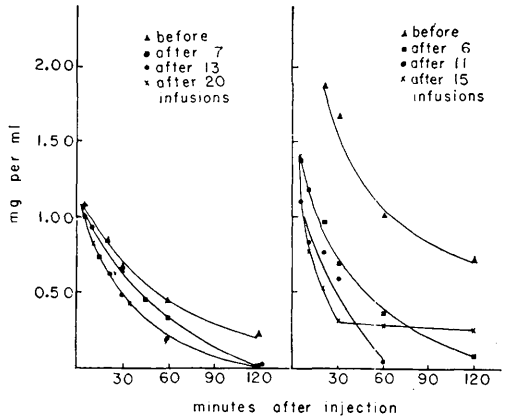

FIG. 1. Glycerol disappearance curves. Left, dog GP; right, dog GQ.

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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TABLE I. Volume of Ascites Fluid Produced and Qualitative Determination of Antibodies.

Days after last antigen inj.		Mouse No. 1	2	3	4	5	6	7	8
14	Vol	10	14	5	2	2	0.5		
	Ring test	4+	4+	2+	2+	+	4+		
25	Vol	13	13	10	4	2	3	5	8
	Ring test	4+	4+	2+	—	+	4+	4+	4+

in mice using homologous antibody, a detailed report of these preliminary observations is given here.

Materials and methods. Swiss Webster female mice weighing from 16-18 g from our own laboratory were used. Each mouse received 2 injections given 2 weeks apart of 1 mg of either bovine serum albumin† or 5 times recrystallized egg albumin mixed in Freund's adjuvant(3). The adjuvant was made as follows: Two parts of paraffin oil ("Vaccinol," obtained from the Pennsylvania Refining Co., Butler, Pa.) containing 2 mg (dry weight) of killed *Mycobacterium phlei* cells per ml were mixed with 1 part of Arlacel A (Atlas Powder Co., Wilmington, Del.), and autoclaved at 15 lb for 20 min. After cooling, 3 parts of sterile adjuvant were added to 2 parts of a saline solution of antigen containing 10 mg/ml. The mixture was emulsified by shaking. Each mouse received two 0.25 ml injections given 2 weeks apart of antigen-adjuvant mixture and was then allowed to develop ascites for at least 3 weeks.

The peritoneal cavity was tapped with an 18 gauge needle and the fluid was allowed to flow into a test tube. On many occasions the needle was removed and peritoneal fluid continued to flow out of the needle puncture site. The fluid was tested for presence of antibodies by the ring test method using a saline solution of the corresponding antigen containing 0.1 mg/ml.

Results. Typical results obtained after 2 injections of 1 mg of 5 times recrystallized egg albumin in adjuvant are given in Table I, where volumes of peritoneal fluid as well as results of ring precipitin test for each of 8 different mice tapped on 2 different occasions are given. The volume of fluid collected on the first tapping, 2 weeks after the last injection of the antigen-adjuvant mixture, ranged from 0.5 ml to 14 ml/mouse with 2 mice having no significant amount of fluid at this time. The second tapping 25 days after the second immunizing injection yielded volumes ranging from 2 ml to 13 ml/mouse, and all mice had some fluid. A third tapping, not included in Table I because the identity of individual mice was lost, was performed 40 days after the second immunizing dose was done on 5 mice and the fluid obtained ranged from 5 ml to 18 ml/mouse. As can be seen from Table I, only one fluid failed to show presence of antibodies to egg albumin. The total amount of fluid collected from these 8 mice in a period of 9 weeks (3 tappings for most mice) was 143.5 ml for an average of 17.9 ml of fluid per mouse.

