The Nature of Antibodies in Mice Injected with Influenza Virus Vaccine.* (28560)

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Several investigations of the heterogeneous nature of antibodies in various species have been reported(1-4). However, the types of antibodies in mice are usually not considered because of the meager amount of information. This report concerns the nature of antibodies synthesized by mice at various stages of the initial response to influenza viral antigen. The findings demonstrate that mice produce antibodies which resemble those found in other species and produce them in about the same sequence following administration of an antigen.

Materials and methods. Mice. Adult albino mice, CF-1 males weighing about 25 g each, were employed in these studies. Each mouse was injected intraperitoneally with a single 0.1 cc dose of aqueous influenza virus vaccine and was bled from the axilla after a suitable interval.

Vaccine. The vaccine consisted of a 1:10 dilution of a stock suspension of type A influenza virus, PR8 strain, which had a chicken red cell hemagglutination titer of 1:10240 by the standard pattern method(5). The viral antigen was made non-infectious by treatment with 1:4000 formalin.

Sera. Individual mouse sera were stored in rubber-stoppered tubes in a mechanical freezer until combined into sera pools. Hemagglutination-inhibiting (HI) titers of sera were measured by the standard pattern method(5) except that the sera were not heated at 56°C for 30 minutes before dilution unless otherwise specified. Sera that had been treated with 2-mercaptoethanol or subjected to centrifugation over a sucrose gradient were employed in the HI test without additional treatment or dialysis.

Treatment with 2-mercaptoethanol. The method employed for treating serum with 2-mercaptoethanol consisted of mixing equal volumes of unheated undiluted serum and a 0.2 molar solution of the chemical and storing the mixture in a rubber-stoppered glass tube at room temperature for 48 hours. Control sera were diluted with a corresponding volume of 0.1 molar phosphate buffered saline pH 7.2 and stored in the same manner. Treated and control sera were tested for HI activity in the usual manner without additional treatment.

Centrifugation. The ultracentrifugal behavior of HI antibodies was ascertained by the sucrose gradient method employing gradations from 10% to 40% aqueous sucrose solutions. Mercaptoethanol-treated serum or saline-treated serum controls in 0.9 cc volumes were layered over 4.4 cc of the gradient and were centrifuged at 35,000 rpm for 18 hours employing a SW-39 rotor in a Spinco model L centrifuge. At the end of the centrifugal run, a small perforation was made in the bottom of the plastic centrifuge tube and serial fractions of the gradient were obtained by collecting successive drops. Finally, the tube was also rinsed with buffered saline to recover rapidly sedimenting components that might have adhered to the bottom of the tube. The rinse fraction was probably also contaminated by materials present at other levels. HI activities of various fractions were ascertained in the usual manner without additional treatment.

Results. I. Biologic half-life of antibody synthesized by mice. There are several reports that antibodies produced by rabbits and guinea pigs during the first week after injection of an antigen are catabolized more rapidly than those appearing after 2 or more weeks (2,3,6). In those studies, rate of metabolic decay or the biologic half-life of antibody was measured as the time required to eliminate 50% of the antibody activity, or 50% of labelled antibody, from the circulation of passively immunized recipients. Ward first

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TABLE I. Elimination of Passively Acquired 4-day and 15-day Anti-influenzal Mouse Antibody in Homologous Recipients.

Time after admin of antiserum	HI titer in recipients of: 4-day antiserum		15-day antiserum	
	(n)*	g.m. + s.e.†	(n)*	g.m. + s.e.
3 hr	(8)	5.0 ± .5	(8)	6.5 ± .2
1 day	(8)	3.0 ± .6	(8)	5.9 ± .1
2 "	(8)	<2.0	(8)	5.5 ± .2
Biologic half-life		.5 day		2.0 days

* No. of mice.
† Geometric mean and standard error: $Log_2 x$.

showed that HI activity appears in the serum of previously unvaccinated adult mice as early as 3 days after a single intraperitoneal injection of formalin-treated influenza viral antigen (7). To ascertain the biologic half-lives of antibodies synthesized during the initial response of mice to this viral antigen, pools of antisera were collected by bleeding actively immunized donors 4 days or 15 days after vaccination. These antisera were administered intraperitoneally in 0.7 cc doses to CF-1 males that had no known previous experience with the viral antigen. The recipients were bled 3 hours, 1 day or 2 days after passive transfer of the antisera and HI activity of individual heated serum samples measured.

