plemented with fetal calf or rabbit serum and 1% L-glutamine, 200 mM. The addition of phytohemagglutinin transformed the lymphocytes into cytologically immature blastoid cells capable of RNA and DNA synthesis and mitotic division. The response began within the first 24 hours of culture and by 72 hours about 75% of the surviving cells were transformed into blastoid cells. Autoradiography with H^3-thymidine disclosed that phytohemagglutinin induced blastogenesis occurs in a population of small lymphocytes which are transformed into large cells prior to the advent of mitotic division.

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Gamma M Synthesis During the Secondary Antibody Response In Mice.* (31133)

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Several studies using rabbits(1-5) and rats (6), have demonstrated the transient appearance of only small amounts of gamma M antibody following secondary antigenic stimulation. The characteristic features of the typical secondary response (*i.e.,* a higher titer of antibody in a shorter period) were not fulfilled with respect to gamma M synthesis, in that titers of macroglobulin appeared at levels only equal to or lower than those demonstrated initially. In one instance in rabbits (3), a single small dose of polio virus stimulated only gamma M synthesis. Reinjection of such rabbits at a critical time not exceeding 3 days after cessation of gamma M synthesis, resulted in a typical secondary response with the prompt appearance of gamma M globulin in higher titers than had been seen initially. This communication reports that gamma M synthesis in the inbred Balb mouse following 2 injections of a purified protein antigen has the characteristics of a typical secondary response.

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Materials and methods. 4-8-week-old male, inbred mice, Balb strain, were injected intraperitoneally with 1.0 mg antigen, human gamma globulin (HGG), lot #7286, Calif. Biochem. Corp., further purified by DEAE cellulose chromatography. The secondary response was induced in the same manner 36 days after the initial exposure. Using high titer rabbit antihuman plasma, only a single band of precipitate was demonstrated on reaction with the purified antigen at a concentration of 5 mg/ml in the Ouchterlony gel diffusion test and by immunoelectrophoresis.

Mice were anesthetized with ether and bled by severing the axillary vessels. Equal volumes of blood were pooled from 3 or 4 mice and the serum separated and kept frozen until antibody titers were determined by the passive tanned cell hemagglutination technique. The red cells were sensitized with 1:20,000 w/v tannic acid in 0.15 M NaCl. After washing and centrifugation the suspension of 2.5% v/v tanned cells was exposed to 250 μg/ml of antigen per ml red cell suspension for 15 minutes at room temperature. After centrifugation, the cells were washed and a

3% suspension made in a 1:250 dilution of normal rabbit serum (NRS) in phosphate buffered saline (pH 7.2). Antibody titrations were carried out in a 1:50 dilution of NRS with serial doubling dilutions of the test serum beginning at 1:10.

Differentiation of the type of immunoglobulin produced during immunization was determined by cleavage with 2-mercaptoethanol (2-ME) and gel filtration. Gamma M globulin has been shown to be sensitive to sulfhydryl compounds which effectively inactivate this globulin(7). A 1:10 dilution of the test serum was mixed in a volume of 1.0 ml with the 2-ME such that the final concentration of the latter was 0.1 M. Serum was also subjected to fractionation in 1.0 ml quantities on Sephadex G-200 employing a column 4.5 × 100 cm in size utilizing 0.1 M Tris buffer in 1 M NaCl (pH 8.0) as the eluting buffer(8). The protein concentration obtained in the fractions was determined by spectrophotometric absorption at 280 mμ. The fractions comprising each peak were combined and subjected to concentration against polyethylene glycol† to a volume of approximately 1.0 ml. One-tenth ml portions of these concentrated specimens were then tested by the passive hemagglutination assay to verify the presence of antibody activity. Several of these concentrated specimens were subjected to mercaptoethanol hydrolysis to verify the sensitivity of the antibody in the first peak and the lack of it in the second peak.

Injection of mice with normal saline, as well as hemagglutination-inhibition tests, were used for control purposes and verified the specificity of the response measured. A standard reference serum was included with each test to facilitate comparison of tests done on different days.

