provoke hypertension. Renal artery clamping induces a rise in blood pressure without causing changes in renin content nor JGI, which remain normal. Combination of these 2 experimental procedures is followed by hypertension and a decrease in renin content and JGI. The width of the adrenal zona glomerulosa is reduced under the influence of cortexone and salt, and remains unaffected by renal artery constriction. In the rabbit, therefore, mineralocorticoids seem to play no major role in induction of renal hypertension. Neither does renin appear as a necessary agent in the mechanism responsible for the development of renal hypertension.

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Received October 21, 1963. P.S.E.B.M., 1964, v115.

Some Physical-Chemical Variables Affecting Insulin Migration *in vitro* I. Electrophoresis.* (28819)

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Berson and Yalow(1), using filter paper electrophoresis, have reported that tracer amounts of insulin-I^{131}, alone or added to serum from control subjects (patients never having received insulin), were quantitatively bound to Whatman 3 mm paper at the site of application. However, if the paper was soaked in an insulin-containing solution prior to electrophoresis to saturate the insulin-binding sites on the paper, the radioactivity migrated in the inter alpha-1-albumin area whether or not plasma was present. By contrast, radioactivity in insulin-treated subjects migrated in a zone between the beta and gamma globulins.

It was later reported by Randle and Taylor(2) and Antoniades *et al*(3) that "free" insulin in serum or plasma migrated in this inter alpha-1-albumin zone.

Using similar *in vitro* electrophoretic techniques, we have previously shown that tris (hydroxymethyl) aminomethane (TRIS) buffer was able to release insulin bound to beta-gamma globulins in the sera of treated and insulin-resistant diabetics(4).

To the best knowledge of the authors insulin electrophoresis has not been performed on cellulose acetate, a supporting media introduced by Kohn(5). This material is homogeneous, microporous, and contains extremely low concentrations of heavy metals. In addition the carboxyl groups are acetylated.

Because of these characteristics, it was felt that the use of this paper would minimize insulin-paper interaction. We, therefore, elected to extend our studies and compare

* This work supported in part by a grant from John A. Hartford Foundation, New York, N. Y. and by U.S.P.H.S. grants.

