organisms was extended somewhat beyond the normal 36 hours, but that the subsequent course of infection, the morphology of the parasites, and the number of merozoites per schizont were not affected. My presently recorded observations extend this body of information by the additional fact that local cooling of the skin before, during and after the introduction of sporozoites by the mosquito does not prevent the initiation of the infection. Hence it appears inadmissable to ascribe the non-susceptibility of the albino mouse to this infection to the fact that the skin into which the mosquito introduces the sporozoites in this animal is several degrees cooler than that of the normal host. In his duck embryo *P. cathemerium* infections, an abnormal host-parasite situation, McGhee(5) found that the infection developed more slowly and never reached the peak at 30°C that it did when the incubation was at 37°C. This observation, however, seems non-contributory under the experimental conditions described here.

Summary. The temperature of the biting site for mosquitoes on the shaved belly of the albino mouse was found to be 3.5°C (6.3°F) lower than that of a comparable plucked area on the chick breast. When the temperature of the chick skin was maintained at or below that of the mouse skin during and for some time after potentially infecting bites of *A. aegypti* mosquitoes carrying sporozoites of *P. gallinaceum,* it was found that the procedure did not in any way deter the onset of infection in these chicks.

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Immunochromatography: A Combination of Chromatography and Immunodiffusion on a Micro-scale.* (29518)

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While the techniques of ion-exchange chromatography, on derivatives of cellulose(1) and dextran gel(2), and gel filtration chromatography(3-7) have proved valuable for the fractionation of serologically active proteins, they have not been used previously in direct combination with immunodiffusion. Recently proteins have been separated on a micro-scale by thin layer chromatography on dextran gels(8-10) and by paper chromatography on anion-exchange cellulose(11). This report describes the combination of chromatography and immunodiffusion on a microscope slide to separate and characterize serologically active compounds in a manner analogous to immunoelectrophoresis(12).

Materials and methods. The cross-linked dextran gels, diethylaminoethyl-Sephadex (DEAE) A-50 medium grade, carboxymethyl-Sephadex (CM) C-50 medium grade and Sephadex G-100 (G-100) 140-400 mesh, were prepared for use according to the manufacturer's instructions.§ In the experiments il-

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§ Cross-linked dextran gels and ion-exchange derivatives of these gels can be obtained from Pharmacia, A. B., Uppsala, Sweden under the trade name Sephadex.

lustrated in Fig. 1, DEAE was equilibrated with sodium phosphate buffer (pH 5.8, 0.05 M), CM with sodium acetate buffer (pH 5.5, 0.1 M), and G-100 with sodium phosphate buffer (pH 8.0, 0.1 M in 0.5 M sodium chloride). For diffusion from CM and G-100 columns a 2% agar gel (Difco, Bacto-Agar) with 1% sodium azide in veronal-HCl buffer (pH 7.2, 0.2 M) was used, whereas, for diffusion from the DEAE columns phosphate buffer (pH 5.8, 0.1 M in 0.5 M sodium chloride) was necessary. Molten agar (2 ml) was pipetted onto a standard microscope slide which had been previously coated with 0.1% agar. After the agar had solidified a longitudinal half was removed and a trough 1.4 mm wide × 7.2 cm long was cut 5 mm from the center of the slide in the remainder of the agar. Dextran gel columns of reproducible dimensions were prepared on the vacant half of the slide. A glass spacer (3.5 mm wide × 0.6 mm thick)‖ was laid next to the cut surface of the agar and a trough (5 mm wide × 0.6 mm thick) was formed between the spacer and a similar thickness of glass laminated to a second slide. Settled dextran gel was pipetted into the trough, the excess buffer removed with filter paper and the bed levelled with a stainless steel razor blade. The spacer was removed and the second slide slid away. The slide with the micro-column was placed on the Perspex¶ holder (Fig. 2), the wicks were attached, and the columns equilibrated with 0.1 ml of the respective buffers described above. After all the buffer had drained from the reservoir the sample for separation (2.5 μl) was applied just below the feeder wick.

