by mixing 1 ml of serum with 0.5 ml of a 6-percent dextran solution. All the unmixed as well as the mixed sera were run in duplicate on filter paper strip electrophoresis and stained for lipids. The third filter paper strip was run and stained for proteins. The electrophoresis was performed on filter paper (Whatman No. 1) in veronal-acetate or in phosphate buffer at *p*H 8.6, ionic strength 0.1, in a potential gradient of 3 to 4 volt/cm for 18 hours. The lipid patterns were stained with Sudan Black (*3*) and the protein patterns with Amidoblack (*4*). The densitometric scannings were performed on an Elphor densitometer.

All lipidograms of the human sera mixed with different molecular weights of dextran in colloidal solution without exception showed the dextran-lipid fraction. It is evident from the lipidograms and their densitometric scannings shown in Fig. 1 that the increase of the molecular weight of the dextran added increases the intensity of the dextran-lipid fraction, and at the same time decreases the intensities of the lipids migrating with the beta globulins. With dextran of molecular weight 500.000, the latter lipid fraction disappears almost completely. The intensities of the A-lipoprotein fraction, as well as of the neutral fats adsorbed at the starting point (NF), seems unchanged by colloidal solutions of dextran of different molecular weights in vitro.

The use of approximately equimolar concentrations of the colloidal solutions of dextrans with increasing molecular weights was omitted in these experiments, because the choice of a concentration of a dextran solution which shows just a faint effect of the formation of the dextran-lipid fraction would result, in the experiment with the next higher molecular weight of dextran, in complete disappearance of the beta-lipoprotein fraction and maximal formation of the dextran-lipid fraction.

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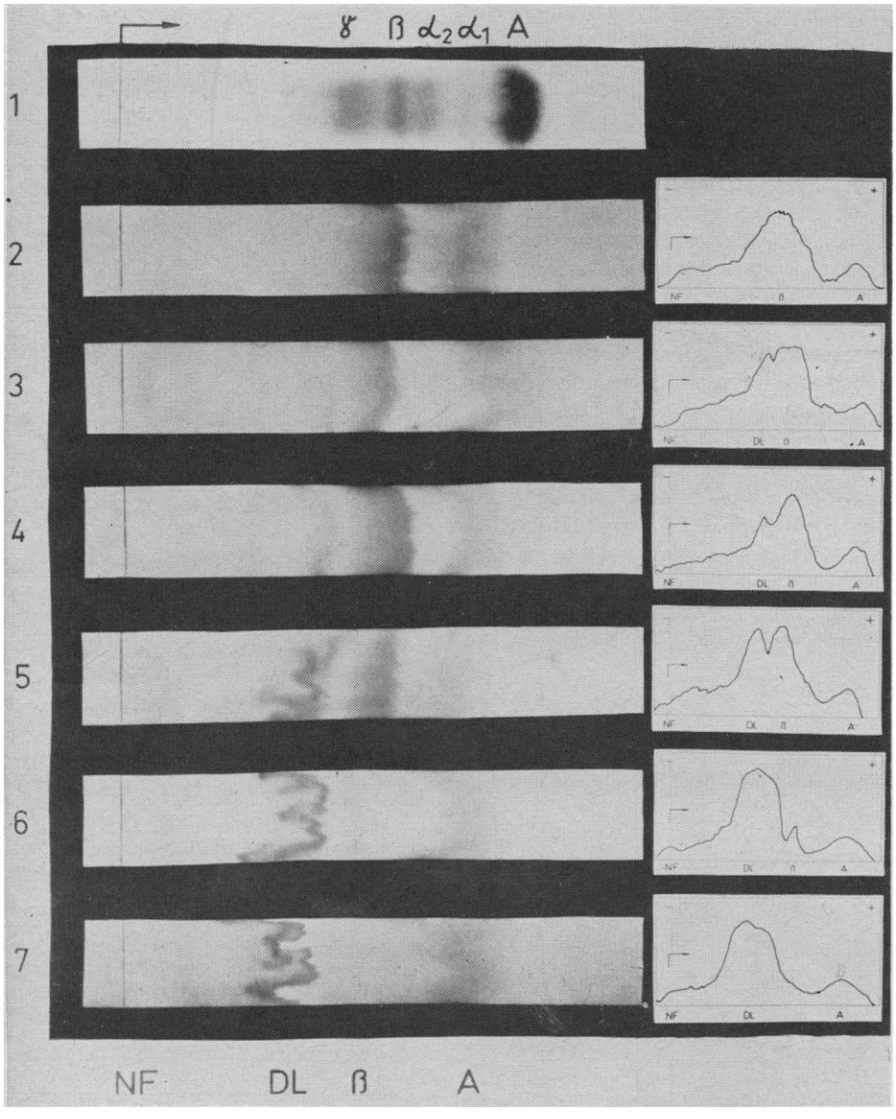