Results. The antigenicity of a single injection of HGG together with the type of antibody evolved is documented in Table I. The response was initiated by gamma M antibody on day 7 with gamma G globulin appearing between days 9 and 12 and continuing at least to day 18.

In Table II are recorded the antibody titers appearing after reinjection of HGG 36 days later, at a time when the hemagglutination titer of antibody had returned to <10. A typical secondary response was manifested with a titer on day 3 of 1:320, which had risen to 15,360 2 days later. Differentiation of the type of antibody responsible for these titers revealed that gamma M globulin was the first immunoglobulin to appear. In contrast to previous studies(1-6) it was evident earlier and in higher amounts than were measurable during the primary response, although gamma G globulin was the predominant globulin by day 5-7.

Differentiation by Sephadex G-200 filtration of immunoglobulins in the sera taken on days 3, 5, 7, 9, 14 after the second injection of antigen are shown in Fig. 1. The Figure relates the curves obtained by gel filtration to the hemagglutination tests performed on whole serum and the combined concentrated fractions obtained from the column. ME hydrolysis performed on several of the concentrated specimens revealed sensitivity of the antibody obtained in the first peak and lack

TABLE I. Primary Antibody Response in Balb Mice Given 1.0 mg Human Gamma Globulin.

Days post injection	Antibody titer*	Antibody titer after 2-mercaptoethanol treatment*
7	80	0
9	160	0
12	320	80
18	320	80
28	0	0

* Expressed as reciprocal of hemagglutination titer.

TABLE II. Secondary Antibody Response in Balb Mice Reinjected with 1.0 mg Human Gamma Globulin.

Days post reinjection	Mean HA titer*	Mean HA titer after 2-mercaptoethanol treatment*
3	320	0
5	15,360	10,405
7	13,650	10,347
9	15,360	7,680
14	3,840	5,760
21	800	1,440

* Reciprocal of titer determined by passive hemagglutination.

† Carbowax, obtained from Union Carbide Chemicals Co., New York City.

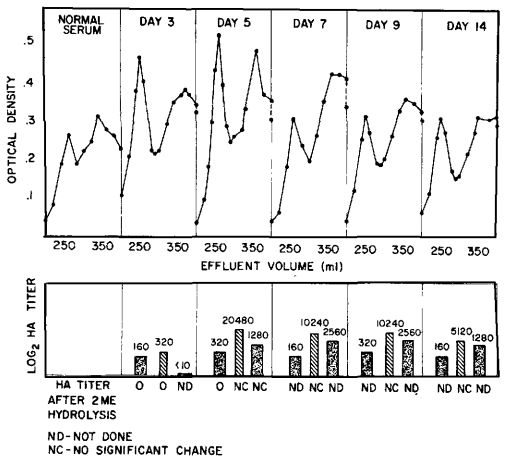

FIG. 1. The secondary antibody response. Comparison of the change in serum protein levels demonstrated by gel filtration with antibody activity demonstrated by hemagglutination on the combined fractions of the respective peak (stippled bars) and the whole serum (striped bars). Results of ME hydrolysis on the fractions are also shown.

of sensitivity with the second peak antibody on days 3 and 5. It is evident that early in the secondary response there was increased protein concentration in the heavier components of the serum proteins along with increased antibody activity. Based on the ME hydrolysis results and the results of others(8, 9) it is unlikely that the antibody titers demonstrated by hemagglutination were due to spill over from the second peak. Though mercaptoethanol sensitivity may not be equated with gamma M antibody there is support for using this method evident in the results obtained with the reliable and accepted method of gel filtration(8,9). The protein with antibody activity recovered from the first peak has been shown to be gamma M in type by ultracentrifugal studies while that recovered from the second peak is primarily gamma G. It is apparent that gamma M globulin with antibody activity appeared earlier and to a higher titer than that seen in the primary response. The macroglobulin represented a significant portion of the total early antibody synthesized. It is of interest to note that although there was a rise in the absorbancy on day 3, the hemagglutination test indicated no antibody present. Indeed no antibody may be present, or as Dreesman *et al*(10) have suggested, the hemagglutination test may not have detected early gamma G synthesis.