Solutions of dog albumin (1.9 g/ml), α globulin (2.3 mg/ml), β globulin (2.3 mg/ml) and γ globulin (1.4 mg/ml), obtained by continuous electrophoresis and tested for protein concentration by standard methods (13), were applied to the micro-columns, fractionated and tested with rabbit anti-whole dog serum (Fig. 1). The lines of pre-

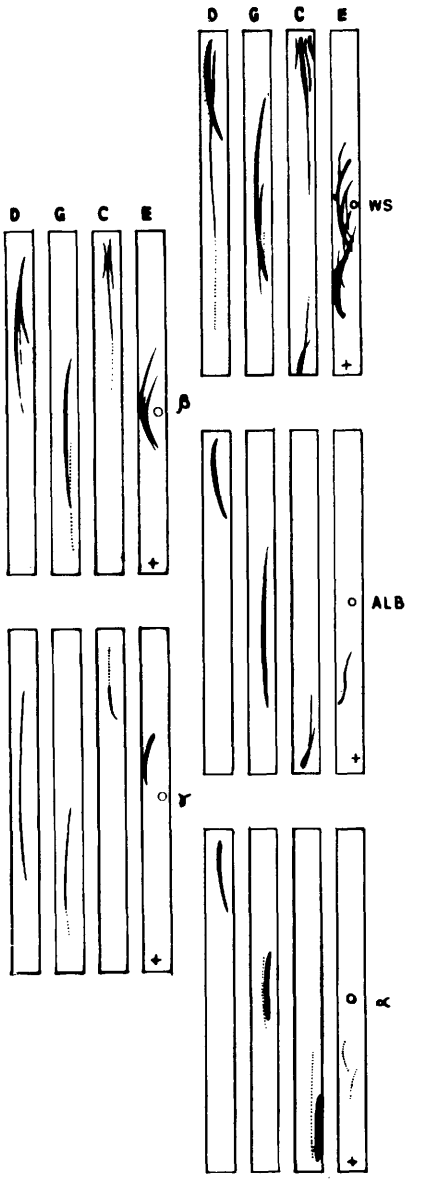

FIG. 1. Precipitin bands in agar from separation of dog serum proteins by immunochromatography, on DEAE-Sephadex (D), CM-Sephadex (C) and Sephadex G-100 (G), and by microimmunoelectrophoresis (E) in veronal buffer (pH 8.6, 0.06 M) with 6 volts/cm. Dog serum (WS) was a 1:4 dilution in the above buffer. Purified proteins were from continuous electrophoresis of dog serum in veronal buffer (pH 8.6, 0.025 M) on a 30 cm curtain with 13 volts/cm; γ-globulin (6 cm from the cathode 1.4 mg protein/ml), β-globulins (10 cm, 2.3 mg/ml), α-globulin (16 cm, 2.3 mg/ml) and albumin (20 cm, 1.9 g/ml); 2.5 μl samples were applied. Letters indicate tops of micro-columns which were applied to right of the agar.

‖ Spacer was constructed from cover slips (No. 1, 76 x 25 mm) laminated with "Araldite" glue from Ciba Ltd., Switzerland.

¶ Perspex is the British equivalent of Lucite.

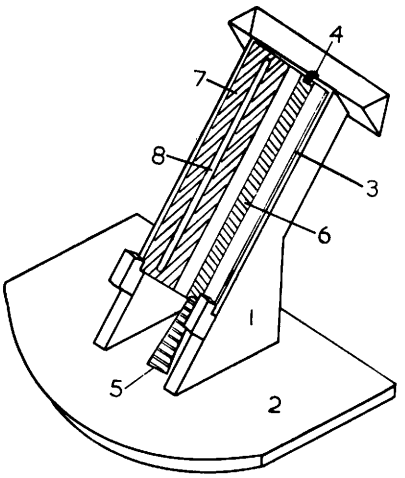

FIG. 2. Apparatus for immunochromatography: (1) Perspex holder and buffer reservoir, (2) Perspex base plate, (3) microscope slide, (4) feeder wick (3 mm × 1.4 mm) Whatman 3 MM paper, (5) effluent wick Whatman 3 MM paper, (6) micro-column 7.6 cm × 5 mm × 0.6 mm, (7) agar gel, (8) trough 1.4 mm wide. The apparatus was covered with a 1.5 l beaker during equilibration and development of the chromatogram.

cipitate noted in these reactions were compared with those that resulted when 1:4 dilution of whole dog serum was treated in the same manner and tested with anti-whole dog serum. The anti-serum used was prepared in a rabbit with 3 subcutaneous injections, each of 1 ml of dog serum (18 mg protein/ml) in Freund's adjuvant (incomplete)(12), at weekly intervals. The rabbit was bled by cardiac puncture 8 days after the last injection.