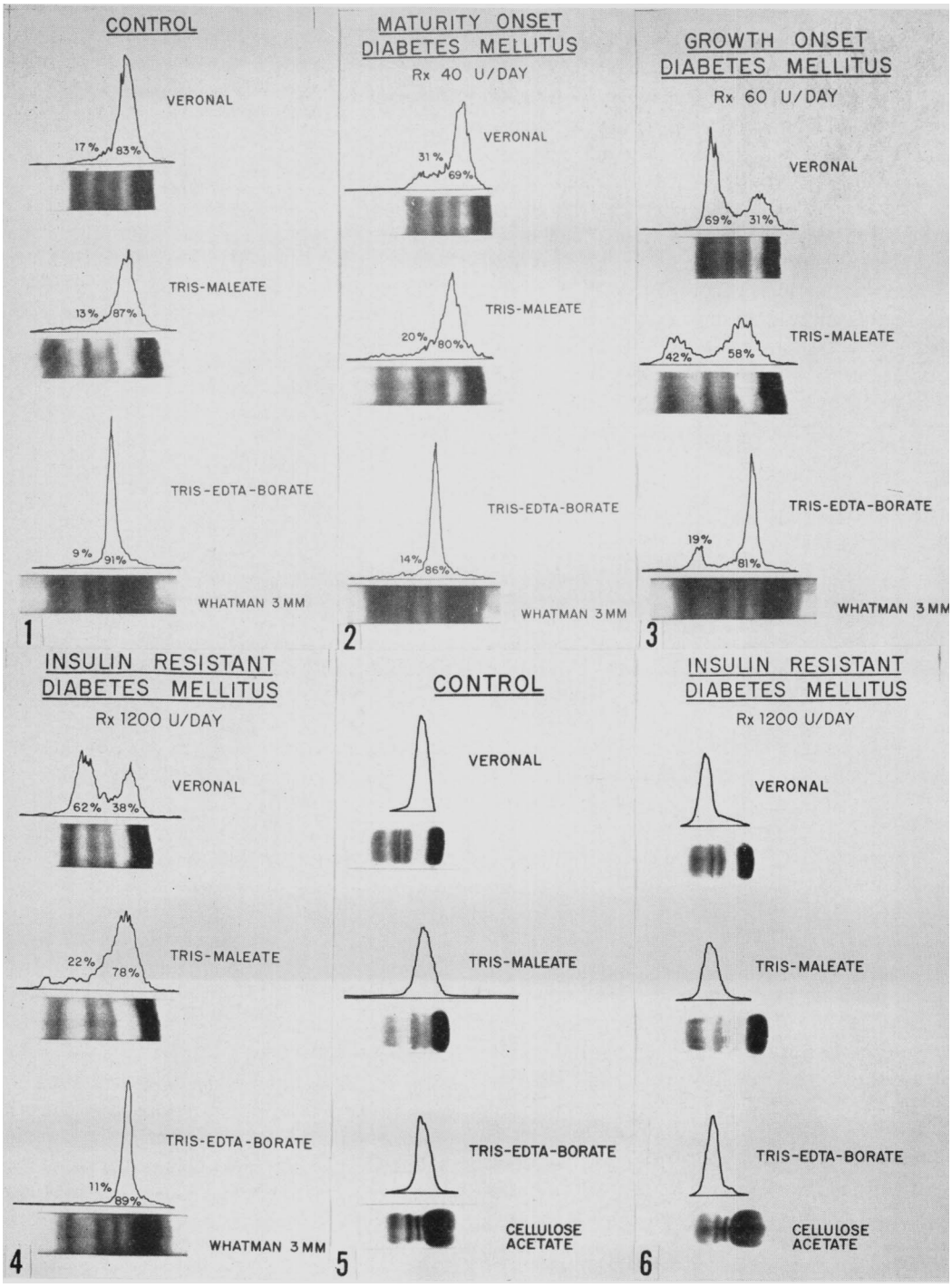

FIG. 1. Control serum equilibrated with insulin-I^{131} demonstrates major localization of activity in the alpha-1-albumin interzone in all buffer systems.

FIG. 2. Serum from insulin treated maturity onset diabetic demonstrates 31% of activity in beta-gamma globulin region in veronal buffer; 20% in TRIS-maleate buffer, and only 14% in TRIS-EDTA-borate buffer.

FIG. 3. Serum from insulin treated growth onset diabetic demonstrates 69% of activity

in beta-gamma globulin region in veronal buffer; 42% in TRIS-maleate buffer, and only 19% in TRIS-EDTA-borate buffer.

FIG. 4. Serum from insulin resistant diabetic demonstrates 62% of activity in beta-gamma globulin region in veronal buffer; 22% in TRIS-maleate buffer, and only 11% in TRIS-EDTA-borate buffer.

FIG. 5. Control serum equilibrated with insulin-I^{131} demonstrates major localization of activity in the alpha-1-albumin interzone in all buffer systems. Note similarity of cellulose acetate to Whatman 3 mm.

FIG. 6. Serum from insulin resistant diabetic demonstrates major localization of activity in the beta-gamma globulin interzone in all buffer systems. Note the marked difference between cellulose acetate and Whatman 3 mm.

our results on Whatman 3 mm paper to cellulose acetate and to relate the migration and distribution of radioinsulin to both the papers and the buffer systems employed.

Methods and materials. Serum samples used in this study were obtained in the fasting state from patients at the Joslin Clinic, New England Deaconess Hospital, and Peter Bent Brigham Hospital, Boston, Mass., and were classified into 4 categories: (a) controls (patients who had never received insulin, who had no family history of diabetes mellitus, and who demonstrated normal oral and intravenous glucose tolerance); (b) insulin-treated, growth-onset diabetics; (c) insulin-treated, maturity-onset diabetics; and (d) insulin-resistant diabetics (patients with insulin requirements greater than 200 units per day). Serum was studied from 6 patients in each category.

