ways. Since such grafts appear incapable of secreting detectable amounts of either FSH or LH the problem of possible interference by endogenous LH in ovarian ascorbic acid depletion is removed. Likewise, in these animals the possibility of ovarian ascorbic acid depletion being due in part to LH-releasing factors as opposed to LH *per se* is eliminated. For these reasons, it would seem desirable to utilize such animals in conjunction with the usual assay animal in testing various agents and biological materials for LH-releasing substances. In this connection there is disagreement in the literature as to whether vasopressin and adrenaline will affect ovarian ascorbic acid in the hypophysectomized animal(2,4). Finally, it should be emphasized that for routine assay of LH we consider the procedure described by Parlow(1) using intact, pseudopregnant animals to be quite satisfactory. It will of course, be necessary to use intact animals as test objects in further search for LH-releasing substances.

Summary. Immature female rats were made pseudopregnant in the manner prescribed for the Parlow LH assay. Hypophysectomy of such animals on the sixth day of pseudopregnancy caused a gradual decline in ovarian weight over a 5-week period but concentration of ovarian ascorbic acid was well maintained. At periods of a week or more after hypophysectomy intravenous injection of LH produced no decline in ovarian ascorbic acid. In other animals the pituitary gland was autografted to the renal capsule on sixth day of pseudopregnancy. Ovarian weight was well maintained in these animals for 4 weeks although at 4 weeks there was a significant decrease in level of ovarian ascorbic acid. Intravenous injection of LH in pituitary graft-bearing rats led to depletion of ovarian ascorbic acid even greater than that found in intact, pseudopregnant rats. It is suggested that animals bearing pituitary autografts should prove valuable in the search for LH releasing factors.

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Studies of Serum Protein Abnormalities in Kala Azar.* (26340)

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Patients with kala azar have long been known to have hyperglobulinemia. Recently, moving boundary and filter paper electrophoresis of serum proteins from such patients have shown a nonspecific diffuse hypergammaglobulinemia and hypoalbuminemia without significant alterations in alpha or beta globulins(1,2,3,4,5). A few workers, however, have reported what they believed to be specific abnormalities. Benhamou *et al.*(6)

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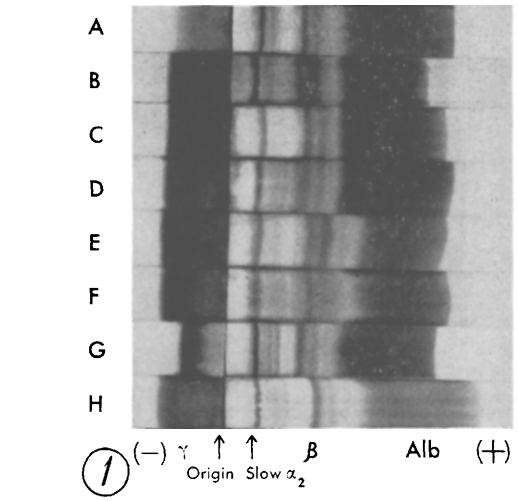
noted the presence of an "X fraction" with a more rapid mobility than albumin, as well as a gamma globulin of slower mobility than that of normal gamma globulin, the latter also identified by Cooper *et al.*(7). Sen Gupta(8) also found this slow moving gamma globulin, and emphasized that it is not seen in several other conditions characterized by hypergammaglobulinemia. Because of these conflicting reports and because the demonstration of a specific protein abnormality in kala azar may bear a relation to other dysproteinemias, the present studies were performed using starch gel and filter paper electrophoresis, immunoelectrophoresis, and the Ouchterlony gel diffusion technic.

Method. Sera from 5 patients with a diagnosis of kala azar established by bone marrow aspiration were obtained in Ceará, Brazil, a region where this disease is endemic. The sera were frozen and shipped to New York for study.

