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## Studies on the Structure of Mouse Antibodies.* (28681)

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Mammalian 7S gamma globulins have been shown to be made of two types of polypeptide chains which can be separated by reduction in 8M urea and starch gel electrophoresis in 8M urea(1). They have been called Light (L) and Heavy (H) chains(2). In human immune globulins, the H chains characterize the different antibody types $\gamma_2$, $\gamma_{1A}$, $\gamma_{1M}$(2). The heterogeneity of $\gamma$-globulins within a given type appears to depend in part on the heterogeneity of the L chains. Normal human or guinea pig 7S $\gamma$-globulin, upon reduction and alkylation and electrophoresis in 8M urea starch gel, yield patterns showing a strong slow band corresponding to the H chains and a faster moving smear corresponding to the L chain components(1,3). In contrast, human myeloma(4) and purified guinea pig anti-hapten antibodies from individual animals(3), similarly treated, show distinctive sharp bands with well-defined mobility corresponding to L chains. Furthermore, in the case of guinea pig antibodies the number and mobility of the bands yielded by antibodies against non-cross-reacting haptens seem to be characteristic of the immunological specificity(5).

Guinea pig 7S antibodies against a single specificity have also been found to consist of 2 families of antibodies $\gamma_1$ and $\gamma_2$ with different electrophoretic mobility(6). These antibodies have been shown to mediate different biological activities in the guinea pig; $\gamma_1$ antibodies being responsible for anaphylactic sensitivity(7) and $\gamma_2$ antibodies for such phenomena which depend upon the fixation of hemolytic complement such as cell lysis and the Arthus reaction(8). $\gamma_1$ and $\gamma_2$ guinea pig antibodies share common antigenic determinants of their S fragments (Piece I and II of Porter) and possess distinct antigenic determinants of their F fragments (Piece III of Porter)(9).

The present study attempts to characterize the antibodies which are produced by individual mice hyperimmunized against well-defined haptens or foreign proteins, as was done for the guinea pig.

Purified antibodies from individual mice appear also to contain at least 2 families of molecules, with slightly different electrophoretic mobility, possessing similar as well as distinct antigenic determinants. Purified anti-hapten antibodies from single mice, when reduced and alkylated and subjected to electrophoresis in 8M urea, yielded distinct bands corresponding to L chains, while mouse $\gamma$-globulin or purified anti-ovalbumin antibodies similarly treated showed a smear corresponding to the zone of L chain migration.

*Methods. Animals.* Random bred Swiss Webster and pure strain $C_3H$ mice were used.

*Antigens.* Ovalbumin 2 $\times$ recrystallized, Worthington Biochemical Corp.

Beef $\gamma$-globulin, Armour, Chicago, Ill.

2,4-dinitrophenyl bovine $\gamma$-globulin (DNP-BGG). Prep. I; 8 groups/mole, prep. II; 45 groups/mole.

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Para-iodobenzenesulfonyl bovine γ-globulin (pipsyl BγG).

The conjugates were prepared with 2,4-dinitrofluorobenzene and from para-iodobenzenesulfonyl chloride according to the methods described(10).

*Immunization.* The animals were hyperimmunized to yield high titer antibody in their peritoneal fluid according to the technique of Munoz(11). They were injected intraperitoneally initially with 1 mg of antigen emulsified with an equal volume of complete Difco adjuvants. Booster injections were given every 2 weeks—an amount of antigen varying from 0.2 to 1 mg was used. After 2 months, the mice with ascites were tapped. (Swiss mice were immunized with ovalbumin, pipsyl BγG and DNP-BGG. $C_3H$ mice with pipsyl BγG.) It should be stressed that only a few mice out of groups of 25 mice survived the procedure and produced a sufficient amount of ascitic fluid with a high enough antibody content to allow isolation from single mice.

*Isolation of antibodies.* Antibodies were isolated from the peritoneal fluid from individual mice. Anti-DNP and anti-pipsyl antibodies were isolated as previously described (6). The ascitic fluids were first decomplemented with immune precipitates of ovalbumin and rabbit anti-ovalbumin at equivalence, using about 10 mg of immune precipitate per 10 ml of peritoneal fluid. The antibody preparations were precipitated with highly conjugated DNP beef fibrinogen or pipsyl fibrinogen. After washing 3 times with cold saline, the precipitates were dissociated with ε-DNP lysine or with pipsyl ε-aminocaproic acid at a concentration of $2 \times 10^{-3}$ M. Thirty-five mg of streptomycin per ml was added to precipitate the derivatized fibrinogen, and the excess hapten was dialyzed in dilute phosphate buffer pH 7.4.

