the elastic fibers of the connective tissue and blood vessels were very well stained.

Chromatography: Perhaps the most interesting observation was the presence of two phosphoinositides which were in greater relative concentration than in the CSF (Fig. 2). The lower inositide appeared to be diphosphoinositide on the basis of a direct chromatographic comparison with guinea pig brain extract and with fraction I isolated from guinea pig brain by the Folch technique(3). Although the choroid plexus contained cardiolipin (Fig. 2) as expected from the strong reaction for succinic dehydrogenase indicating the presence of mitochondria, unlike CSF it was negative for plasmalogen.

Discussion. We suggested earlier that lipids of the CSF may be contributed by the choroid plexus. The similarities of the respective lipid patterns, especially the 2 inositides (not examined for in the earlier CSF work) may strengthen this suggestion. The plasmalogen positive cardiolipin of CSF remains unexplained.

Summary. Some histochemical features of human choroid plexus are described and the lipid pattern of chloroform:methanol extracts of choroid plexus are compared with CSF. Two phosphoinositides were demonstrated in both specimens, and although cardiolipin is present in choroid plexus it is plasmalogen negative.

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Measurement of Gamma Globulin in Germfree Guinea Pigs by Hemagglutination Inhibition. (28678)

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During experiments with germfree guinea pigs it became necessary to measure the gamma globulin of large numbers of animals with low serum gamma globulin concentrations. Because electrophoretic methods are not accurate for estimation of low levels of gamma globulin(1), a search was made for an immunochemical method which would be accurate at low levels, require only small amounts of serum, and could be carried out with minimum effort. Inhibition of the Coomb's reaction of human erythrocytes coated with specific anti-Rh antibodies, has been reported to be an accurate method(2-4) for measurement of low levels of gamma globulin in human sera. Latex particles coated with specific anti-human gamma globulin have been used to estimate serum gamma globulin concentrations, but this technique requires a specific anti-gamma globulin antibody, a complicated standardization procedure and has a graded endpoint(5). This report describes a hemagglutination inhibition procedure which is sensitive at low gamma globulin concentrations, can be applied to various species, has a sharp endpoint and, when combined with the microtiter dilution technique of Takatsy(6, 7), permits a rapid measurement of gamma globulin levels in large numbers of animals.

Materials and methods. Preparation of gamma globulin. Guinea pig gamma globulin was obtained by fractionation of pooled normal guinea pig serum by diethylaminoethyl cellulose chromatography(8). The first peak of protein was concentrated by ultra-filtration, dialyzed against 0.85% NaCl and tested for protein content by double diffusion in agar(9) and immunoelectrophoresis(10) against potent rabbit anti-whole guinea pig serum. Only a single gamma globulin precipitation line was obtained.

Rabbit anti-guinea pig gamma globulin serum. Antiserum was produced in rabbits by immunizing them with an aliquot of the

guinea pig gamma globulin in complete Freund's adjuvant. The adjuvant contained 0.85 ml Bayol F, 0.15 ml Arlacel, and 0.75 mg killed *Mycobacterium tuberculosis H37Rv* per ml. The adjuvant was emulsified with an equal volume of 0.85% NaCl containing 0.75 mg protein per ml. Four-tenths ml of the emulsion was injected intradermally into each rear foot pad of 2 rabbits. The same volume was injected in each flank 2 weeks later so that each animal received a total of 1.6 ml of antigen-adjuvant mixture containing a total of 0.6 mg of protein and 0.6 mg of *M. tuberculosis*. At weekly intervals following the second injections, 40 ml of whole blood was collected from the ear vein of each rabbit. The serum was separated by centrifugation, 1:10,000 merthiolate added, and each sample frozen separately.

The antiserum produced a single precipitin line against whole guinea pig serum that formed a line of identity with gamma globulin preparations in double diffusion in agar. The antiserum also formed a line consistent with gamma globulin upon immunoelectrophoresis against whole guinea pig serum.

