Summary. A modified heparin has been prepared. This heparin derivative has undergone slight methylation and partial de-sulfation. Anticoagulant activity was retained, and limited absorption from duodenum of dogs as measured by systemic anticoagulant effect occurred following instillation of the modified heparin in buffer solutions of pH 5, 6, and 7.

1. Schanker, Lewis S., Tocco, D. J., Brodie, B. B., Hogben, C. A. M., *J. Pharmacol. and Exp. Ther.,* 1958, v123, 81.

2. Hogben, C. Adrian M., Schanker L. S., Tocco, D. J., Brodie, B. B., *J. Pharmacol. & Exp. Therap.,* 1958, v125, 275.

3. Loomis, T. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 447.

4. Warner, R. C., Schubert, M., *J. Am. Chem. Soc.,* 1958, v80, 5166.

5. Walkenstein, Sidney S., Knebel, Cornelius M., *Anal. Chem.,* 1957, v29, 1516.

6. Caley, E. R., Rodgers, L. B., *Ind. Eng. Chem. Anal.,* 1943, v15, 32.

7. Lee, R. I., White, P. D., *Am. J. Med. Sci.,* 1913, v145, 495.

8. Lange, N. A., *Handbook of Chemistry,* 1949, p124, Handbook Publisher, Sandusky.

9. Wolfrom, M. L., Montgomery, Rex, Karabinos, J. V., Rathgeb, P., *J. Am. Chem. Soc.,* 1950, v77, 1597.

10. Foster, A. B., Martlew, E. F., Stacey, M., Abstracts Papers, Am. Chem. Soc., 1954, v126, 6D.

11. Wilander, O., *Skand. Arch. Physiol. Suppl.,* 1939, v15, 89.

12. Loomis, T. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 444.

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Further Observations on Mechanism of Hypoglycorrhachia in Experimental Meningitis.* (25728)

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We have demonstrated that incubation of CSF containing polymorphonuclear leukocytes with pneumococci at 37°C resulted in greater consumption of glucose(1) than when organisms were incubated in cell-free CSF. CSF containing a comparable number of leukocytes in absence of bacteria displayed little glycolytic activity. These findings pointed to definite synergistic effect between polymorphonuclear leukocytes and bacteria in depressing CSF glucose. The reaction was dependent upon number of bacteria and white cells in the incubation mixture but was not altered when erythrocytes were added to the medium. The mechanism of synergistic action of leukocytes and bacteria in enhancing glycolysis in the CSF was not clear, but it was suggested that bacterial cells multiplied more rapidly in the presence of leukocytes resulting in increased consumption of glucose, or that white cells engaged in phagocytosis exhibit greater glycolytic activity. Evidence will be presented here that the synergistic effect between cells and bacteria in producing hypoglycorrhachia is a function of enhanced phagocytosis in the subarachnoid space of intact animals.

Methods. Aseptic meningitis was produced in mongrel dogs by injection of 4 ml of sterile pyrogen-free, 0.85% NaCl into the cisterna magna. Four hours later 4 ml of CSF which regularly contained 2000 to 4000 WBC/mm^3 (95-99% polymorphonuclear leukocytes) was removed by cisternal puncture. The animals were then given 4 ml of physiological saline containing 10^8 viable pneumococci intrathecally and 3 hours later CSF was again removed for quantitative determinations of bacteria, leukocytes and glucose. One group of control animals was given physiological saline at beginning of experiment and 4 hours later,

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and in another, pneumococcal meningitis was produced without antecedent aseptic meningitis. In other studies heat-killed pneumococci, India ink particles and a purified lipopolysaccharide obtained from *Salmonella abortus equi* (Pyrexal) were administered to animals with aseptic meningitis. Seed cultures of type III pneumococci were stored at −70°C. To prepare a culture for infection of animals, the contents of one tube were thawed and added to 9 ml tryptose phosphate broth. After incubation at 37°C for 8 hours, the broth culture was diluted with 0.85% NaCl solution. Inocula consisted of 4 ml suspension containing approximately 10^8 viable bacteria. Number of organisms in inocula and CSF was determined by colony counts of serial 10-fold dilutions, a single colony being counted as one viable unit. Leukocytes were counted in standard manner and CSF glucose was determined on cell-free supernates by the glucose-oxidase method(2).