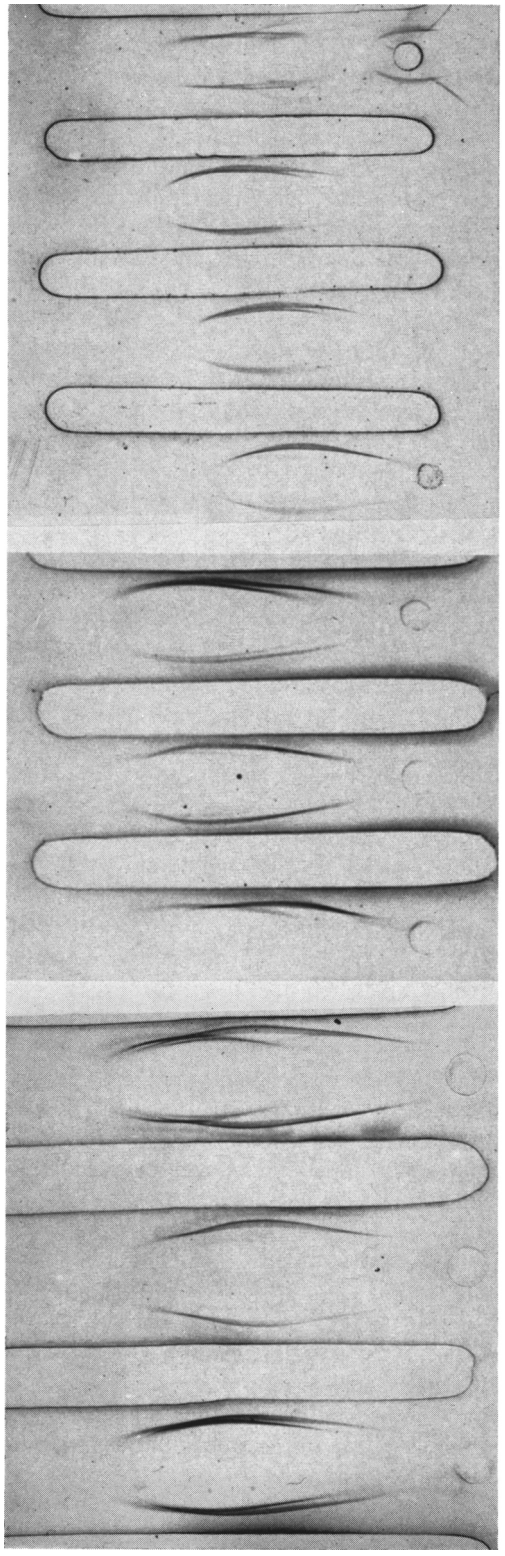
The anti-ovalbumin antibodies were isolated according to the method of Singer(12). Decomplementation was carried out as above with a rabbit anti-bovine γ-globulin system. The antibody preparations were lyophilized before use.

*Immunoelectrophoresis.* Rabbits were immunized with normal mouse serum in complete adjuvants. The serum containing the largest amount of antibodies against mouse γ-globulin was chosen to develop the immunoelectrophoretic patterns. A modification of the Scheidegger technique of immunoelectrophoresis was used(6). Since the zone of migration of the immune globulin at pH 8.6 is towards the cathode, the wells were placed towards the anode to provide better separations.

*Starch gel electrophoresis in 8M urea.* 1 to 2 mg antibody samples were dissolved in 8M urea containing 0.2 M mercaptoethanol, at a concentration of 10 mg/ml. After 2 hours, 12 mg iodoacetamide were added to each sample and starch gel electrophoresis in 8M urea performed in formate buffer pH 3.1 as described(13).

*Results.* The results of immunoelectrophoresis of the isolated antibody preparations from individual mice anti-DNP-BGG, anti-pipsyl BγG and anti-ovalbumin developed by a rabbit serum anti-mouse serum, are shown in Fig. 1. Irrespective of the immunological specificity, all preparations from random bred Swiss mice or $C_3H$ mice show 2 precipitin arcs with slightly different electrophoretic mobility corresponding to 2 distinct antibody populations. The antigenic relationship between these 2 antibody types seems to be one of partial immunological identity, with common as well as distinct antigenic determinants. The relative intensity of the 2 precipitin lines and therefore the respective concentrations of the 2 antibody types vary with individual antibody preparations regardless of specificity. With 2 of the purified antibody preparations, DNP 5 and pipsyl $C_3H$ C, some small added spurs can be seen which cannot now be interpreted.