A combination of double diffusion in agar and immunoelectrophoresis using special reagents(11) was used to demonstrate that this antiserum reacted with the common antigenic determinants of both the fast (γ_1) and slow (γ_2) moving portions of 7s gamma globulin, but not with the determinants unique for the fast component (Fig. 1). Normal guinea pig serum was concentrated by ultracentrifugation and subjected to immunoelectrophoresis. By reaction with specific antiserum (G-20, upper trough)* the precipitin bands of both the fast (γ_1 or β_2A) and slow (γ_2) portions of 7s gamma globulin in the normal serum, as well as a beta globulin, were formed. These lines were extended laterally by addition of guinea pig gamma globulin isolated by block electrophoresis to the lower trough. A well at the end of the slide was filled with the antiserum to be tested (AS). The reaction of the antiserum (AS) with both fast and slow gamma globulin

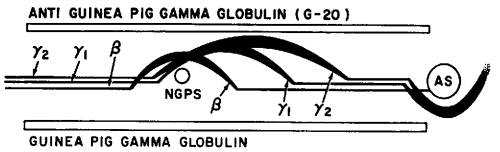

FIG. 1. Composite drawing of immunoelectrophoretic patterns demonstrating the reaction of the rabbit anti-guinea pig gamma globulin antiserum (AS) with the common antigenic determinants of both the fast (γ_1) and the slow (γ_2) moving components of guinea pig gamma globulin (see text).

components was demonstrated by the fusion of both the γ_1 and γ_2 lateral lines with the circular precipitin line formed by antiserum AS. A lateral precipitin line formed by a protein not reacting with AS would have extended through the circular precipitin line around the well containing AS. This effect was observed by adding a gamma globulin preparation containing beta globulins to the lower trough. The resulting beta precipitin line extended laterally from the beta arc (β) and passed through the circular precipitin line around the lateral (AS) well.

Test Procedure. Formalinized(12), tanned sheep erythrocytes were sensitized with the guinea pig gamma globulin preparation (0.5 mg guinea pig gamma globulin per ml of packed cells) by the method of Stavitsky (13). Dilutions of known amounts of guinea pig gamma globulin or guinea pig sera were made in a 1:100 saline dilution of normal rabbit serum using a modification of the microtiter technique of Takatsy(6,7). Twofold serial dilutions of the unknown guinea pig sera were made in 2 rows; one beginning with a 1:2 dilution, the other with a 1:3 dilution, in order to obtain staggered endpoints. To each well was then added a constant dilution of the rabbit anti-guinea pig gamma globulin serum. After 30 minutes at room temperature, to permit the rabbit antiserum to react with the gamma globulin present, one drop (0.025 ml) of a 1% suspension of the sensitized erythrocytes was added to each well. The plates were then agitated to suspend the erythrocytes, placed at 4°C overnight, and read the following morning.

Results. Addition of guinea pig gamma globulin antiserum inhibited the agglutination globulin to rabbit anti-guinea pig gamma

* Kindly supplied by Dr. Howard C. Goodman, Laboratory of Immunology, NIAID.

of erythrocytes sensitized with guinea pig gamma globulin. In the wells that contained sufficient gamma globulin to react completely with all of the rabbit anti-gamma globulin antibody added (antigen excess), no antibody remained to react with the sensitized erythrocytes subsequently added, and no agglutination occurred. However, in those wells that contained excess anti-guinea pig gamma globulin antiserum, there was agglutination of the erythrocytes sensitized with guinea pig gamma globulin. Thus, inhibition of agglutination depended directly on the amount of guinea pig gamma globulin added to each well. The lowest concentration of a known solution of gamma globulin which caused hemagglutination inhibition when added to each of the 4 constant antiserum dilutions was considered the endpoint (extinction concentration) for the given antiserum dilution. The results with serial dilutions of known amounts of guinea pig gamma globulin are shown in Fig. 2 and Table I. As the dilution of the rabbit antiserum used was increased, smaller amounts of gamma globulin were detectable. Thus, the extinction concentration in the 1:50 dilution of the rabbit anti-gamma antiserum was

