Fate of Intravenously Administered Glycerol.* (23354)

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Although glycerol is one of the most widely used pharmaceutical products very little work has been done on the fate or metabolism of intravenously administered glycerol solutions. Perhaps this is due to the fact that early investigators found aqueous glycerol quite hemolytic when given by vein(1). However, this was shown to result from the fact that glycerol penetrates the red cells very rapidly and thus does not exert much osmotic action across the red cell membrane. Deichmann (1) and Miner and Dalton(2) have reviewed the toxicity of glycerol solutions and concluded that glycerol given intravenously is not very toxic when administered in isotonic saline.

In our own studies on intravenous fat emulsions we became interested in the use of glycerol as a dispersing medium for oil and lecithin. We were able to prepare an anhydrous fat emulsion in this manner which could be diluted with 5% glucose to furnish an acceptable high caloric fluid for intravenous use. In the present study we have investigated the disappearance from blood and the urinary excretion of glycerol administered in aqueous solution by vein.

Methods. Mongrel dogs maintained on Purina chow were used for this study. Glycerol solutions were prepared for injection by diluting USP glycerol with 5% aqueous dextrose or 0.9% aqueous NaCl. Plasma glycerol was determined by the method of Lambert and Neish(3) on an aqueous dilution of the supernatant after the addition of 9 ml 10% trichloroacetic acid to 1 ml plasma. Urine glycerol was determined directly. Both urine and blood values were corrected for formaldehydogenic materials present in preinjection samples.

Results. In previous experiments with dogs infused with intravenous fat emulsions containing 10 to 20% glycerol it was noted that the secretion of urine increased markedly during the 24 hr period following injection. This agrees with previous observations that glycerol might act as an osmotic diuretic(1,2). In the first experiments of this series we measured the disappearance of intravenously injected glycerol from the bloodstream and its appearance in urine. Dog A (Table I) received 12 g glycerol dissolved in about 50 ml isotonic saline. During the first 30 min. after injection the plasma glycerol level stayed above 2 mg/ml and the urine contained 2.5 g glycerol. After 1 hr, however, when the plasma levels reached 1 mg/ml or below, the amount of urinary glycerol fell to negligible quantities. Thus in the next 2 dogs when the administered dose was reduced to 6 g, plasma levels as early as 5 min. after injec-

TABLE I. Plasma Glycerol Concentrations and Total Urinary Glycerol at Various Intervals after a Single Intravenous Dose of Glycerol.

		Plasma glycerol		Urine glycerol	
Dog	Dose, g	Time after inj., min.	mg/ml	Time interval, min.	g
A 10.5 kg	12	30	2.08	0- 60	2.500
		60	1.19	60-120	1.330
		120	1.25	120-180	.351
		180	.35	180-270	.086
		240	.23	270-330	.032
		300	.15		
		360	.13	0-330	4.300
B 10 kg	6	5	.600	0- 60	.250
		10	.552	60-120	.158
		15	.413	120-240	.048
		33	.149	240-360	.024
		60	.095		
		120	.111		
		220	.027		
		360	.035	0-360	.480
C 11.4 kg	6	5	1.33	0-120	.476
		11	1.02	120-270	.120
		17	.82	270-420	.002
		30	.54		
		65	.21		
		135	.15		
		225	.13		
		310	.19		
		375	.19	0-420	.598

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TABLE II. Fate of Glycerol in Chronically Infused Dogs.

Dog	Wt, kg	Glycerol infused, g/day	Urine vol, ml/day	Urine glycerol, g/day	Increase in serum glycerol during infusion, mg/cc	Remarks
GP	9	75	1207 ± 55*	32.7 ± 1.9	7.3 ± .5	Infused for 21 days. Convulsed on 15th day.
GQ	8.5	58	1039 ± 51	27.4 ± 4.2	7.7 ± .6	Infused 16 days. No convulsions.
GL	10.9	75	1305 ± 53	42.2 ± 4.3	8.1 ± .7	Infused for 3 days, convulsed. After 1 mo rest, infused for 4 days and convulsed again.

* Stand. error.

tion were down to 0.6 and 1.3 mg/ml and there appeared to be very little excretion of glycerol in the urine.

The next set of experiments was concerned with the fate of glycerol administered in large quantities by daily intravenous infusions. Three dogs received 58-75 g glycerol daily as a 20% solution in isotonic glucose by intravenous drip over a period of 1 hour. The results on these animals are summarized in Table II. Blood glycerol concentrations were determined immediately before, and again at the end of the infusion period. The initial values may have measured the presence of formaldehydogenic materials other than glycerol and in any case were less than 0.2 mg/ml. Twenty-four hour specimens of urine collected during preinfusion periods were less than 200 ml. Infusion of glycerol increased this volume 5-6 fold and it is thus obvious that the administered glycerol solution which amounted to approximately 300 ml exerted a powerful diuretic effect. Immediately after each infusion the dogs were very thirsty and drank a large quantity of water. The amount of glycerol contained in the 24 hr urine specimens was determined daily and appeared to be rather constant throughout the experiment. The 3 animals excreted 44, 47 and 56% of the administered glycerol in the urine and thus utilized for metabolic purposes no more than half of the administered dose.