Fig. 1. Paper strip electrophoresis of human serum and mixtures of 1 ml of serum and 0.5 ml of a 6-percent colloidal solution of dextrans with increasing molecular weights, and densitometric scannings of the lipidograms. 1, Serum unmixed, protein staining. 2, Serum unmixed, lipid staining. 3–7, Mixtures of serum and dextran (Molecular weights of dextran: 3, 10.000; 4, 40.000; 5, 80.000; 6, 150.000; and 7, 500.000). Note the appearance of the dextran-lipid fraction (DL-fraction) on the lipidograms and densitometric scannings.

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14 February 1961

Myelin-Binding Antibodies in Experimental "Allergic" Encephalomyelitis

Abstract. Antibodies with a specificity for myelin, as demonstrated by immunofluorescence, are present in the sera of rabbits injected with spinal cord. These antibodies react with both adult rabbit and human spinal cord but not with unmyelinated areas of neonatal human spinal cord.

Complement-fixing antibodies are known to be present in the sera of animals with experimental "allergic" encephalomyelitis (EAE) (*1*). Moreover, Bornstein and Appel (*2*) have recently shown that the sera of rabbits with "allergic" encephalomyelitis are capable of producing demyelination of rat cerebellum in tissue culture. The latter finding prompted us to study the sera of such animals for the presence of myelin-binding factors. Fluorescent-antibody studies by Beutner *et al.* (*3*) suggested that the myelin sheath may be one of the antibody combining sites.

"Allergic" encephalomyelitis was induced in seven of a total of ten rabbits

by the injection of an emulsion consisting of one part rabbit spinal cord and two parts Freund's adjuvant. A single 0.4-ml dose was given intradermally, divided among the four foot pads. The affected rabbits developed severe paresis of the hind limbs, incontinence, and ataxia between the 16th and 18th days and were bled and killed at that time. Histological examination revealed the characteristic lesions of "allergic" encephalomyelitis in all the affected animals. The three unresponsive rabbits were bled at 21 days and kept under observation.

Control rabbits received single injections of adjuvant alone (two rabbits) or of adjuvant emulsified with other materials, such as bovine serum albumin (two rabbits) or rabbit liver (four rabbits). An additional two rabbits were injected with a suspension of 10 percent spinal cord in saline without adjuvant. The latter received 0.4 ml intradermally three times a week for 5 weeks. None of the rabbits in any of these groups exhibited clinical evidence of disease, and all were bled 21 days after the last injection. All sera were absorbed with rabbit erythrocytes taken from three normal rabbits which subsequently served as the source of normal tissue. All tissues were frozen in liquid nitrogen and kept at −20°C. The globulin fractions of the rabbit sera were separated by ammonium sulfate precipitation (50 percent saturation). Anti-rabbit gamma globulin was produced in chickens, and the globulin fraction of this antiserum was similarly prepared. The latter was then conjugated to fluorescein isothiocyanate (*3*). The conjugate was absorbed with rat liver powder prior to use.

Unfixed frozen sections of normal rabbit spinal cord washed in phosphate-buffered saline (*p*H 7.2) were incubated with either the absorbed whole EAE rabbit serum or its globulin fraction for 45 minutes at room temperature. They were then washed with buffered saline and incubated with fluorescein-conjugated anti-rabbit gamma globulin for an equal period of time. Following a final washing they were mounted in buffered glycerine and studied by means of a fluorescence microscope unit. Brilliant specific fluorescence was observed in the myelin sheaths (Fig. 1), which could be identified in both transverse and longitudinal sections by phase-contrast microscopy. The fluorescein-labeled anti-rabbit gamma globulin produced bright fluorescence even when the EAE sera were diluted 25-fold. The EAE serum reacted equally well with sections prepared from autologous spinal cord. Similar specific fluorescence was observed with the sera of the three unresponsive rabbits. Rabbits which received multiple injections of spinal cord alone exhibited fluorescence which was just perceptible in undiluted sera.

