ml were detected. In each of 8 specimens obtained 4 hours after the last preceding dose, concentrations of 30 to 100 μg per ml were measured.

Concentrations in cerebrospinal fluid. In single specimens of cerebrospinal fluid obtained from 2 subjects 2 hours after a single intramuscular dose of 3 g, concentrations of viomycin of 10 and less than 4 μg per ml were obtained, while the corresponding serum concentrations were 100 and 125 μg per ml respectively. In a third specimen of cerebrospinal fluid obtained after 60 days from a patient who had received 1 g of viomycin intramuscularly 3 times daily continuously during this time, the concentration of viomycin was 12.5 μg per ml, while the concentration in the serum specimen which had been obtained simultaneously was 100 μg per ml.

None of these subjects had evidence of meningeal involvement.

Comment. The results of this study, which has demonstrated that viomycin was rapidly absorbed and that high concentrations were achieved in the serum after intramuscular injection, may bear only a very loose relationship to the successful utilization of the drug in antimicrobial therapy. It has been encouraging to note, however, that the serum concentrations of viomycin which were attained in the present study were well in excess of the minimal inhibitory concentrations of viomycin required *in vitro* to suppress strains of *M. tuberculosis* isolated from patients with active tuberculosis(4). The pattern of absorption and urinary excretion of viomycin was similar to that of streptomycin, and the serum concentrations which were attained following the intramuscular administration of viomycin have compared favorably with the concentrations of streptomycin which have been achieved following comparable doses of the latter drug(6).

Summary. A study has been made of the absorption and excretion of viomycin following intramuscular administration in humans. Maximum serum concentrations of 82 to 125 μg per ml were attained within 2 hours after a single dose of 25 to 50 mg per kilo. Reasonably high concentrations were achieved in the serum of patients receiving daily maintenance doses of the drug. Small concentrations of viomycin, 10 to 12.5 μg per ml, were detected in the cerebrospinal fluid in the presence of high serum concentrations of the drug, 100 to 125 μg per ml. Viomycin was excreted in the urine in high concentrations, and from 65 to 100% of the administered dose was recovered in the urine within 24 hours.

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Abnormalities of the Eye Occurring in Young Vitamin E-Deficient Rats. (18468)

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In the course of some studies with vit. E-deficient rats, unusual abnormalities of the eye were observed in the young of mothers deficient in this vitamin. Such lesions had not been encountered before in this laboratory, and have not been mentioned by other investigators to our knowledge.

Although the data are limited, it seems of importance to report them, in view of the recently published studies of Owens and Owens(1) indicating that vitamin E may play an important role in the prophylaxis of retrolental fibroplasia, a disorder which appears frequently during the early months of life in small, prematurely born infants. This condition has been recognized for many years, and has been ascribed to a variety of etiological factors; however, Owens and Owens (2) are the first investigators to follow the progress of the disease from the initial stages of dilatation of the retinal arteries and formation of fibrous bands extending into the vitreous to the final fusing of these bands with folds of the swollen retina to form the characteristic membrane behind the lens. The membrane may be complete, causing total blindness or it may be partial, allowing some vision to be retained. Symptoms accompanying this condition noted by these and other workers(3) were eyeballs smaller than normal and hemorrhages behind the lens. Once the

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3. Krause, A. C., *Arch. Ophth.*, 1946, v36, 387.