Paper electrophoresis of parotid saliva of the rat can provide patterns which can be of value in a semi-quantitative analysis of protein components.

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Site of Formation of the Group-Specific Component and Certain Other Serum Proteins.* (29101)

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Since the initial observations of Hirschfeld(1) on the group-specific component (Gc) considerable work has been performed to identify and characterize this a_2 globulin. The demonstrated polymorphism of this system has been shown to be under genetic control(2), and population studies have given additional information concerning the existence of uncommon genetic variants(3). Schultze(4) and Cleve and Bearn(5), have isolated and partially characterized, the group-specific components. Partial characterization of the two inherited components of this system was reported recently from this laboratory(6). However, there is still little information concerning the role of this protein in health or disease. In this study, an attempt has been made to determine the site of synthesis of the group-specific component. Using the techniques elaborated by Hochwald, Thorbecke, and Asofsky(7) various human tissues were cultured *in vitro* in the presence of radioactive amino acids. The incorporated label was studied in newly synthesized serum proteins by autoradiography following immunoelectrophoresis.

Materials and methods. Biopsy specimens of normal human liver, spleen, lymph node, stomach, jejunum, pancreas, skeletal muscle, were obtained at time of surgery and placed in the culture medium (T. C. No. 199 with added bicarbonate—Microbiological Associates). Fifty mg wet weight of each tissue was cut into small fragments under sterile conditions and placed in roller tubes containing 2 ml reinforced Eagle's culture medium deficient in either lysine or isoleucine(8). The pH of the medium had previously been adjusted following addition of 2-5 μc/ml of either C^{14} lysine or C^{14} isoleucine (Schwartz 1000 μc/mg) to the tube lacking that particular essential amino acid. The tubes were then placed either on a shaker platform or in a roller tube apparatus at 37°C for 24-48 hours, at which time the tubes were frozen quickly, thawed, and centrifuged at 4° to remove sediment. The supernatant was dialyzed against 0.9% NaCl at 4°C for 48 hours, using Visking tubing of 8/32 diameter

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TABLE I.

Human tissue	Serum proteins incorporating C^{14} isoleucine
Liver	albumin α_1 globulin group-specific component ceruloplasmin α_2 macroglobulin transferrin haptoglobin
Lymph node	α_2 globulin transferrin
Spleen	α_1 globulin
Pancreas	α_1 globulin transferrin
Skeletal muscle	α_2 macroglobulin
Jejunum	none
Stomach	"

(Visking Co., Chicago, Ill.). The material was concentrated by burying the dialysis sack in Sephadex G-25 (Pharmacia, Uppsala).

Immunoelectrophoresis was carried out according to the method of Scheidegger(9) as modified by Hirschfeld(10) using as a carrier 1 μl human serum of phenotype Gc 1-1 or Gc 2-2. Concentrated tissue culture supernatant (6-12 μl) was added to the antigen well and electrophoresis carried out for 120 minutes prior to addition of anti-human serum (Pasteur Institute No. 306). Specific anti-α_2-macroglobulin serum (Behringwerke AG) was also used for identification purposes.

In several experiments highly purified Gc prepared in this laboratory was used as a carrier and added to the supernatant. After a 24 hour diffusion period the slides were washed for 12 hours in 0.9% NaCl and dried at 38°C prior to staining with azocarmine. Identification of the precipitation arcs was carried out by the use of specific staining methods, specific antisera, and comparison with purified proteins(11).

The dried slides were exposed to Kodak Royal Pan film sheets and placed at 4°C for from 2 to 8 weeks. The autoradiographs were developed after varying exposure periods. The time required for the appearance of adequate autoradiographs depended upon the amount of added radioactive label, the final volume of culture medium supernatant, and the volume of culture supernatant applied for immunoelectrophoresis.

Results. Table I contains a summary of the various serum proteins that incorporated C^{14} isoleucine during the incubation period. Liver, spleen, and lymph node tissues were twice cultured with this labeled amino acid, and the other listed material once. Recent work in this laboratory on the amino acid composition of purified Gc has revealed a content of 5 times as many lysine as isoleucine residues(6), thus, a third set of experiments was carried out using C^{14} lysine in a lysine deficient medium. In addition to the serum proteins listed in Table I an α_1 globulin, an α_2 globulin (both unidentified) and 19S gamma globulin were noted in autoradiographs from liver tissue cultures.

