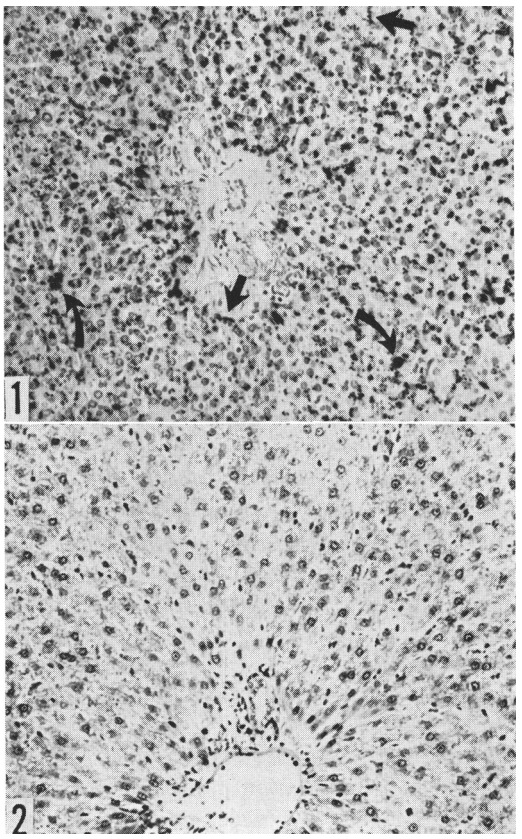

FIG. 1. Section of liver from rat given iron supplement for 130 days after construction of an end-to-side portacaval shunt. Note large amount of iron in Kupffer cells (bent arrow) and hepatocytes (straight arrow). Perl's reaction. Nuclear fast red counterstain (× 90).

FIG. 2. Section of liver from sham operated rat given iron supplement for 130 days, showing absence of stainable iron. Perl's reaction. Nuclear fast red counterstain (× 90).

increased pinocytic activity and mitochondrial damage.

Summary. To determine the role of portacaval shunts in the development of hepatic siderosis, rats were subjected to end-to-side and side-to-side portacaval shunts. Hepatic siderosis developed only in rats with end-to-side shunts, while visible liver cell damage and cirrhosis were absent. It is concluded that shunting of the portal blood flow *per se* results in hepatic siderosis, even in the absence of cirrhosis.

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An Antibody with Alpha-1 Globulin Mobility. (28911)

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Antibodies in man and in most animal species are considered to be generally associated with a group of serum proteins structurally related, known as gamma-globulins (1-3). Their electrophoretic mobility is generally slow and many workers today consider that antibodies with higher mobility reported in the literature(2,3,6,7), are actually gamma-globulins with different mobilities, but belonging to the same antigenetic family (4, 5). There are few data in the literature describing, by indirect methods, antibodies with

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alpha-globulin mobility(8-10). Some workers found them in the alpha-2 subfraction (11,12).

It is considered today(13,14) that antibody activity is associated with 3 serum proteins: the gamma (S = 7), the beta-2M (S = 19) and the beta-2A (S = 7) globulins. The experiments described below, demonstrate by immunoelectrophoresis the existence of a precipitating antibody with alpha-1 globulin mobility.

Materials and methods. Antigens. Three strains of insect flagellates namely: *Leptomonas culicidarum*† (L.C.), *Herpetomonas muscidarum*† (H.M.) and *Strigomonas fasciculata*‡ (S.F.) were grown in a diphasic medium(15) to which we brought several slight modifications. The flagellates were incubated for 7 days at 26°C, harvested and washed 3 times in saline. They were then disrupted in a M.S.E. ultrasonic unit for 150 seconds at 1.4 A. The sonic disintegration was carried out in a Na-barbitone—HCl buffer at pH 8.2 and ionic strength 0.05. The sonic extracts were centrifuged at 2500 r.p.m. for 10 minutes and the supernatants stored at 4°C.

Antisera. Groups of 2 male rabbits weighing 2000-2500 g were used for each strain. Three intramuscular inoculations of 10^9 cells each, in incomplete Freund's adjuvant, were performed at 10-day intervals and 6 intravenous inoculations each of 4×10^8 cells in saline, at 3-day intervals.

The rabbits were bled 12 days after the last injection and the sera stored at −20°C until used.

Protein determinations of antigens and serum fractions were performed according to Lowry(16).