The results of starch gel electrophoresis in 8M urea of the reduced and alkylated antibody preparations from 2 experiments are presented in Fig. 2. The pattern of normal mouse 7S γ-globulin shows a strong slow band and a faster migrating smear similar to what is observed with other mammalian 7S γ-globulins. Mouse anti-ovalbumin antibodies of which 2 preparations have been studied show patterns similar to that of normal mouse γ-globulin. In contrast, anti-pipsyl



and anti-DNP antibodies present banded patterns in the zone of migration of L chains. Anti-pipsyl antibodies from 2 Swiss Webster mice show 3 distinct fast bands. Anti-pipsyl from 3 additional mice showed also the same banded pattern but with the slowest of the 3 fast bands weak or missing. Anti-pipsyl antibodies produced by $C_3H$ mice showed 2 of the 3 fast bands. In one of the preparations $C_3H$ C one of the bands appears to be split into two. Most anti-DNP antibodies showed one strong band with a tendency to smear. Some material migrating more slowly than the strong slow band observed in all preparations, represents probably incompletely reduced molecules. The urea starch gel electrophoretic patterns were therefore different for anti-pipsyl antibodies, anti-DNP antibodies and anti-ovalbumin antibodies.

*Discussion.* As has been reported for guinea pig antibodies of different specificities(6), anti-pipsyl and anti-DNP mouse antibodies when reduced and alkylated in 8M urea yield starch gel electrophoresis patterns that differ in fast bands corresponding to L chains. Antibodies with the same specificities obtained from different mice show similar patterns. The lack of distinct banding with anti-ovalbumin antibodies may be due to the numerous antigenic determinants which protein antigens possess. Besides these species similarities, some differences must be noted. Dissociated guinea pig anti-DNP antibodies yield more numerous and sharper bands than reduced and alkylated mouse antibodies with the same specificity since as many as 5 fast bands are observed with guinea pig antibodies instead of one strong band in the mouse with a tendency to smearing. The significance of these differences is not clear.

Isolated mouse antibodies from hyperimmunized mice consist principally of 2 cross-reacting antibody populations with slightly

FIG. 1. Immunoelectrophoresis patterns of purified mouse antibody preparations developed with rabbit anti-serum #1. From top to bottom: mouse serum diluted 1:5, anti-DNP-BGG S. W. 3, anti-DNP-BGG S. W. 4, anti-ovalbumin S. W. 1, anti-pipsyl-BGG $C_3H$ A, anti-pipsyl-BGG $C_3H$ B, anti-pipsyl-BGG $C_3H$ C, anti-DNP-BGG S. W. 5, anti-DNP-BGG S. W. 6, and anti-pipsyl-BGG S. W. 1.

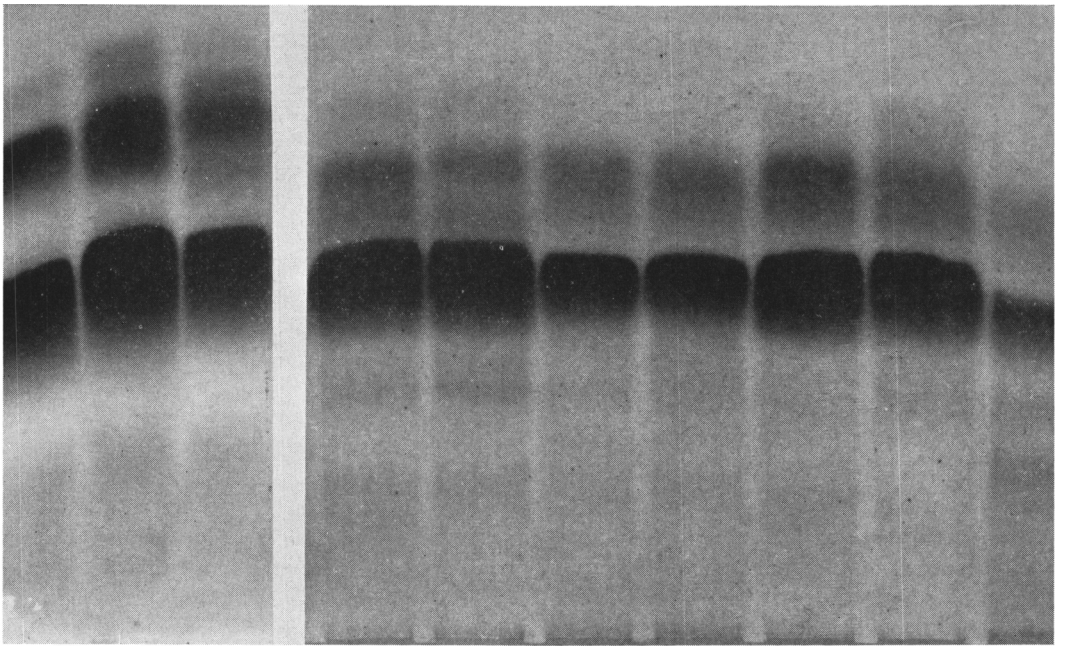


FIG. 2. Starch gel electrophoresis patterns in 8M urea of purified mouse antibody preparations after reduction and alkylation. From left to right: anti-pipsyl $C_3H$ A, anti-pipsyl $C_3H$ B, anti-pipsyl $C_3H$ C, anti-pipsyl S. W. 1, anti-pipsyl S. W. 2, anti-ovalbumin S. W. 1, anti-ovalbumin S. W. 2, anti-DNP-BGG S. W. 1, anti-DNP-BGG S. W. 2, and mouse $\gamma$-globulin.