Since all the mice do not develop ascites the following experiment was performed to establish the frequency with which mice receiving 2 injections of Freund's adjuvant-antigen mixture develop this condition. Fifty mice received 1 mg of crystalline bovine serum albumin in adjuvant per injection. Two weeks after the second injection mice were tapped and fluid volumes recorded. Of 50 mice, 38 or 76% gave from 0.5 ml to 13 ml of fluid each. In another group of 50 mice receiving egg albumin instead of bovine serum albumin the number of mice giving significant amounts of fluid was somewhat smaller. This latter group was tapped on 3 different occasions, and the results obtained are summarized in Table II. On the first tapping 25 mice gave from 1 ml to 15 ml each for a total of 180 ml and an average of 7.2 ml/mouse. On the second tapping, again 25 mice gave from 1 ml to 14 ml each for a total of 156 ml of fluid and an average of 6.2 ml/mouse. During last tapping 16 mice gave from 3 ml to 23 ml each, for a total of 156 ml and an average of 9.7 ml/mouse. From these mice a to-

† Purchased from Armour & Co., Chicago, Ill.

TABLE II. Number of Mice Developing Marked Ascites of 50 Injected.

Day after last inj.	No. mice tapped	Total vol of fluid collected (ml)	Vol range (ml)	Avg vol of fluid/mouse tapped
14	25	180	1-15	7.2
25	25	156	1-14	6.2
40	16	156	3-23	9.7

Total vol collected 492.

tal of 492 ml of peritoneal fluid has been collected. The mice still appeared healthy after the third tapping. Most of the fluids gave intense precipitation with egg albumin. Relatively few mice failed to respond with production of antibodies in the peritoneal fluid. A pool of the fluids from the first tapping was found to contain 213 μ of antibody nitrogen/ml, as determined by the quantitative precipitin technic(4).

Discussion. The findings here reported may prove to be of great value for investigations of mouse antibody as well as anaphylaxis using homologous antibody. These studies have previously been complicated by the limited amounts of serum that one can obtain from a mouse. Use of peritoneal fluid as a source of antibody would overcome this obstacle.

Fig. 1 shows a mouse that developed marked distension of the abdomen compared to one of its mates of the same age that failed to give any appreciable amount of ascites. Before tapping this mouse weighed 52 g of which 28 g was due to ascites fluid. This tremendous accumulation of fluid does not occur in all mice, but usually 2 or 3 of 10 mice do give 10-15 ml each. Mice do not seem to be adversely affected by removal of such large amounts of liquid and this material, after clotting and clearing by centrifugation, is not toxic to other mice. Since there are relatively few cells in this ascites fluid as compared to blood, little volume is lost after breaking the clot that forms and centrifuging at 4,000 rpm for ½ hour.

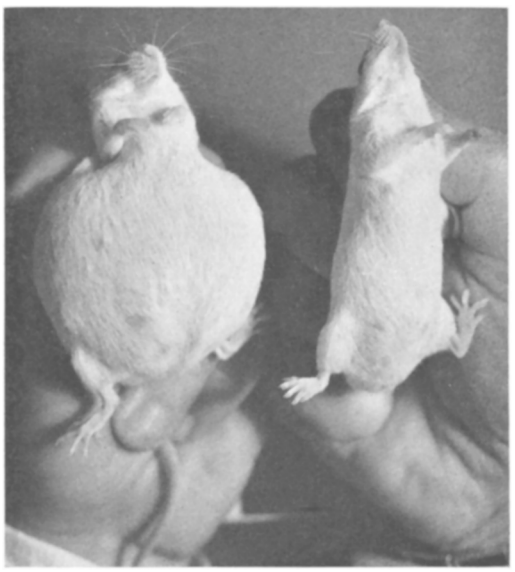

FIG. 1. These 2 mice, of same age, received 2 inj. of adjuvant-antigen mixture. The one on the left developed marked ascites (28 ml of fluid) while the one on the right did not accumulate any fluid.

As little as 0.05 ml of peritoneal fluid containing 10 μ of antibody nitrogen has been sufficient to produce passive anaphylactic deaths in pertussis-treated mice. This points out the importance of using homologous antibody in such studies, since a much greater amount of rabbit antibody is required(5). These studies will be reported later.

Summary. Mice injected intraperitoneally with either egg albumin or bovine serum albumin mixed in Freund's adjuvant develop large amounts of peritoneal fluid containing antibodies to the antigen injected. As much as 28 ml of fluid has been collected from a single mouse on a single tapping. Most mice produced from 0.5-14 ml of fluid each. About 50% of the mice respond with accumulation of fluid in the peritoneum.

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