Discussion. It has been demonstrated in mice that on reinjection of the soluble protein antigen, human gamma globulin, gamma M globulin, as well as gamma G globulin was synthesized in higher titers and appeared in a shorter time than after the first injection. Thus, the secondary response in mice to this protein antigen was the result of augmented synthesis of both immunoglobulins. Memory, or capacity for accelerated gamma M synthesis in our experiments in mice, appeared to be more persistent than its transient appearance in rabbits against polio virus(3). However, it has been reported just recently that gamma M synthesis during the secondary response to protein antigens in rabbits does occur over an extended period(11).

The reason for the differences in our results and those discussed previously(1-6) may lie in basic differences between antigen-antibody systems, or in the level of antibody present at time of reinjection. It is known that gamma G antibody acts to suppress gamma M synthesis(12); consequently our mice were reinjected only after their antibody levels, as determined by the sensitive passive hemagglutination assay, had subsided to <10.

Summary. The secondary response in mice was characterized by the appearance first of gamma M globulin followed in 3 to 7 days by the appearance of gamma G globulin which then remained the principal circulating antibody. The titers of both gamma M and gamma G antibody appeared more rapidly and rose to higher levels after the second injection than after the initial antigenic exposure.

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Diuresis in Newborn Rat Given Intravenous Water or Salt Solution.* (31134)

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Diuresis in response to intragastric administration of water or salt fails to occur in newborn rats(1), dogs, or humans(2). Absence of diuresis has been attributed to the state of certain renal structures and to non-functioning of the antidiuretic hormone system or its renal effects. The present work aimed to explore factors of manipulation that might influence diuresis. Route of administration proved to be one factor, for intravenous fluid aroused diuresis.

Procedure. Rats were mostly used at 3-26 hours after birth. Each was taped to a board and kept in an incubator after preparation. Fluids were injected into the femoral vein (I.V.) through a cannula finely drawn from No. 10 polyethylene tubing and inserted during temporary ether anesthesia. A urethral catheter (No. 10 tubing) was inserted into the urinary bladder, outside a thin glass probe which was then withdrawn. The catheter led the urine into a calibrated glass capillary to measure its flow. Fluid was injected 0.5 to 1 hour after preparation was completed. Chloride analyses of 4 μl volumes of urine or plasma employed a microburette and the method of Natelson(3). In other tests, fluids were injected into the peritoneal cavity (I.P.). Radioactive Na^{22} was sometimes added to these fluids so that movement of injected Na could be traced.

Results. After I.P. injection of water, only a small diuresis was discerned; urine flow in the second half hour was significantly greater ($P = .02$) than the mean of all control periods. This urine flow did not differ from that following loading by stomach tube (S.T.) (reported in(1)). Diuresis was almost as small when 1 M NaCl instead of distilled water had been given (Table I). Rats 3 days of age similarly treated produced little more urine in 1.5 hours after water had been given ($1.3 \pm .14$% b.w.), (b.w. = body weight), and no more urine after 1 M NaCl had been given. Peritoneal fluid equalled the plasma in chloride concentration at 0.6 hour. During that period of time the intraperitoneal volume, as collected in sacrificed animals, had not diminished. Na^{22} recovery then showed 1% of the dose in urine, and only 6% in peritoneal fluid. Cl in peritoneal fluid equalled 9% of the amount injected. Plasma chloride concentration remained greatly elevated for at least 18 hours.

After I.V. injection, completed in 6 minutes, diuresis began about 4 minutes later (Fig. 1). The small delay was the same whether the injecta were hypotonic or hypertonic to the blood plasma.

Administration I.V. of 1 M NaCl resulted in (a) the full return of the volume of the injecta (4% of b.w.) in the urine of the newborn within 1.5 hours, and (b) even greater urinary volume at 2-5 days of age, 5.2% b.w. (Fig. 1, B). Urine production after I.V. loading was 4-fold greater when 1 M NaCl was given than when water was given.

Maximal concentrations of chloride in urine

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