Iodinated† crystalline beef insulin was resuspended in 1% human serum albumin with a final specific activity of either 4-7 or 20-30 millicuries per milligram insulin. For filter paper electrophoresis, 2.0 microcuries of the iodinated insulin was added to one milliliter of serum, following which the mixture was shaken and allowed to remain at 4°C for periods of 30 minutes to 4 days.‡ Twenty microliters of this solution were applied to Whatman 3 mm paper strips, 3.0 × 30.6 cm, previously saturated in a solution of distilled water containing 160 units of crystalline zinc insulin§ per 100 ml. Electrophoresis was performed in a Spinco-Durrum cell at room temperature using a constant current of one milliampere per strip for a period of 16 to 18 hours. Immediately following electrophoresis, the strips were dried at 120°C for 30 minutes. I^{131} distribution was assayed by both radioautography and by scanning dried paper with a Nuclear-Chicago Actigraph II Counter prior to protein staining with aqueous bromphenol blue. Protein distribution was determined visually or with a Beckman model RA Analytrol employing a B-1 balancing cam and two 500 millimicron interference filters in the front and rear filter holder.

For cellulose acetate electrophoresis, 8.0 μc of the iodinated insulin were added to one milliliter of serum and incubated as described for filter paper electrophoresis. Five microliters of this solution were applied to oxoid‖ cellulose acetate strips 2.5 × 12 cm previously saturated in buffer solution without added insulin and blotted. Electrophoresis was performed in a Shandon‖ electrophoresis cell at room temperature, using a constant current of one milliampere per strip for 3 hours. Immediately following electrophoresis, the strips were dried at 80°C for 10 minutes. I^{131} distribution was assayed as described for filter paper electrophoresis prior to protein staining with 0.2% Ponceau S in 3% trichloroacetic acid. Protein distribution was determined visually.

Thyroxine-I^{131} employed in comparing labeled thyroxine and labeled insulin distribution in this study was provided through the courtesy of Dr. Sidney Ingbar.

Results and discussion. Results obtained using thyroxine-I^{131} were identical to those previously reported(4) confirming the data of Ingbar and Freinkel(6).

Representative results obtained with insulin-I^{131} on Whatman 3 mm paper presaturated with insulin are shown in Fig. 1-4.

† Abbott Laboratories, North Chicago, Ill.

‡ We have not observed any difference in distribution of radioinsulin following electrophoresis during this time interval.

§ E. R. Squibb and Sons, New York.

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

Both insulin-I^{131} alone and insulin-I^{131} added to normal serum migrated in a zone between the alpha-1-globulins and albumin in all buffer systems employed (Fig. 1).

The distribution of radioactivity in the sera of the insulin-treated diabetics (Fig. 2, 3, 4) differed significantly from the controls. In veronal buffer (pH 8.6, ionic strength 0.075) an appreciable amount of the radioactivity was seen to migrate between the beta and gamma globulins in the sera of the maturity-onset diabetics. Sera from the growth-onset and insulin-resistant diabetics showed almost complete binding of the radioinsulin in this area.

In a TRIS-maleate system, (pH 8.6, 0.075 molar TRIS) on the other hand, a significant portion of the label in the sera of the growth-onset diabetics, which migrated in the beta-gamma globulin interzone in veronal buffer, was observed to migrate in the alpha-1-albumin interzone. Qualitatively similar observations were made in the sera from maturity-onset and insulin-resistant diabetics, and it is to be noted that there was a quantitative increase in the shift of the distribution of radioinsulin from the beta-gamma globulin interzone to the alpha-1-albumin interzone in the TRIS-maleate system as compared to the veronal system.

Furthermore, in studies with the TRIS-EDTA-borate buffer of Aronsson and Grönwall(7) (pH 8.9, 0.50 molar TRIS) it was observed that greater than 90% of the radioactivity in the sera of the growth-onset diabetics, which migrated in the beta-gamma globulin interzone in veronal buffer, was now found to be associated with the alpha-1-albumin fraction. The radioinsulin distribution in the sera from both maturity-onset and insulin-resistant diabetics employing the Aronsson and Grönwall buffer were identical to those obtained in the TRIS-maleate system.

When cellulose acetate was used as the supporting medium for zone electrophoresis, both insulin-I^{131} alone and insulin-I^{131} added to normal serum, migrated in a zone between the alpha-1-globulins and albumin in all buffer systems employed (Fig. 5).

In veronal buffer, sera from maturity-onset, growth-onset and insulin resistant diabetics showed distribution of radioinsulin similar to that observed on Whatman 3 mm paper; however, in distinct contrast, there was no alteration in distribution of insulin-I^{131} in these sera on cellulose acetate in either the TRIS-maleate or TRIS-EDTA-borate buffer systems (Fig. 6).

