nously into pregnant hamsters on the 8th day of gestation at levels of 10 mg/kg produces a fetal mortality of 50%. Gross congenital malformations, consisting of microphthalmia, anophthalmia, umbilical hernia, exencephaly, and skeletal anomalies are found in a significant number of survivors.

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## Insulin Binding Proteins in Human Serum. *In vitro* Experiments. (28168)

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Quantitative determination by means of the rat diaphragm or fat pad technique of insulin-like activity of human serum proteins indicates the insulin-like activity to be localized in the $\alpha$- and in the $\gamma$-areas of preparative starch-block electrophoresis(1). The insulin-like activity can be localized by euglobulin precipitation in the precipitate; thus most probably a linkage exists between insulin and one or more serum proteins. Data given below which support this view can be obtained by means of immuno-electrophoresis. This method, when combined with autoradiography, can reveal protein binding of drugs, vitamins and hormones(2,3).

*Material and methods.* Immuno-electrophoresis was performed as described by Scheidegger(4). Autoradiography was made as described by Clausen & Munkner(2,3). Crystalline human insulin, labeled with $J^{125}$ was produced by iodination in an acid medium(5). No significant amounts of radioactive impurities were present in the labeled insulin since: 1) when mixed with carrier pig insulin and subjected to starch-block electrophoresis the protein corresponded to the radioactivity pattern; 2) when mixed with carrier human insulin and subjected to recrystallizations the specific radioactivity remained constant.

The labeled insulin-$J^{125}$ had after recrystallization a specific activity of about 0.6 mc/mg. One milliliter of normal human serum was mixed with about 10,000 micro units of insulin-$J^{125}$. The mixture was left for 4 days either at 4°C or at 22°C. Afterwards the serum was kept deep frozen until use. A similar experiment, performed with another batch with specific activity of about 8 mc/mg (but less purified through the preparation and hence containing considerable amounts of radioactive impurities), was carried out by adding 1000 $\mu$ units/ml serum.

Immuno-electrophoresis was performed in veronal buffer (pH = 8.6, ionic strength $\mu$ = 0.05). The immuno-electrophoreses were developed with a horse antiserum against normal pooled human serum from the Pasteur Institute, Paris, a goat antiserum against normal pooled human serum described earlier (6) and finally with specific antisera against $\alpha$-2- and $\beta$-2-macroglobulin from Behring-