In these experiments, DEAE chromatograms were developed with 0.06 ml phosphate buffer (pH 5.8, 0.1 M in 0.2 M sodium chloride); CM chromatograms with 0.06 ml sodium acetate buffer (pH 8.0, 0.1 M in 0.5 M sodium chloride). When all the buffer had drained from the reservoir the wicks were removed and a drop of hot agar, of the same pH and ionic strength as the bed of agar, was run along the edge of the bed. A microscopic slide with a ground glass edge lightly smeared with silicone grease was used to push the dextran gel evenly into contact with the agar. The trough was filled with anti-serum and the slides were left in a humid chamber for 30-40 hours. After this period the agar was washed and the precipitin bands stained with amido-black 10B (12). Dilute solutions of hemoglobin and of bromphenol blue were used to determine the approximate volumes of buffer required to develop the columns. With the buffers used, hemoglobin was not adsorbed on DEAE and bromphenol blue was not adsorbed on CM. The volume of buffer used to develop the columns of G-100 was calculated from Pedersen's results(14). Experiments were carried out at room temperature (28 to 31°C, relative humidity 70-80%).

Results. The results of immunoelectrophoresis and immunochromatography of dog serum proteins are shown in Fig. 1. With the particular development conditions used, albumin remained close to the origin whereas γ-globulin formed an elongated band towards the foot of the column on DEAE; the reverse was observed with CM. Although β-globulins were retained on both ion-exchangers three precipitin bands were observed in the agar. The α-globulin preparation contained some albumin which did not separate on DEAE but 2 bands were observed when CM was used. The proteins retained by DEAE did not diffuse into the pH 7.2-agar used for the CM immunochromatograms but did diffuse into the pH 5.8-agar. On G-100 two strong precipitin bands were obtained with dog serum. The faster corresponded to γ-globulin and the other to a mixture of albumin, α-globulins and β-globulin.

Discussion. The above findings correspond in general to results obtained on larger columns by others(2-4). Although inspection of bands formed on the immunochromatograms of whole dog serum suggested coalescence of some antigen-antibody systems (Fig. 1) some proteins difficult to resolve by immunoelectrophoresis were clearly separated by varying the pH and ionic strength of the buffers used for immunochromatography. For example, when serum was applied to a micro-column of DEAE equilibrated with phosphate buffer (pH 5.8, 0.1 M in 0.5 M sodium chloride) and developed with phosphate buffer (pH 5.8, 0.1 M), albumin and the globulins moved rapidly down the column leaving an

α-globulin type at the origin. Whereas, with the conditions shown in Fig. 1 albumin was not separated from α-globulin and was only partially separated from β-globulins. Sober and Peterson(15) showed that α-globulin was more strongly adsorbed than were albumin and other globulins on DEAE-cellulose.

Although Sephadex G-200 has been used to separate human serum into macroglobulins, 7S γ-globulins and albumin(3), it was too fluid to remain on the microscope slide. Chromatograms with this material were prepared by imbedding G-200 in a 5 mm wide trough cut in the center of the agar. This yielded better separation of γ-globulin from albumin. Hemoglobin controls showed that there was little lateral diffusion into the agar during the development of the chromatograms. Immunochromatography on G-100 and G-200 may prove useful for the characterization of serologically active polypeptides from partial hydrolysates of antigens and antibodies (c.f. 15).

The technique of imbedding the chromatographic material in agar was not considered advisable for the ion-exchange Sephadexes because of possible interference by the stronger buffers in the agar with the development of the chromatograms. After chromatography, a mixture of antigens can be tested with 2 antisera by placing the column in a slot 2 mm wide between 2 troughs for antisera. Transfer of the column was most easily done on the edge of a microscope slide lightly coated with silicone grease. A drop of hot agar should be run along the slot before the column is introduced.