Fig. 1 shows the immunoelectrophoresis of a sample of serum of phenotype Gc 1-1 and purified Gc 1-1 both serving as a carrier for C^{14} isoleucine liver culture medium supernatant. The drawing of the autoradiograph of this slide is illustrated below and indicated radioactivity in precipitation arcs corresponding to an unidentified α_1 protein, Gc 1-1, two α_2 proteins identified as ceruloplasmin and haptoglobin, and transferrin. The precipitation arc of the isolated Gc showed marked radioactivity. Synthesis of this serum protein was not demonstrated in any of the other organs studied.

The presence of C^{14} labeled α_2 macroglobulin in skeletal muscle culture supernatant was an unexpected finding which was confirmed using specific antiserum (Fig. 2).

Discussion. The results obtained with other serum proteins agree well with the findings of Hochwald, Thorbecke and Asofsky(7), who also were able to demonstrate the formation of albumin, α_1 globulin, ceruloplasmin, α_2 macroglobulin, transferrin and haptoglobin in cultured human tissue. Synthesis of an α_2 globulin and transferrin in lymph nodes, and an α_1 globulin by spleen tissue was also noted by these workers. The finding that trace amounts of transferrin and α_1 globulin were formed in pancreatic tissue was previously undescribed and cannot easily be explained; it is possible that lymphoid elements in this tissue are responsible for its formation.

The apparent formation of α_2 macroglobulin

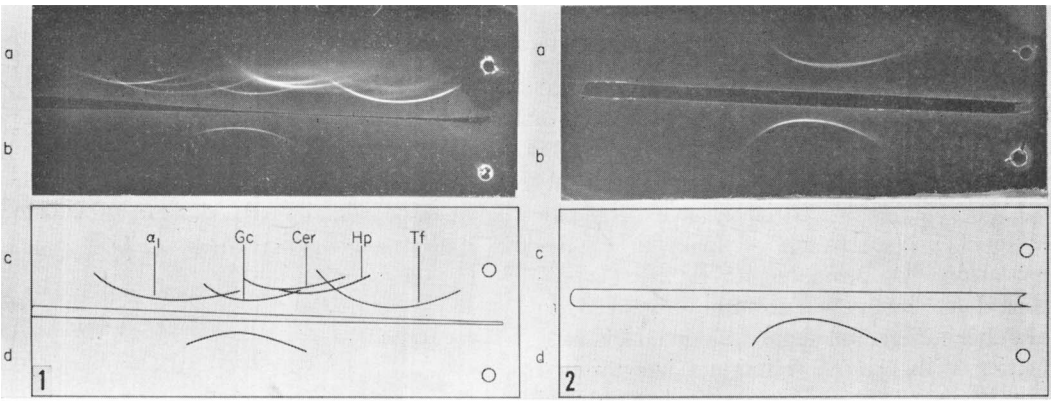

FIG. 1. Immunoelectrophoresis of normal human Gc 1-1 serum (a) and purified Gc 1-1 (b) with 6 μl concentrated liver culture media supernatant added to each antigen well. The pattern was developed with antihuman horse serum. Corresponding autoradiograph developed 6 weeks later showed radioactivity in total serum (c) corresponding to a α_1 globulin, Gc 1-1 (Gc), ceruloplasmin (Cer), haptoglobin (Hp), and transferrin (Tf). Gc 1-1 is identified in lower pattern (d).

FIG. 2. Immunoelectrophoretic pattern of normal human serum (a) and same serum as carrier with added skeletal muscle culture supernatant, (b) using specific α_2 macroglobulin antiserum. Autoradiograph of slide developed after 14 days demonstrated C^{14} isoleucine radioactivity in the precipitation arc of α_2 macroglobulin.

in cultured muscle was noteworthy and requires further investigation. It is possible that the radioactivity associated with the α_2 macroglobulin indicates that the α_2 macroglobulin may bind a protein synthesized by the muscle tissue, rather than evidence that the α_2 macroglobulin can be synthesized by muscle itself. Indeed it seems possible that additional studies will show that the α_2 macroglobulin can bind and transport a variety of proteins synthesized in different tissues.

Studies on the site of formation of the Gc serum protein were unambiguous. In 3 separate experiments a clearly demonstrable precipitation arc corresponding to Gc was observed in cultures of liver tissue. In those experiments in which other tissues were cultured, no Gc precipitation arc could be demonstrated.

Summary. In vitro studies using C^{14} isoleucine and C^{14} lysine indicate that the liver is the site of synthesis of the group-specific component. The possibility that the α_2 macroglobulin may bind proteins synthesized in various tissues is discussed. Synthesis of certain additional serum proteins in other human tissues cultured *in vitro* also has been demonstrated.

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