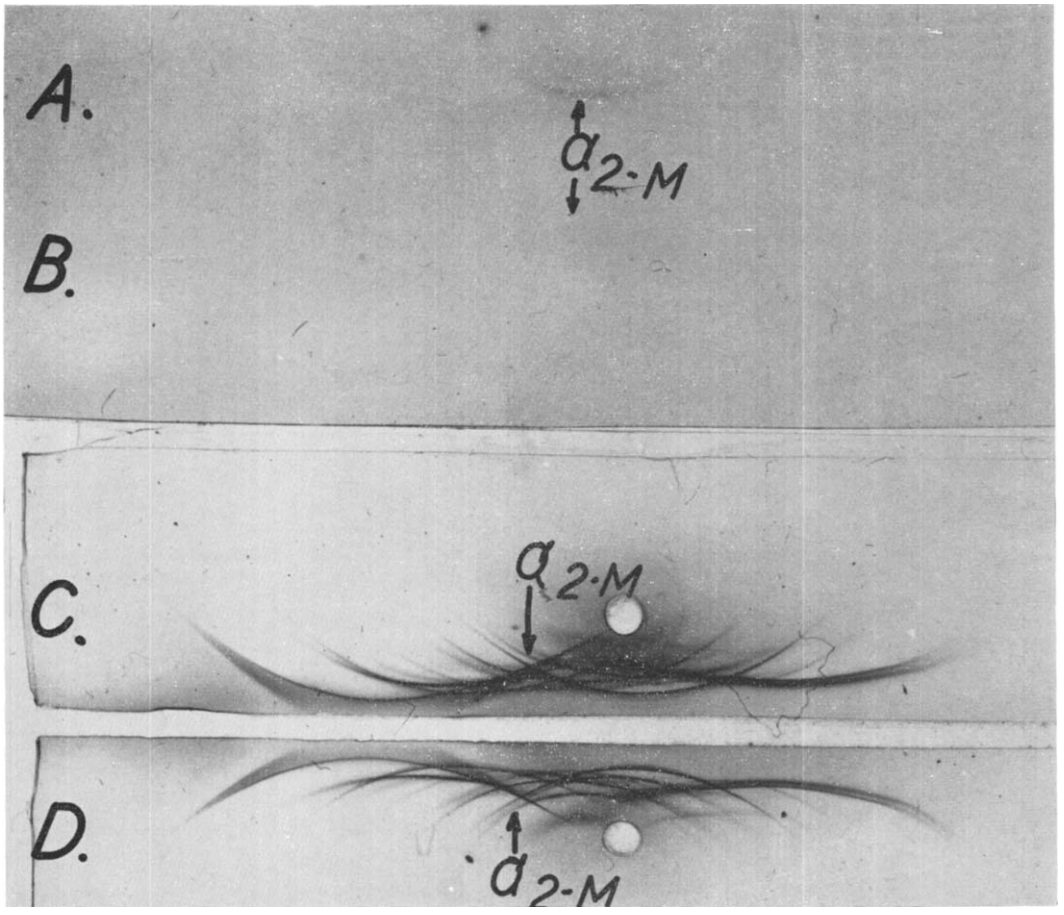


FIG. 1. A. and B. Autoradiography of C. and D. C. and D. Immuno-electrophoresis of normal human serum incubated (4°C) with 10,000 micro units insulin-$J^{125}$/ml (specific activity 0.6 mc/mg) and of control untreated serum respectively. The immuno-electrophoresis is developed with a horse antiserum against normal, pooled human serum. After the immuno-diffusion the immuno-electrophoresis was washed with saline for 2 days to remove non-bound insulin and non-reacting antigen and antibody. Radioactivity is localized to the α-2-M-globulin precipitation line.

werke, Marburg, Germany.* Autoradiography was performed on Kodak-X-ray film, which on the opposite side was in contact with a 100 μ thick goldlayer on a brass plate. This arrangement increased the blackening.

*Results.* Fig. 1 and 2 demonstrate that α-2-macroglobulin must bind insulin, because the corresponding precipitation line in the immuno-electrophoresis is radioactive. Furthermore, in sera incubated at 22°C, 3 experiments revealed both the α-2-M- and the β-2-M (γ-1-M)-globulin to bind insulin. Similar results were obtained with the impure radioactive insulin of high specific activity.

*Discussion.* Originally Berson *et al.*(7) showed that free insulin does not migrate on paper-electrophoresis, and claimed that radioactivity, sometimes localized to the α-area, originated from altered insulin. However, *in vivo* experiments indicate the turnover of labeled insulin to be different from that of radioactivity precipitable with trichloracetic acid(8). The data presented here support the view that insulin can occur in serum bound to serum proteins. This view is also supported by electrophoresis on ion-exchange-

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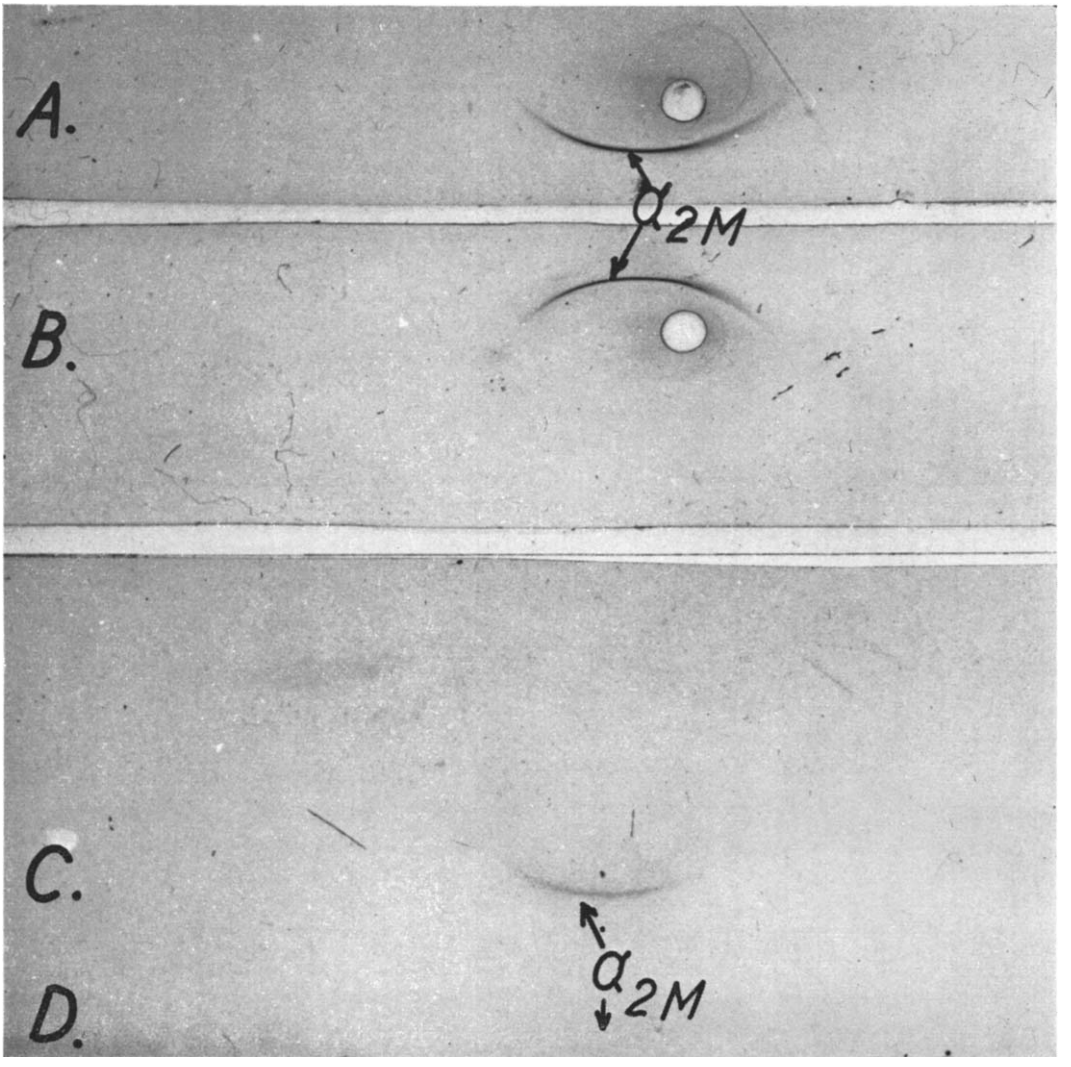