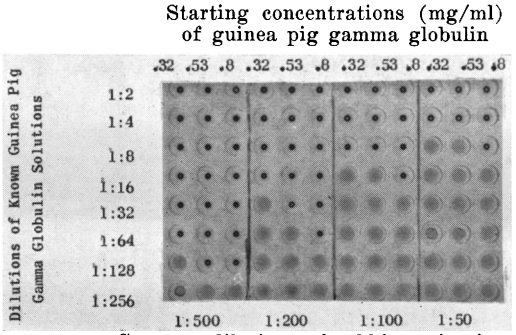

FIG. 2. Calibration of hemagglutination inhibition test. An even distribution of cells indicates agglutination; a button indicates no agglutination. Serial dilutions of known concentrations of guinea pig gamma globulin start at the top of each column. When enough gamma globulin is present to combine with all of the rabbit anti-gamma globulin antiserum, no agglutination of the gamma globulin coated erythrocytes occurs. The highest dilution of gamma globulin giving inhibition is the end-point. By multiplying the end-point dilution by the starting concentration, the lowest effective concentration is calculated and is defined as the extinction concentration.

TABLE I. Calibration of Extinction Concentrations and Absolute Quantities of Guinea Pig Gamma Globulin Detected by Hemagglutination Inhibition Titration Against Constant Dilutions of Rabbit Anti-Guinea Pig Gamma Globulin Antiserum.

Dilution of rabbit anti-guinea pig gamma globulin antiserum	Lowest inhibitory conc* (mg/ml)	Highest non-inhibitory conc (mg/ml)	Absolute quantity detected† (μg)
1:50	.080	.068	2.0
1:100	.044	.034	1.1
1:200	.0125	.010	.36
1:500	.004	.0031	.1

* Extinction concentration.

† Smallest absolute amount of guinea pig gamma globulin detectable. Calculated by multiplying the extinction concentration by the volume added (.025 ml).

0.080 mg/ml, 0.044 in the 1:100 dilution; 0.0125 mg/ml in the 1:200 dilution and 0.004 mg/ml in the 1:500 dilution. Since only 0.025 ml of each dilution of gamma globulin was added to each well, the absolute amount of guinea pig gamma globulin detectable at the 1:50 dilution of rabbit antiserum was 2.0 μg; 1.1 μg at the 1:100 dilution; 0.36 μg at the 1:200 dilution; and 0.1 μg at the 1:500 dilution. However, as the dilutions of the antiserum increased the endpoints became less distinct. This did not interfere with endpoint readings until dilutions greater than 1:500 were used.

For determinations of unknown amounts of gamma globulin in sera, a 1:100 dilution of the antiserum was chosen. This dilution provided clear endpoints, satisfactory resolution, and extinction titers within a convenient range of the dilution system used. The gamma globulin concentrations of the unknown sera were calculated by multiplying the reciprocal of the highest effective dilution (endpoint) of the test serum by the extinction concentration of the 1:100 antiserum dilution. For example the gamma globulin level of an unknown serum with an inhibition endpoint of 1:16 was determined by multiplying 16 by 0.044 mg/ml (Table I) giving a serum gamma globulin concentration of 0.70 mg/ml or 70 mg%. Table II shows the serum gamma globulin levels in 6 weeks' old germfree(14) and normal guinea pigs obtained by this

TABLE II. Gamma Globulin Levels in Germfree and Normal Guinea Pigs Determined by Hemagglutination Inhibition and Paper Electrophoresis.

Test	Germfree (12)* Mean	Germfree (12)* Range	Normal (8)* Mean	Normal (8)* Range
Hemagglutination inhibition	51.3†	35.2– 70	370	282–422
Paper electrophoresis	174	134 –242	310	231–404

* No. of animals.
† mg of gamma globulin/100 ml of serum.

method and by paper electrophoresis and the biuret reaction(15). The average serum gamma globulin level of normal animals was 310 mg% by paper electrophoresis and 370 mg% by hemagglutination inhibition. At low serum concentrations, the hemagglutination inhibition technique had markedly greater accuracy. The serum gamma globulin concentrations of the germfree animals obtained by this method were one-third to one-fourth (51 mg%) of the levels indicated by paper electrophoresis (174 mg%).