Human spinal cords were obtained from autopsies within 3 hours of death. When the sections were incubated with the EAE rabbit serum a similar pattern of fluorescence was observed. Transverse sections were prepared from the spinal cord of a 10-day-old infant in order to determine whether the EAE globulins would bind to areas of white matter which had not yet undergone myelination. These areas were demonstrated in an adjacent block, which was fixed in Formalin and stained with Luxol blue. Fluorescence was observed in regions such as the dorsal roots and, to a lesser extent, in the posterior columns, but not in the region of the pyramidal tract, which is known to myelinate at a later stage of development. The pattern

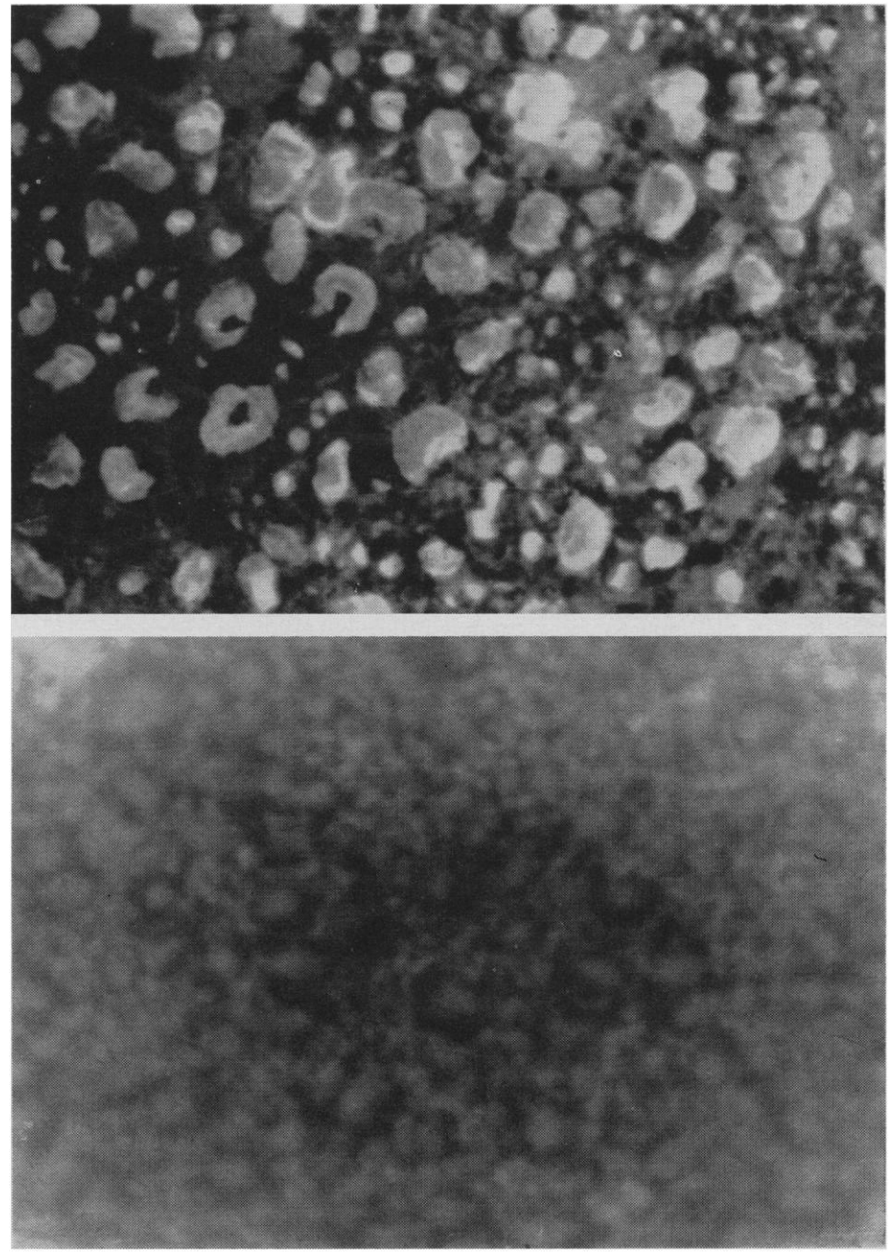

Fig. 1. (Top) Frozen transverse section of normal rabbit spinal cord incubated with EAE rabbit serum and washed and stained with fluorescein-labeled anti-rabbit gamma globulin. Note the fluorescence of the myelin sheaths. (Bottom) A control section incubated with normal rabbit serum. Note the absence of specific fluorescence (about × 275).

and degree of binding of the EAE globulins corresponded to the distribution of myelin in the sections stained with Luxol blue, thus suggesting that the presence of myelin is required for binding to occur.

In control studies, the sera of animals with "allergic" encephalomyelitis did not bind to sections of rabbit liver or kidney. Prior absorption of EAE sera with rabbit spinal cord removed the specific fluorescence, whereas similar treatment with rabbit liver had no effect. Specific fluorescence was not obtained with any of the sera from rabbits injected with liver or bovine serum albumin in combination with Freund's adjuvant or from rabbits injected with the adjuvant alone. Negative results were also obtained with the sera of rabbits with acute nephrotoxic nephritis and with the potent anti-rabbit-kidney serum (chicken) used to produce the nephritis. Fluorescein-labeled anti-chicken gamma globulin was used for detection of the latter.