Paper electrophoresis was performed on 30/4 cm Whatman no. 1 filter paper strips. The runs were carried out in a Na-Barbitone-Na-Acetate buffer pH 8.8, ionic strength 0.06. The electrophoretic separation was finished in 7 hours at 140 V and 0.5 mA/cm width; then the electrophoregrams were fixed and stained(17).

Characterization of glyco- and lipoproteins was performed according to Uriel(4), in a borate buffer pH 8.9, ionic strength 0.08.

Preparative electrophoresis was performed according to Osborn(18) with certain modifications. The eluates were dialyzed against a 0.15 M phosphate buffer, pH 7.2 and subsequently concentrated by dialysis against polyvinylpyrrolidone (P.V.P.).

Immunoelectrophoresis(4) was carried out on microscopic slides(19), in a Na-barbitone-HCl buffer pH 8.2 ionic strength 0.05. The electrophoretic separation was carried out for 2 hours at 50 V and 10 mA for the whole system (6 slides). After addition of the reference system the slides were incubated for 24-36 hours in a wet chamber, perfectly closed. At the end of this period precipitin-lines were fully developed and photographs were taken.

Agar-gel double diffusion(20) was carried out on microscopic slides.

Results. The rabbit antisera (anti-L.C., anti-H.M. and anti-S.F.) were tested by micro immunoelectrophoresis against their homologous antigens. The results are shown in Fig. 1. It was obvious that while the anti-L.C. and anti-H.M. sera gave marked precipitin arcs in the gamma globulin zone only, both anti-S.F. sera gave clear precipitin lines in the alpha-globulin zone, and one serum also a weak line in the gamma area.

To check the presence of heterologous precipitations the anti-S.F. sera were tested by immunoelectrophoresis also against the heterologous antigens (L.C. and H.M.). In no case were precipitin arcs observed.

To determine to which fraction of the alpha-globulins, alpha-1 or alpha-2, this unexpected antibody belonged, the anti-S.F. sera were separated by preparative electrophoresis in agar and the fractions as well as a whole anti-S.F. serum were tested by a slide agar double diffusion test, against the S.F. ultrasonic extract, which contained 1.5 mg protein/ml. As a control all the fractions as well as the whole serum were developed on paper electrophoresis (Fig. 2).

From the electropherograms it could be

† Kindly supplied by Prof. S. Adler, Hebrew Univ., Jerusalem.

‡ Kindly supplied by Dr. Colas Belcour, Pasteur Inst., Paris.

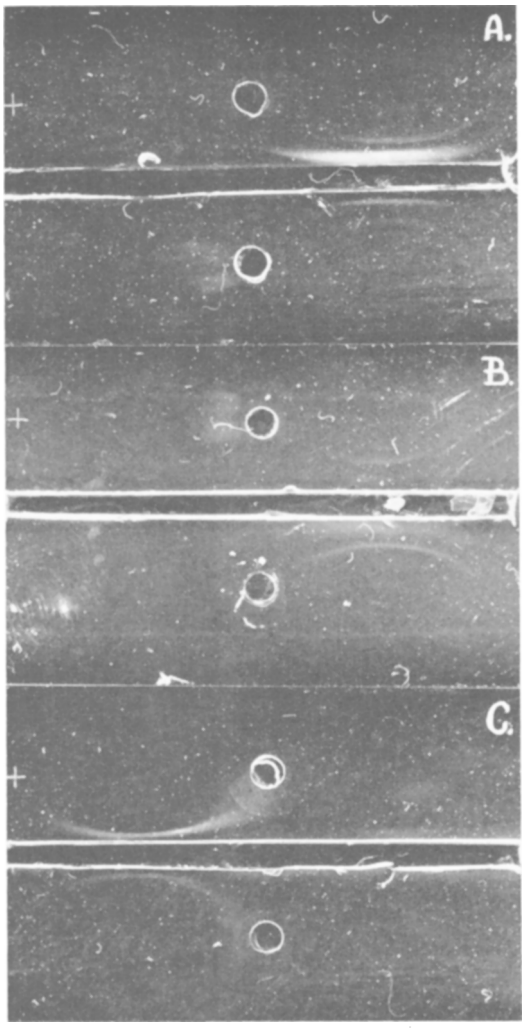

FIG. 1. Immunoelectrophoretic patterns of rabbit antisera: A. anti-L.C., B. anti-H.M., C. anti-S.F., developed with their homologous sonic extracts.

seen that while fractions 1, 2, 3 and 5 are fairly homogenous, fraction 4 is a mixture of alpha-1 globulin and albumin.