Immunochromatography on CM might also be useful in the study of antibodies. One advantage of immunochromatography on ion-exchange Sephadexes over immunoelectrophoresis is that with the former much larger volumes of sample solution can be applied to the column provided the pH and ionic strength of the sample solution are compatible with the conditions necessary for adsorption of the proteins under investigation. For example 10 μl of rabbit anti-dog serum was applied to a column of CM equilibrated with sodium acetate buffer (pH 5.5, 0.1 M). The chromatogram was developed with 0.06 ml sodium acetate buffer (pH 5.5, 0.25 M). The trough was filled with dog serum (1:4, in veronal buffer pH 8.6, 0.06 M. After 24 hours a narrow and a broad precipitin band were observed in the gel in the γ-globulin region.

Immunochromatography may rapidly determine the chromatographic properties of a few micrograms of serologically active compounds as a preliminary to fractionation on a large scale by conventional procedures. As an additional method of characterization based on adsorptive properties, molecular size and charge on serologically active proteins it should prove a valuable adjunct to immunoelectrophoresis. Moreover, since the diffusion into agar of a mixture of antigenic proteins from the ion-exchange Sephadexes can be modified by altering the pH and ionic strength of the buffer in the agar to permit or prevent the diffusion of the more strongly absorbed proteins from the ion-exchanger, the applicability of immunochromatography to characterize serologically active polypeptides (16,17) as well as other compounds appears great.

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Response of the Superficial Lymphatics of the Arm of Intact Man to Synthetic Vasopressin.* (29519)

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Within the past few years advances in the field of polypeptide chemistry have permitted identification and synthesis of a number of very potent vasoactive polypeptides(1). During studies with 2 synthetic vasopressins phenylalanine^2-lysine^8-vasopressin (PLV-2)† and lysyl^8-vasopressin (Lys-Vp)†, an unusual response to the intradermal injection of these substances was noted.

Material and methods. PLV-2. PLV-2 was injected intradermally on the medial aspect of the volar surface of the forearm approximately 15 cm proximal to the wrist of 10 human subjects varying from 24 to 66 years of age. In addition, PLV-2 mixed with patent blue V dye was injected intradermally at a similar site on the volar aspect of the forearm of another 15 subjects ranging from 24 to 53 years of age. Control injections of dye diluted with 0.85% sodium chloride solution were made in the opposite forearm. The technic for studying the superficial lymphatics with vital dyes, such as patent blue V, was first described by McMaster(2) and has been used for many years in this laboratory(3).

In the experiments in which PLV-2 was injected without dye, a solution containing 5,000 milliunits (mU) of PLV-2 per cc was used. The volume of fluid injected varied between 0.01 cc (50 mU of PLV-2) and 0.1 cc (500 mU of PLV-2). In the majority of experiments 0.01 cc of fluid was injected. In the experiments in which PLV-2 was mixed with patent blue V dye, 0.5 cc of an 11% solution of patent blue V dye was added to 0.5 cc (2,500 mU) of PLV-2 to make a solution containing 2,500 mU of PLV-2 per cc in 5.5% patent blue dye. From 0.01 cc (25 mU of PLV-2) to 0.1 cc (250 mU of PLV-2) of this mixture was injected intradermally. Control injections of dye were made with a 5.5% stock solution of patent blue V; the volume injected being equal to the volume of the test fluid injected in the contralateral forearm.

All injections were made with a specially constructed microsyringe which made it possible to introduce small volumes of fluid with accuracy(4). After an intradermal wheal was raised records of the responses to the polypeptide and the dye were made by placing a clear strip of exposed X-ray film over the arm and tracing directly onto the film the various responses observed at 5 minute intervals. Color and black and white photographs of the forearm were also obtained at various intervals to record the rate of the responses to the intradermal injections.

All subjects were studied in a climatic room maintained at 22° to 24° Fahrenheit and 46% relative humidity. The subjects' arms were supported passively at heart level throughout the observations.

Lys-Vp. Lysyl^8-vasopressin (Lys-Vp) was injected intradermally on the forearm as de-

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