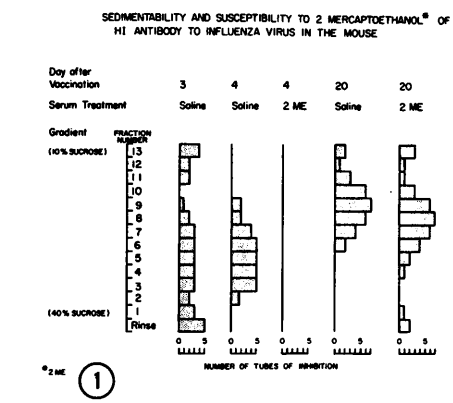
HI titers in recipients of antiserum collected 4 days after vaccination fell approximately 4 times more rapidly than those in recipients of 15-day antiserum (Table I). The biologic half-life of 4-day antiserum was 0.5 day while that for 15-day antiserum was 2.0 days. The latter figure is in close agreement with that value given for the biologic half-life of mouse γ-globulin in mice(8).

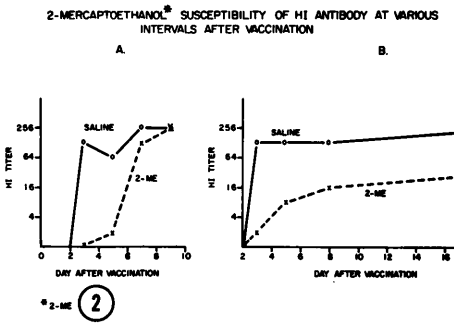
II. Sedimentability and susceptibility to 2-mercaptoethanol. Antibodies in human, rabbit and guinea pig sera reportedly consist principally of particles that sediment in the ultracentrifuge in the manner of 18s macroglobulins and 6.5s γ-globulins. The more rapidly sedimenting particles lose their specific biological activity after treatment with 2-mercaptoethanol, but slow sedimenting antibodies appear to resist the action of that chemical. Although Fahey and Humphrey reported the occurrence of 18s antibodies in mice injected with sheep red cells and 6.5s antibodies in mice injected with haemocyanin and pneumococcal polysaccharide, SSS III(9), they did not ascertain whether sedimentability and susceptibility to 2-mercaptoethanol were related.

Sedimentation properties of HI antibodies obtained at various times after injection of antigen are illustrated by the ultracentrifugal behavior of pooled antisera from mice bled 3, 4, or 20 days after vaccination. Sedimentability of different antibody molecules was ascertained from the amount of HI activity in various fractions recovered after centrifugation. In addition, the finding that mouse hemoglobin sedimented chiefly to fractions 8, 9 and 10 provided an internal control for estimating sedimentability.

Most of the anti-influenzal antibodies in 3- and 4-day antisera were found in fractions obtained from the denser portions of the gradient at the bottom half of the centrifuge tube. Treatment of aliquots of these antisera with equal volumes of 0.2 molar 2-mercaptoethanol for 48 hours destroyed their HI activity. The destructive action of 2-mercaptoethanol on sedimentation fractions of 4-day antiserum employed to assess the rate of elimination of early antibody is clearly shown in Fig. 1. The brief biologic half-life and susceptibility to 2-mercaptoethanol plus the degree of sedimentation indicate the likelihood that antibody in these samples consisted principally of 18s macroglobulins. In contrast, most of the HI activity in the 20-day antiserum was found in fractions obtained from less dense portions of the gradient at the upper half of the centrifuge tube. This group of antibodies sedimented to approximately the same level as mouse hemoglobin. Treatment of 20-day antiserum with an equal volume of 2-mercaptoethanol for 48 hours before centrifugation had little influence on these slower moving antibodies (Fig. 1). The location of these antibodies on the sucrose gradient and their resistance to 2-mercaptoethanol indicate the likelihood that they consist chiefly of 7s γ-globulins, and, to some extent of β_2A globulins.

III. Sequential appearance of different HI antibody molecules after injection of viral antigen. Bauer and Stavitsky reported that 18s antibodies precede 7s antibodies and predominate during the early portion of the primary





antibody response of rabbits to a variety of antigens(3). The same sequence of appearance of antibody molecules has also been seen in sera from man, guinea pigs and chickens at various times after vaccination(2,10,11). Fahey and Humphrey presented evidence that this order of appearance probably occurred in mice injected with sheep red cells, but not after injection of haemocyanin or pneumococcal polysaccharide SSS III.