There is considerable evidence, as summarized by Waksman (*4*), that the pathological lesions of "allergic" encephalomyelitis are a manifestation of delayed hypersensitivity and associated with cell-bound antibodies. However, it is likely that circulating antibodies may play some role in its pathogenesis. Bornstein (*2*) demonstrated in vitro a circulating toxic factor, and Paterson *et al.* (*5*) have recently presented evidence for a circulating factor in the serum of rats that have recovered from "allergic" encephalomyelitis which, when passively transferred, protects other rats against developing the disease. This factor may be an anti-brain antibody, with properties similar to the "enhancing" factor demonstrable in tissue-transplantation studies, which appears to protect the transplant against rejection by the host animal (*6*). The sera of our rabbits with experimental "allergic" encephalomyelitis possessed a factor capable of binding specifically to myelin. This serum factor exhibits all the properties of an organ-specific antibody. The localization of the antibody to myelin assumes added significance in view of the fact that the encephalitogenic antigen is considered to be a myelin constituent (*4, 7*).

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Effect of Estradiol on the Transfer Rate of Small Molecules into the Rat Uterus

Abstract. The uterus-to-serum distribution ratio has been studied for urea-C^{14} as a function of time, and for sucrose-C^{14} at an early time after injection of the substance into rats. For both substances, estrogen influences the rate of passage between plasma and uterus.

One mechanism proposed for the biological action of estrogens has been an effect on the permeability of the uterus. There has been little experimental work that bears directly on this theory, largely because of the many variables present when the target organ is studied in its natural environment. At least three physical changes occur in the rat uterus soon after estrogen treatment: there is an increase in water content (*1*); there appears to be an increase in capillary permeability (*2*); and there is an increase in blood-flow rate (*3*). In a recent paper (*4*), Halkerston *et al.* concluded that no changes in permeability of the uterus could be found 1½ hours after injection of estradiol into ovariectomized, nephrectomized, and adrenalectomized rats. Their measurement of "permeability" was the distribution ratio (tissue to plasma) of C^{14}-labeled materials (including sucrose-C^{14}) 90 minutes after the injection of the tracer. Since their work has been quoted in the recent literature (*5*), it seems worth while to re-examine the problem before the proposed effect on permeability is discarded.

The critical process is really the "flux" (*6*) of a substance across a biological barrier, and not the "steady-state" relation between levels in blood and tissue which is achieved some time after the tracer is injected. Since the small molecules used in this type of study enter the uterus very rapidly, it is almost impossible to obtain tissue levels that increase with time; the die-away curve in plasma is so steep that a downhill gradient may exist between tissue and plasma which favors loss of the tracer material from the tissue. Nevertheless, one can get tissue-to-plasma ratios quite early after the administration of a tracer substance and examine these as a function of time.

Ovariectomized Long Evans rats weighing 200 to 250 g were given 2 μg of estradiol in saline intravenously 1 hour before sacrifice; the controls received saline. Experimental and control animals were anesthetized with pentobarbital (50 mg/kg, injected intraperitoneally) and then given urea-C^{14} intravenously (3 to 5 μc) from 0 to 10 minutes before sacrifice. In one set of experiments rats received 3 to 5 μc of sucrose-C^{14} 3 minutes before sacrifice. At the time of sacrifice a blood sample was obtained by heart puncture, the aorta was severed, and the uterus was removed. The serum was collected from each blood sample, the serum proteins were precipitated with 75-percent ethanol, and a sample of the supernatant was saved for assay of radioactivity. After weighing, the uterus was placed into a vial containing 1 ml of distilled water, and the vial was stored overnight in the refrigerator. A sample of the water from this vial was used to assay for radioactivity in the uterus. (It was demonstrated that equilibrium between tissue and aqueous phase had occurred by homogenizing

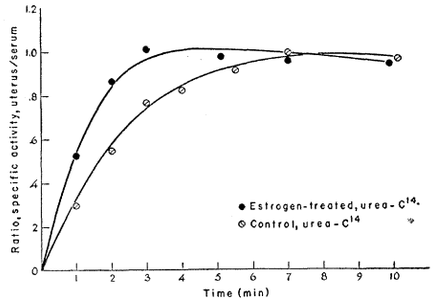

Fig. 1. Distribution ratios between uterus and serum for urea-C^{14} as a function of time after injection of tracer. Open circles, animals that received saline 1 hour before time of sacrifice; solid circles, animals that received estradiol 1 hour before time of sacrifice.