Discussion. The hemagglutination inhibition technique described above provides a rapid, convenient, and accurate method for measuring low levels of serum gamma globulin in the guinea pig. This method could be applied to gamma globulin determinations in other species by varying the antigen-antibody system. In addition, any serum protein component which can be isolated in purified form lends itself to quantitation by this method. It is not necessary to obtain a specific antiserum, although this was done in the present case. A dilution of an anti-whole serum antibody could serve just as well as the specific anti-protein antibody so long as the material coating the cells is a single protein and the anti-whole serum antibody contained antibodies to this same protein. In fact, hemagglutination inhibition has been used for detection of thyroglobulin(16,17), and liver protein(18) in serum, as well as gamma globulin(19).

Immunochemical methods are more accurate than electrophoretic procedures for measurement of minute amounts of antigens(1). Quantitative precipitin(20-22), simple gel-diffusion(23) and precipitation inhibition (24) have also been used to measure levels of gamma globulin too low to be determined by electrophoretic methods. Electrophoretic methods may not detect concentrations as low as 50 mg% and may have an error of 100% at a concentration of 100 mg%(1). Paper electrophoretic determination of gamma globulin levels has shown less than a 2-fold difference between the germfree and normal animal(25), but as shown here, paper electrophoresis does not reflect the true 5- to 6-fold difference. Similarly, greater differences were found in levels of gamma globulin in germfree and normal rats(22) when measured by the quantitative precipitin technique, than when measured by paper electrophoresis. Thus, although there are theoretical reservations to quantitation by immunochemical methods(1,26), these methods are generally more sensitive and accurate than electrophoretic analysis at low levels of gamma globulin.

The validity of the method is directly related to knowledge of the specific immunological reaction. By careful evaluation of the reaction of the antiserum used for quantitation and the antigen coating the erythrocyte, a fairly good estimate of exactly what proteins are being measured can be made. The antiserum used in the present study was tested and shown to react with the common antigenic determinants of the fast (γ_1) and slow (γ_2) gamma globulins of the guinea pig. This test demonstrated that: 1) the fast and slow gamma globulins share common antigenic determinants in the guinea pig as in other species(11,27,28), and 2) since reactions with other serum proteins were not detectable, the antiserum must have measured only the 7s gamma globulins.

The error arising from dilutions starting with a single concentration was decreased by running parallel serial dilutions starting with 2 different concentrations. At low concentrations of gamma globulin small differences between sera were easily distinguished by hemagglutination inhibition, but at normal concentrations of gamma globulin the same small differences were not detected. Thus, differences between 20, 30 and 40 mg% were resolvable, while differences between 320, 330 and 340 mg% were not.

Summary. Guinea pig serum or gamma

globulin inhibits agglutination of erythrocytes coated with guinea pig gamma globulin when added to rabbit anti-guinea pig gamma globulin antiserum. This inhibitory effect provides a convenient, accurate, and readily interpreted technique for measurement of low concentrations of serum gamma globulin. The serum gamma globulin concentrations of germfree guinea pigs are about one-fifth those of normal guinea pigs when measured by this method, which is much lower than previously reported. The quantitative hemagglutination inhibition technique is more accurate than paper electrophoresis, and its principle is potentially applicable to measurements of many proteins or other antigenic materials.

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Stimulation of Prolactin Secretion by Perphenazine in Pituitary-Hypothalamus Organ Culture.* (28679)

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Transplantation of the anterior hypophysis into the kidney capsule of the rat maintains functional corpora lutea for prolonged periods(1,2) and initiates lactation in estrogen-primed rats(3). A similar effect resulted from infliction of a specific hypothalamic lesion in the estrogen-primed rabbit(4). Initiation of lactation was also reported following administration of chlorpromazine to women(5) and estrogen-primed rats(6) and of reserpine to estrogen-primed rabbits(7,8). Sulman and Winnik(11,12) have postulated that these effects are the result of hypothalamic depression by the above drugs, but no direct proof of this is available.

Recently Meites *et al*(13) succeeded in

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