HI antibody appeared by the 3rd day after intraperitoneal injection of influenza virus. This early antibody was easily destroyed by treatment with 0.2 molar 2-mercaptoethanol for 48 hours and probably consisted of 18s macro-globulins. Antisera obtained 5 or more days after vaccination contained progressively larger amounts of HI activity that was resistant to the action of 2-mercaptoethanol and probably consisted of 7s γ-globulins. In some experiments, the 7s type antibody molecules appeared to replace the 18s types antibody by the 9th day (Fig. 2a). In others, 18s type remained predominant for 20 days at least (Fig. 2b).

Discussion. Table II summarizes several important characteristics of hemagglutination-inhibition antibody found in sera of mice during the first and second week after a single intraperitoneal injection of an aqueous preparation of formalin-treated influenza virus. It is evident that these findings describe at least 2 types of antibody molecules. The earlier antibody has characteristics of macroglobulins, probably of the 18s variety. However, in the absence of more precise measurements of sedimentation coefficients, the presence of some molecules intermediate to 18s and 7s globulins is also possible. The γ-globulins appeared to comprise a major portion of the HI activity in serum obtained during the second week after vaccination and were not particularly important before then. It was apparent that the 7s γ-globulin molecules were not completely refractory to the action of 2-mercaptoethanol. Preliminary findings in this laboratory indicate that the destructive effect of 2-mercaptoethanol on HI activity during the second week after vaccination depends, in part, on whether the antiserum was heated or diluted before adding the sulfhydryl reducing agent. Apparently, reactivity with 2-mercaptoethanol should not be routinely employed as the sole criterion for classification of various antibody molecules.

TABLE II. Major Properties of HI Antibodies in Mice.

	Early	Late
Induction period	2 days	3-4 days
Biologic half-life	0.5 day	2 "
Reactivity with 2-mercaptoethanol	High	Low
Sedimentability	Fast	Slow

Direct measurement of rates of catabolism of different antibody molecules revealed striking differences between the biologic half-life of early and late antibodies. Due to the rapidity with which early antibody was eliminated from the circulation of passively immunized mice it is possible that there was insufficient time for equilibration of antibody between blood and other body fluids. This might have caused an error in the direction of underestimating the biologic half-life. However, unpublished observations in actively immunized mice exposed to x-rays after the appearance of circulating antibody indicate that such

an error would be small since antibody responses in these animals set an upper limit of 0.7 to 0.8 day for the half-life of macroglobulins. On the other hand, later antibody was eliminated at a rate approximating published values for the biologic half-life of mouse antiserum in mice. Apparently, equilibration did not appear to be an important factor in determining the rate of elimination of γ-globulin type antibody.

Differences in biological half-lives of early and late antibodies provide unmistakable evidence of some relationship between structural and biological properties of these molecules. In view of the fact that the specificity of immunologic phenomena is sensitive to small structural alterations of the reactants, it seems likely that the immunologic activities of early and late HI antibodies would differ. Indeed, in the rabbit, early and late hemolysins for sheep red cells appear markedly dissimilar in their hemolytic properties(12).

Summary. Hemagglutination - inhibition (HI) antibodies appeared in the serum of mice 3 days after injection of influenza virus vaccine. The initial antibody was sensitive to 2-mercaptoethanol, sedimented as an 18s macroglobulin, and had a biologic half-life of 0.5 days. Antisera obtained approximately 2 weeks after vaccination contained antibody that resisted treatment with mercaptoethanol, sedimented as 7s γ-globulin, and had a half-life of 2 days.

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Radiation Dose-Response Characteristics of Leucocyte Recovery in the Mouse. (28561)

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Early recovery of cell counts in peripheral blood has long been associated with protective and therapeutic treatment effects in irradiated animals(1,2) but few studies have employed these recovery times quantitatively. Although the survival end point is most generally used, in irradiated mice this is, at best, sensitive over a range of only about 250 rads. In practice, virulent infections such as *Pseudomonas* are frequently encountered(3) and they introduce major variations in survival, the cause of which may go undetected in laboratories which do not make routine bacteriological examinations. On the other hand, granulocyte and lymphocyte recovery times may be dose-related over a wider range and, importantly, at sublethal doses where variations introduced by infections do not interfere. Since there is much evidence of a causal relationship between granulocyte recovery and ability to survive(4,5) it is possible that, under certain circumstances, time of granulocyte recovery to a given level can be substituted for survival in studies of protective and therapeutic agents. While these remarks emphasize the problems arising from infection, clearly there are numerous other occasions in