different electrophoretic mobility. These observations are consistent with the described immunoelectrophoretic patterns of mouse $\gamma$-globulins which show several spurs originating in the main $\gamma$ arc(14). The relationship of these antibody types with the mouse $\beta_{2A}$ (15) has not been investigated, but the slight difference of mobility between the 2 precipitin arcs observed in these experiments suggest that both antibodies may be considered as $\gamma$ antibody types. Considering that in the guinea pig two 7S antibody types with different electrophoretic mobility have been also described and have been found to mediate different biological reactions(7,8), the possibility that the 2 mouse antibody populations also mediate different reactions, should be investigated.

*Summary.* Anti-DNP, anti-pipsyl and anti-ovalbumin antibodies have been isolated from the peritoneal fluids of individual hyperimmunized mice. On immunoelectrophoresis, the antibody preparations appeared to contain, regardless of specificity, at least 2 families of molecules with slightly different electrophoretic mobility, possessing similar as well as distinct antigenic determinants. After reduction and alkylation in 8M urea and starch gel electrophoresis in 8M urea, the anti-hapten antibodies yielded distinct bands corresponding to L chains, in contrast normal mouse 7S $\gamma$-globulin and purified anti-ovalbumin antibodies showed smears corresponding to the zone of L chain migration.

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## Relationship of Endogenous Pyrogen to Lysozyme.* (28682)

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Endogenous pyrogen (EP) is a material found in the blood stream of animals made febrile with bacterial endotoxins, certain colloids, viruses of the influenza group, following induction of experimental streptococcal, pneumococcal and staphylococcal infections and production of experimental hypersensitivity reactions(1). EP shares many of the biological properties of leukocytes or leukocytic extracts derived from inflammatory exudates, including ability to produce fever, and it has been assumed that EP is a product of leukocytes injured by a variety of pyrogenic stimuli(2).

EP can only be assayed by its ability to produce fever in a recipient of the same species. This method is relatively insensitive, cumbersome and expensive, imposes the limitation of species specificity, and involves the hazard of contamination by bacterial pyrogens which are ubiquitous in the environment. Because of these difficulties it seemed desirable to equate the biologic activity of EP with lysozyme, an enzyme which is easily measured by its action on a specific substrate, *Micrococcus lysodeikticus*. Lysozyme was chosen because of its high concentration in leukocytes which are presumed to be the major source of EP. In the present experiments alterations in concentration of lysozyme in serum were compared with changes in activity of EP under experimental conditions which are known to affect the quantity of EP in serum.

*Materials and methods.* Female mongrel dogs weighing 6-12 kg were housed unrestrained in air-conditioned rooms. Food and water were withheld on the day of experiment. Temperatures were measured rectally with clinical thermometers at 30-minute intervals for 4 to 6 hours. Endogenous pyrogen was prepared by intravenous injection of 3.0 ml typhoid vaccine containing approximately $10^9$ killed organisms into normal dogs. Animals were exsanguinated 120 minutes after injection of vaccine by femoral artery or cardiac puncture under pentobarbital anesthesia. Blood was permitted to clot, serum obtained by centrifugation, and cultured in thioglycollate broth. If found to be sterile, sera were stored at 4°C until used. Sera were never kept more than 2 weeks. EP was assayed by injecting 20 ml of serum into 3 normal recipient dogs. Animals used for pyrogen assay were permitted 3 hours for acclimatization and were not used if temperatures fluctuated more than 0.2°C. Fever curves were plotted on standard graph paper, the area beneath the curve for the first 2 hours after injection was measured by planimetry (Keuffel-Esser 423 M-9788) and the resulting area was defined as the fever index. Animals were made tolerant by daily injections of 3.0 ml typhoid vaccine for 9 days, and EP assayed from the same donor on day 1, 2, 5, and 9. Adrenal hormone used in all studies was Δ-1 hydrocortisone. Hypothermia was induced by immersing anesthetized animals in an ice bath and monitoring temperatures with a rectal thermister (Telethermometer, Yellow Springs Instrument Co.).

Lysozyme was determined prior to injection of endotoxin in each animal and on each sample of EP by the method of Kerby

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