Total protein, albumin, and globulin were determined by a modified Kingsley method (9). Smithies' method was used for starch gel electrophoresis(10). Soluble potato starch was obtained from the Connought Laboratories. Gels were prepared at a concentration of 12.2 g % in 0.021 M borate buffer at pH 9.05. Electrophoresis was performed for 16 hours at 10°C with a potential of 4.5 volts per cm using a 0.30 M borate buffer. Filter paper electrophoresis was performed with the Spinco apparatus using Veronal buffer, pH 8.6, ionic strength 0.05. The paper and the gel strips were stained with naphthalene black B 200. Gel diffusion technics were performed by the Ouchterlony method as described previously by Korngold and Lipari(11).

Scheidegger's procedure(12) for micro immuno-electrophoresis was modified as follows: lantern slide covers were coated with a thin layer of Ion Agar No. 2§ at 1.2% in phosphate buffer (ionic strength 0.05, pH 7.6). The same buffer at ionic strength 0.1 was placed in the electrode vessels of the Spinco model R paper electrophoresis apparatus. After the antigen solutions were applied to the agar with a micro burette, the plate was inverted and the edges of agar on the plate were pressed against the filter paper wicks to assure good contact between them(13). A constant current of 45 mA was applied for 2 hours, during which the albumin migrated 12 mm from the origin. At the end of the run, 0.1 ml of an anti-human serum‖ was introduced into the troughs. Shadowgraphs were made 24 hours later.



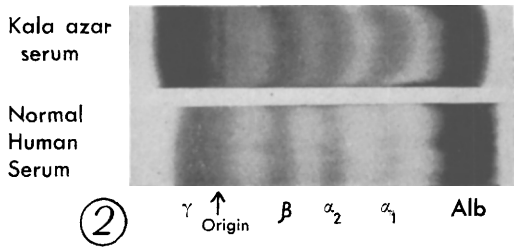


FIG. 1. Starch gel electrophoresis patterns of serum proteins. The origin is marked. A, normal; B, C, D, E, F, kala azar; G, multiple myeloma; and H, cirrhosis of liver.

FIG. 2. Filter paper electrophoresis patterns of serum proteins. The origin is marked. Upper pattern is from patient with kala azar and lower pattern is normal control serum.

Results. All serum globulin values were elevated. Analysis of each of the 5 sera by starch gel electrophoresis revealed a marked hypergammaglobulinemia (Fig. 1). There

§ Consolidated Laboratories, Inc., Chicago Heights, Ill.

‖ This antiserum, produced by immunizing goats with human serum fractions, was kindly supplied by Dr. R. T. Fisk, Hyland Laboratories.

was a concentration of protein in the slow gamma region of all 5 samples. In 3 of these samples, there was an additional protein concentration in the fast gamma region. Thus, one or 2 moderately well-defined, deeply-stained bands appeared in all of the samples. These bands were not as distinct as those commonly seen in multiple myeloma, but they were considerably more pronounced than those found in other forms of hypergammaglobulinemia, such as Laennec's cirrhosis. The remaining area of gamma globulin distribution appeared essentially normal. Variations in other components of the serum protein pattern after starch gel electrophoresis were related to differing haptoglobin types. One sample, B, showed a markedly elevated slow alpha-2 component.

Filter paper electrophoresis showed a diffuse elevation of the gamma globulin without any discrete narrow bands (Fig. 2). In 3 of the samples, a small amount of protein remained at the origin. These were the same 3 sera in which the fast-moving gamma component was observed after starch gel electrophoresis.

The Ouchterlony gel diffusion study revealed a reaction of identity when the 2 antigens, kala azar serum and normal serum, were compared using rabbit antisera prepared against Cohn fraction II (gamma globulin) as antibody. This indicated that the gamma globulins were not of the type seen in multiple myeloma(14). Each kala azar serum, however, contained increased amounts of macroglobulins.

Immuno-electrophoresis of the sera (Fig. 3) also showed a marked hypergammaglobulinemia, which is apparent from the diffuse nature of the zone of precipitate produced by the gamma globulin and by the fact that these zones moved to the antiserum troughs. Moreover, the gamma-1 macroglobulin was markedly elevated when compared with the normal sera run simultaneously, a finding which is in agreement with the results obtained by the Ouchterlony method. Only one serum, C, had an elevated beta-2A globulin.