Large doses of glycerol were administered in this study in order to throw some light on one of the toxic manifestations previously observed, *viz.* tremors and convulsions. These signs appeared in most dogs receiving more than 5 g glycerol per kg body weight, but only after repeated injections of the glycerol. In several animals it appeared that the occurrence of convulsions sensitized the animal to subsequent infusions. For example, one dog convulsed after 9 daily glycerol infusions of 6.8 g/kg(4). When given a rest period of 3 days the animal convulsed on the first injection. After another 2 day rest convulsions occurred again after a single injection. During the rest periods the animal appeared quite normal.

At the time it was postulated that during a prolonged course of injections an animal might store glycerol by an inability to excrete or metabolize glycerol and thus gradually build up a convulsive level. This, however, was not found to be the case in the present study. Urinary excretions did not show any trend to diminish over the injection period. Nor did the daily preinjection blood levels show any tendency to increase throughout the experiment. To make an additional test on this point, 2 animals were given on 4 separate occasions during the course of injections a single dose of 6.3 g glycerol and the disappearance from blood was determined. The results are shown in Fig. 1. Dog GP was tested prior to infusion and again after 7, 13, and 20 infusions. All disappearance curves fell within a narrow range. Indeed dog GP was tested in this manner on the day prior to the severe convulsions and again at the end of the infusion series without any noticeable changes in disappearance rates. Dog GQ showed only one disappearance curve suggesting some retention but this was not apparent

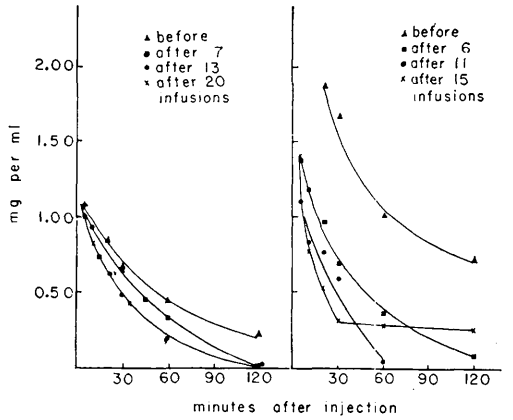

FIG. 1. Glycerol disappearance curves. Left, dog GP; right, dog GQ.

on later tests and this animal never convulsed. The third animal in Table II was chosen for its earlier demonstrated sensitivity to glycerol. About a year before these tests this animal had received 6.5 g glycerol/kg/day and convulsed on the 19th day; after a rest period of one week in a second series of infusions of 5.8 g/kg/day the dog convulsed after the 10th infusion. Nine months later convulsions occurred once on the third day after infusing 6.9 g/kg/day and again after a one month rest on the 4th day of infusing the same amount. This animal did not demonstrate any retention of glycerol, as evidenced by adequate urinary excretion, and preinjection blood levels on the days of convulsion of 0.06 and 0.08 mg/ml which were even lower than the average preinjection levels in other dogs. Thus there appears to be no evidence that during a course of glycerol infusions the animal develops an inability to dispose of this material from the bloodstream.

Summary. Intravenously administered glycerol rapidly disappears from the bloodstream. Single injections of 6 g glycerol did not produce excessive urinary excretion of glycerol but 12 g led to excretion of one-third the dose. Daily infusions of large doses of glycerol produced marked polyuria and in some animals led to tremors and convulsions. These disturbances were not caused by accumulation of glycerol in the blood.

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Production in Mice of Large Volumes of Ascites Fluid Containing Antibodies. (23355)

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Studies of mouse antibody have been limited by the difficulty of producing serum of high titer as well as by the limited amount of blood that can be obtained from each mouse. Recently Lipton, Stone and Freund(1) have shown that a high titer of antibody can be obtained in the serum of rats immunized with antigens mixed in Freund's adjuvant, and Stone(2) has obtained similar results in mice. However, the problem of collecting large amounts of serum in mice still remains. Recently it has been observed that mice injected intraperitoneally with antigens mixed with Freund's adjuvant develop large amounts of peritoneal fluid that has been found to contain specific antibody in high concentration. Due to the practical importance of this observation for investigation of mouse antibody as well as the study of anaphylactic reactions

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