FIG. 2. A. and B. Immuno-electrophoresis of the same serum as used in Fig. 1. Immuno-electrophoresis was developed with specific rabbit antiserum against human α-2-macroglobulin. C. and D. Autoradiography of A. and B. It is seen that the α-2-macroglobulin precipitation line is radioactive.

paper, which suggests the insulin-protein complex to migrate as an $\alpha$- or $\beta$-globulin(9). Thus the electrophoretic medium may be of significance for demonstration of a protein-insulin complex in serum. Agar-gel electrophoresis gives free migration conditions for proteins *i.e.*, no interaction with proteins, when these have a molecular weight below 200,000(10). For proteins with a molecular weight above this value, frictional forces between the agar-micell and the migrating molecules exist, but the magnitude of resistance depends on the shape of the molecule. Thus globular-shaped proteins have lower friction than spindle-shaped proteins. On the other hand paper-electrophoresis does not give free migration conditions, as strong binding reactions occur between paper and proteins. Furthermore, immuno-electrophoresis in agar-gel after the diffusion period, in which the antigen-antibody complex is established, is followed by a washing procedure, by which non-reacting antigen and antibody are washed out by standing in saline for 2 days. This procedure also causes the free insulin to be removed. Thus only bound insulin remains in the agar-gel.

On the basis of these circumstances, it was found in the present work that α-2-macroglobulin always binds insulin, when the insulin is incubated in serum at 4°C or at 22°C. Moreover the β-2-M-globulin probably can bind insulin, when the incubation occurs at 22°C.

*Summary.* Immuno-electrophoresis combined with autoradiography revealed insulin-$J^{125}$ by *in vitro* experiments to be bound to α-2-macroglobulin at 4° and 22°C and to β-2-M (γ-1-M) at 22°C.

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## Strain Differences in Seizure Responses and Brain Cholinesterase Activity in Rats.* (28169)

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Strain and sex differences in electroshock and picrotoxin seizure responses in 2 strains of rats have been reported(1,2). Greater brain excitability, as shown by lower seizure thresholds, was observed in both males and females of one ($S_1$) strain than in males and females of another ($S_3$) strain. Also, within the same strain, females demonstrated greater convulsability than males. Because the $S_1$ strain has higher brain acetylcholine (ACh) concentration and cholinesterase (ChE) activity than the $S_3$, it was suggested that strain differences in convulsability might be related to differences in either ACh concentration or ChE activity or both(1). To explore further the relation between convulsability and brain ACh-ChE levels, electroshock seizures were measured in additional strains of rats specifically bred for differences in cortical ChE(3) and also characterized by differences in brain ACh(4). Seizure responses were correlated with brain ACh and ChE in males only, as the latter data were not available for females. However, because of the strong central excitatory action of estradiol in rats(5,6), seizures were also measured in females to determine if sex, as well as strain, differences could be detected. Objectives of this investigation then were to compare electroshock convulsions in 6 strains (3 pairs of strains) of rats, to determine if sex differences are present, and to try to correlate convulsive responses with corresponding values of brain ACh-ChE.

*Methods. Animals.* Six strains of rats—$S_1$, $S_3$, RCH, RCL, RDH, and RDL—known to differ in brain ChE activity and ACh concentration were used. The first 2 strains are descendants of Tryon's maze-bright ($S_1$) and maze-dull ($S_3$) rats(7). The remaining strains were selectively bred by Roderick

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