Discussion. Our studies employing filter paper electrophoresis suggest that no specific

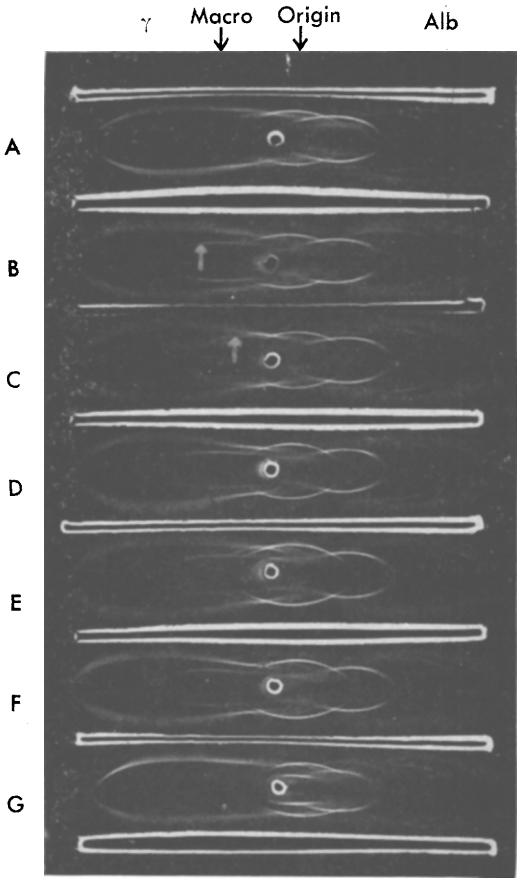


FIG. 3. Strips A and G contain normal sera; patients' sera are in the other strips. Gamma-1 macroglobulin is indicated by an arrow in strip B. The same band can also be seen in strips C, D, E, and F. Beta-2A globulin is indicated by an arrow in strip C which is the only one which shows this band.

protein abnormalities are demonstrable in the sera of patients with kala azar. The nonspecificity of the electrophoretic pattern on filter paper thus coincides with the majority of the previous results reported with this technic in this disease. The band remaining at the origin in 3 of the specimens probably represents fibrinogen or denatured protein rather than any specific protein alteration.

On the other hand, the definite protein concentration in the slow gamma region apparent in all the starch gel patterns differs from that seen either in multiple myeloma or in other conditions associated with hypergammaglobulinemia, such as cirrhosis and sarcoidosis. Simply stated, slow gamma

peaks in the patterns of the sera from patients with kala azar were more diffuse than those in multiple myeloma, and more discrete than those in other hypergammaglobulinemias. The 3 bands in the fast gamma region were found in the 3 samples showing the concentration at the origin on filter paper electrophoresis, and probably represent the same protein. Analysis of the filter paper and starch gel patterns revealed nothing that could be interpreted as the "X fraction" described by Benhamou.

We believe that the band in the slow gamma region reflects a protein abnormality in kala azar not found in several other pathologic conditions also characterized by hypergammaglobulinemia. It is conceivable that these bands represent a particular antibody response to one or another antigen derived from *L. donovani*. We should like to emphasize that with the technics used in this study, it is not possible to state whether the protein abnormality results from an increase of normal gamma globulins or from the appearance of abnormal proteins.

The observation that in kala azar a precipitation occurs when serum is added to water (Sia water test), together with the finding of cryoglobulinemia(15,16) suggested that increased concentrations of macroglobulins might also be present. This was found to be so in our study. Analysis in the ultracentrifuge was not performed to verify this, since results with this technic are generally unsatisfactory when frozen sera are used. Moreover, gel diffusion and immuno-electrophoresis are equally satisfactory for the detection of elevated macroglobulins.

Summary. Study of sera from 5 patients with kala azar revealed an abnormality in the starch gel electrophoretic pattern not seen in other diseases associated with generalized hypergammaglobulinemia. This abnormality consisted of a definite protein concentration in the region of slow gamma globulin. An elevation of macroglobulins was also found.

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Thiocyanate Secretion in Sweat in Cystic Fibrosis.* (26341)

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Cystic fibrosis is a disease of various exocrine glands, particularly the pancreas, bronchial mucus glands, and sweat glands. Sodium and chloride concentrations in the sweat in cystic fibrosis are approximately 5 times as high as in normal individuals(1).

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