applied stress. Different degrees of inherent susceptibility to EAE(9) and to stress may influence results also.

Suppression of EAE by intraperitoneal adjuvant was probably due to stressful effects of granulomatous peritonitis. The fact that adjuvant in the peritoneal cavity caused far more suppression of EAE than adjuvant in the skin suggested a non-specific rather than immunologic mechanism. Suppression of EAE by granulomatous peritonitis may explain the low incidence of EAE in rats following intraperitoneal challenge with encephalitogenic emulsions. Stress may be involved also in the action of drugs or other treatments on EAE (for example, stressful effects of intraperitoneal salicylate(14) and infantile stimulation(15).

Summary. Stress, in the form of restraint repeated throughout the experiment or during its first half, suppressed development of clinical and histologic signs of experimental allergic encephalomyelitis (EAE) in rats after intradermal challenge with encephalitogenic emulsion. Clinical signs, but not histologic lesions, were suppressed by restraint when the highly effective foot pad route of challenge was employed. Intraperitoneal injections of adjuvant also suppressed EAE in rats simultaneously challenged with encephalitogenic emulsion in the skin. It is suggested that suppression of EAE by intraperitoneal adjuvant was due to the stressful effects of the consequent sterile granulomatous peritonitis. Thus, the occurrence of granulomatous peritonitis may explain the failure to produce EAE when rats are challenged by intraperitoneal route with encephalitogenic emulsions that contain adjuvant.

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Heparin-Like Substances in Cement Lines of Vascular Endothelium of Guinea Pigs.* (27184)

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Recently, Samuels and Webster demonstrated vascular endothelial cement lines by treating large blood vessels in stretch preparations with heparin followed by toluidin blue staining(1). This suggests an affinity

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in vitro of these lines for heparin as well as for other compounds containing sulfonic acid groups(2). Subsequently, electron microscopical observations confirmed that such lines represent the cell borders(2,3). Therefore, we attempted to demonstrate a heparin-like substance in the cement lines *in vivo*.

Materials and methods. The jugular vein, inferior and superior vena cava, portal vein, aorta and mitral valve of guinea pigs were removed under ether anesthesia and briefly washed with Tyrode solution immediately after opening. They were pinned out on thin wooden boards 0.1 cm in thickness under Tyrode solution, avoiding excess extension(4). The specimens were stained with 0.1% toluidin blue in pH 5.0 McIlvaine buffer solution for 2 min., followed by treatment with 10% ammonium molybdate solution for 40 min. They were then removed from the boards and mounted in the same solution without subsequent dehydration and clearing. The same procedure was carried out following pretreatment with 0.1% heparin sodium in Tyrode solution for 15 min., as described by Samuels and Webster(1).

Guinea pigs from the laboratory stock weighing 400 to 500 g were divided into 5 groups. Group 1 (25 animals) was used for demonstration of metachromatic material in the cement lines as outlined above. The 5 guinea pigs of group 2 were injected with 0.3 ml of 0.1% heparin solution into the perivascular sheaths of the jugular veins of one side. The vessels were removed 15 to 20 min. later. The 5 animals in group 3 received intracardially 1.5 ml of 0.1% heparin in Tyrode solution; the vessels were removed 30 min. later. Group 4 (20 animals) was given intracardially 1.5 ml of 20% Ishizu peptone† in saline to produce peptone shock; to prevent death from shock, animals were given artificial respiration, and 15 min. later the large vessels were obtained. Furthermore, plasma from the shocked animals, which contained a high content of heparin-like substance as measured by the Serafini method (5), was used for pretreatment of normal vessels followed by toluidin blue staining. Group 5 consisted fo 15 guinea pigs; the veins of 5 animals were stained with 0.1% toluidin blue in a McIlvaine buffer of both pH 7.0 and 2.4(6); the veins of the 10 other guinea pigs were treated by the colloidal iron method (7) with hyaluronidase digestion(8) prior to toluidin blue staining. Ten guinea pigs serving as controls were subjected to intracardiac or perivascular administration of physiologic saline.