Electrophoretic analysis of the anti-S.F. sera showed an increase of relative concentration of alpha-1 and alpha-2 glycoproteins and a decrease of relative concentration of alpha-1 lipoproteins, as compared with the anti-L.C. and normal rabbit sera.

From the agar diffusion tests (Fig. 3), it was seen that the whole anti-S.F. serum as well as the alpha-1 globulin-albumin mixture gave clear precipitin arcs, while all the other eluates were negative. Since the main albumin fraction gave negative results it was obvious that the antibody was present in the alpha-1 globulin fraction. The results were also confirmed when the anti-S.F. serum was developed with a chicken anti-rabbit serum§ by immunoelectrophoresis.

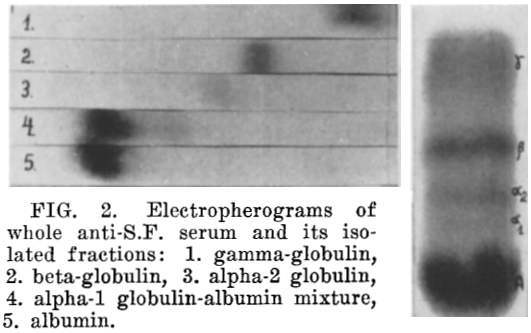

FIG. 2. Electropherograms of whole anti-S.F. serum and its isolated fractions: 1. gamma-globulin, 2. beta-globulin, 3. alpha-2 globulin, 4. alpha-1 globulin-albumin mixture, 5. albumin.

Discussion. Antibodies with great electrophoretic mobility have been described. Among them the skin sensitizing antibodies constitute the most important group. But there is very little evidence that they display alpha-globulin mobility. Most workers describe them as beta-1, beta-2 or gamma-1, but even in these cases opinions are divided(6). In view of the reported higher mobility of skin sensitizing antibodies, it would be of interest to see whether the present antibody has not a stronger affinity for the skin than the classical gamma globulins, since it was recently shown that rabbit antibodies to egg albumin,

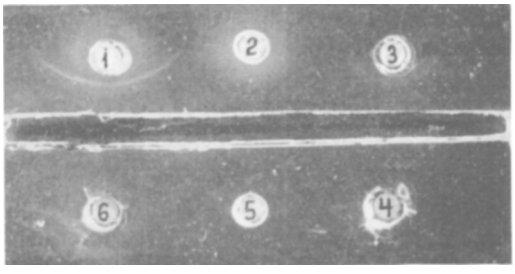

FIG. 3. Agar double diffusion of whole anti-S.F. serum and its isolated fractions, against homologous sonic extract: 1. whole anti-S.F. serum, 2. gamma-globulin, 3. alpha-1 globulin-albumin mixture, 4. beta-globulin, 5. alpha-2 globulin, 6. albumin.

§ Kindly supplied by Dr. D. Nelken, Hadassah Medical School, Jerusalem.

with beta-globulin mobility have a stronger affinity for the skin in passive cutaneous anaphylaxis than the corresponding gamma-globulin antibodies(21).

Although there are data describing antibodies in the alpha-globulin fraction (especially in the alpha-2 globulin), they have never been proved by a direct test. On the other hand the results were not always confirmed when reinvestigated(22,23,24). The present finding is the first instance, to the authors' knowledge, in which an antibody with alpha-1 globulin mobility was demonstrated by agar immunoelectrophoresis when tested against the homologous antigen.

It is well known that the type of antibody is determined by the animal used, the individual response, the method, conditions and number of inoculations. The above described antibody was obtained under experimental conditions in which other antigens induce the formation of typical gamma-globulin antibodies.

Isolation of the antigen involved and analysis of the determinant groups responsible for production of this type of antibody, might uncover a whole class of antigens with similar properties.

Chemical study of the anti-S.F. antibody with alpha-1 globulin mobility, isolated by an immune-specific method, would show whether the chemical composition of this antibody could be related to the reported increase of the relative concentration of alpha-1 glycoproteins and the relative decrease of alpha-1 lipoproteins.

Whether the present antibody with alpha-1 globulin mobility belongs also to the gamma-globulin family as beta-2A and beta-2M antibodies(4,5), or whether it is a new type with completely different antigenic characters has to be clarified. If this last assumption would prove to be correct, a different mechanism and/or a specific type of cell might be involved in its production.

Summary. An antibody with alpha-1 globulin mobility to *Strigomonas fasciculata* was demonstrated by agar micro-immunoelectrophoresis and subsequently isolated by preparative zone-electrophoresis in agar.

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