Summary and conclusions. In confirmation of our previous studies(4), it is again noted that TRIS in this *in vitro* system does not function as an inert buffer, and that physical-chemical interaction with insulin does occur on Whatman 3 mm paper. The degree of interaction is greater in the buffer system containing the greater concentration of TRIS. The beta-gamma globulin binding of radioinsulin in the growth-onset diabetics is not as completely dissociated by TRIS as that observed in the maturity-onset or insulin-resistant diabetic patients. This observation suggests that insulin is more tightly bound to the beta-gamma globulins appearing in the serum of growth-onset diabetics as compared to more freely dissociated binding by the beta-gamma globulins of the maturity-onset or insulin-resistant diabetic patients in this study. An alternative suggestion is that a difference in the nature of the chemical bond which is dissociated by the TRIS occurs in the sera of growth-onset diabetics. Since displacement of radioinsulin from beta-gamma globulin binding sites by TRIS was evident on Whatman 3 mm paper, but not on cellulose acetate, we conclude that there was a network interaction between the radioinsulin, TRIS, and the Whatman 3 mm supporting medium, despite apparent saturation of insulin binding sites with carrier insulin. We wish to present this as a physical-chemical observation in this particular *in vitro* system, and we feel that it would be unwise to attempt to draw physiological conclusions from this data.

The authors wish to express their gratitude to Dr. J. Lawrence Oncley, Professor of Biological Chemistry, Harvard Med. School for many stimulating discussions and expert advice generously given. Without his help much of this work could not have been completed. We are indebted to him for his excellent criticism of this manuscript.

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Received February 19, 1963. P.S.E.B.M., 1964, v115.

Studies on Activation of Intracellular Catalase of Mouse Liver. (28820)

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Recent investigations have indicated that catalase is probably localized within a specific type of particle associated with the mitochondrial fraction. For example, uricase and catalase were found to migrate together during sucrose density gradient centrifugation for 2 hours(1). DeDuve and co-workers(2) also reported that the uricase-containing particle could be separated from lysosomes by means of glycogen equilibration. Catalase activity was found to be associated with the former particle. Furthermore, different particle sites for acid phosphatase and uricase were found after a suspension of the mitochondrial fraction was subjected to sucrose density gradient equilibration(3). Thus catalase was thought to be localized within the so-called uricase particle which differs from both the large mitochondria, site of a variety of metabolic functions including oxidative phosphorylation, and the lysosomes which contain acid phosphatase and other hydrolytic enzymes(4).

The possibility that the particle containing catalase may be surrounded by a membrane through which leakage of this soluble enzyme may occur is indicated by its ease of redistribution following homogenization and isolation of the various subcellular fractions(5,6). The rate-limiting role of this membrane was demonstrated not only by variations in catalase activity of the mitochondrial fraction produced by differences in homogenization, isolation and resuspension procedures but by treatment with detergents (7,8).

Investigations of the behavior of catalase of the mitochondrial fraction* under various conditions of sucrose isolation and suspension were studied and the effect of deoxycholate, phosphate buffer at pH 2 and 1.25 M sucrose on the catalase activity of several cytoplasmic fractions were carried out.

Methods. Preparation of cytoplasmic fractions. Liver from male mice of the IBAH strain was homogenized in 10 volumes of 0.25 M sucrose. The homogenization consisted of 3 strokes at 4500 rpm in a Potter-Elvehjem type homogenizer. The homogenization was carried out over a time interval of 20 to 25 seconds. The homogenates were utilized for isolation of mitochondrial and cell sap fractions according to a procedure previously reported(9).

The mitochondrial and cell sap fractions were also isolated from 0.88 M sucrose. In this case, the liver was again homogenized in 10 volumes of the medium followed by centrifugation at 14,000 $\times$ g for 15 minutes. The supernatant fraction was centrifuged at 21,000 $\times$ g for 30 minutes in rotor #40 (Spinco model L) in order to obtain the mitochondrial fraction.

* The term mitochondria as used in this discussion refers to the mitochondrial fraction as isolated by differential centrifugation from 0.25 M sucrose homogenates. This fraction includes the light and heavy mitochondria as well as lysosomes and uricase particles.