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Results. The heparin toluidin blue method of Samuels and Webster produced clear, smooth, continuous lines on the endothelium, confirming their results. Coarse and sometimes indistinct metachromatic lines were demonstrated in specimens stained with toluidin blue without pretreatment with heparin (Group 1) (Fig. 1). The polysaccharide substance in the lines was soluble in fixatives, absolute alcohol, Carnoy solution and 4% lead acetate solution, which were used prior to toluidin blue staining. After perivascular injection of heparin (Group 2) staining of the lines of the venous endothelium by toluidin blue method was increased and more extensive when compared to the non-injected site (Fig. 2). In heparinized animals (Group 3) (Fig. 3) and shocked animals (Group 4) the lines in the large veins and cardiac valves exhibited a moderate increase in staining intensity. In contrast, the endothelial lines of large arteries in these animals exhibited only a minimal increase in intensity of metachromasia. *In vitro* treatment of normal endothelium with plasma from the shocked animals (Group 4) enhanced intensity of metachromasia of the lines so that they resembled those of the shocked animals, whereas treatment with normal plasma did not increase the staining. Variations of the pH of the buffer (group 5) resulted in only slight differences in color and staining intensity. The metachromatic lines could not be digested by pretreatment with hyaluronidase.

Discussion. Whether the endothelial cell borders contain a heparin-like substance *in* vivo under physiological conditions was not known because of technical difficulties in demonstrating small amounts. Even good fixatives for polysaccharides fail to preserve such a substance in the cement lines. The

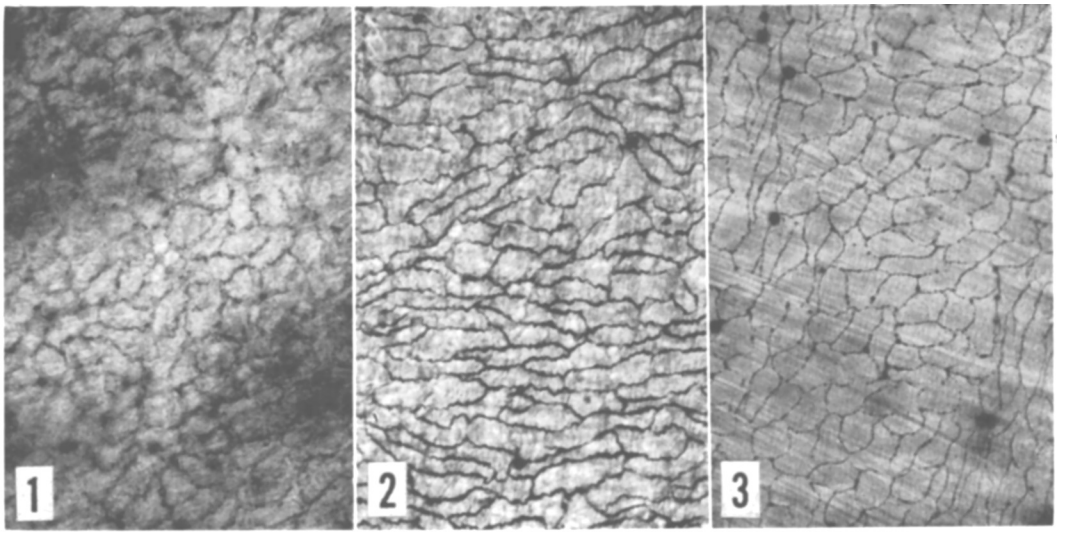

FIG. 1. Lines of the endothelium from guinea pig jugular vein stained with toluidin blue followed by treatment with ammonium molybdate. Some lines are stained poorly.

FIG. 2. Lines in jugular vein after inj. of 0.3 ml of 0.1% heparin solution into the perivascular sheath. Increase in staining intensity. (Toluidin blue method.)

FIG. 3. Endothelial lines of inferior vena cava, obtained 30 min. after an intracardiac inj. with 1.5 ml of 0.1% heparin sodium in Tyrode solution. The lines are more clear and smooth than those in Fig. 1. (Toluidin blue method.)

facts that staining with toluidin blue without previous fixation gives successful results at both pH 2.4 and pH 7.0, and that it is not reduced following pretreatment with hyaluronidase suggest that the lines contain polysaccharides with sulfonic acid groups. This is in agreement with demonstration of the lines by application of sulfonic acid derivatives *in vitro* followed by azur II(2). Since general or regional heparinization moderately increases the metachromasia of the lines as does hyperheparinemia induced by peptone shock, the cement lines seem to absorb a heparin-like substance from the circulating blood and the vascular walls. This is confirmed *in vitro* by the experiments using plasma from the shocked animals. Variations in the intensity and extent of staining of the metachromatic lines under physiological conditions may depend upon differences in structure of the vessels and function of the endothelium. These observations may assist in explaining why platelets do not adhere to normal endothelium.

Summary. A heparin-like substance was demonstrated in the endothelial cement lines of the large vessels and cardiac valves of guinea pigs *in vivo*. Heparin present in blood increases staining of the lines, probably because of absorption of this substance from the circulating blood.

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