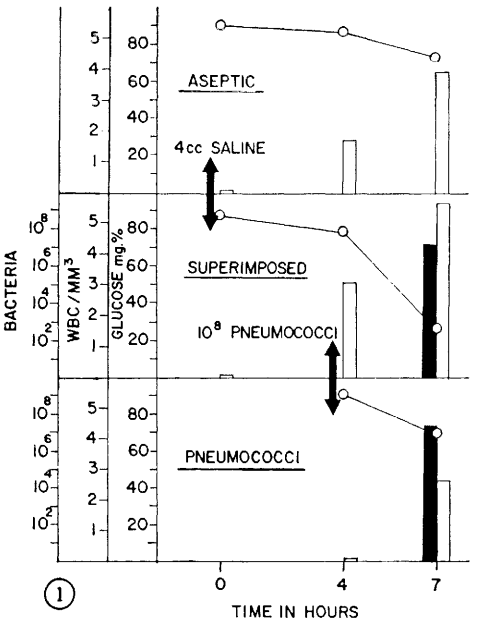
Results. Eight dogs were given 0.85% NaCl intrathecally. Four hours later 2000 WBC/mm^3 had appeared in the CSF and no appreciable decrease in glucose had occurred (Fig. 1, top). A second cisternal puncture 3 hours later, revealed further increase in cells without significant drop in sugar. Administration of a second aliquot of 4 ml 0.85% NaCl 4 hours after beginning of experiment did not alter results. A second group, consisting of 9 animals, was given 10^8 pneumococci suspended in 4 ml 0.85% NaCl, after aseptic meningitis had developed (Fig. 1, center). Three hours later, CSF glucose of these animals had dropped from 82 to 28 mg %. Number of bacteria remained approximately the same as that in the original inoculum. While there had been an increase in number of leukocytes, the increment was no greater than in controls with aseptic meningitis in whom hypoglycorrhachia did not develop. In a third group of 8 dogs without aseptic meningitis, pneumococci were instilled into the cisterna magna and 3 hours later no significant fall in CSF glucose had occurred (Fig. 1, bottom). This contrasted sharply with findings in animals with aseptic meningitis antedating pneumococcal meningitis. Twenty-four hours later this difference was no longer apparent and the sugar was depressed in both groups of animals.

These observations indicate that a combination of pneumococci and leukocytes possesses greater glycolytic activity than either of its constituents *in vivo* as well as *in vitro*. Serum sugars were determined along with CSF sugars in all experiments and showed no appreciable deviation from normal.

To determine whether the decrease in CSF glucose could be attributed to alteration in metabolic requirements of the bacteria, the experiment was repeated employing pneumococci which had been rendered non-viable by exposure to 60°C for 30 minutes (Fig. 2). Six animals with antecedent aseptic meningitis, and approximately 2000 WBC/mm^3 in the CSF, experienced a marked fall in sugar when 4 ml suspension containing 10^8 non-viable pneumococci was injected (Fig. 2, bottom). On the other hand, hypoglycorrhachia did not ensue when the same number of bacteria were administered to animals without aseptic meningitis.

In another experiment 4 animals with aseptic meningitis were given 4 ml of 1% suspension of sterile, pyrogen-free India ink, and 4 ml of saline containing 1 μg of purified lipopolysaccharide Pyrexal was administered to 4 others. All 8 animals demonstrated a fall in glucose of from 50-90% of base-line values. In controls without aseptic meningitis injected with India ink and Pyrexal, there was a reduction in CSF glucose of only 10-20%.

Discussion. These data illustrate that glycolysis in CSF is brisker when bacteria are introduced into CSF containing leukocytes than when organisms are administered to animals with normal spinal fluid. Identical results were obtained when non-viable organisms were administered. This eliminates from consideration the possibility that viable bacteria consume glucose more avidly in presence of leukocytes, and suggests that some alteration in metabolism of the white cells is responsible for the hypoglycorrhachia. Introduction of both viable and non-viable pneumococci into CSF of animals with aseptic meningitis was associated with marked rise in *number* of leukocytes, but the relative increase was no greater than that observed dur-



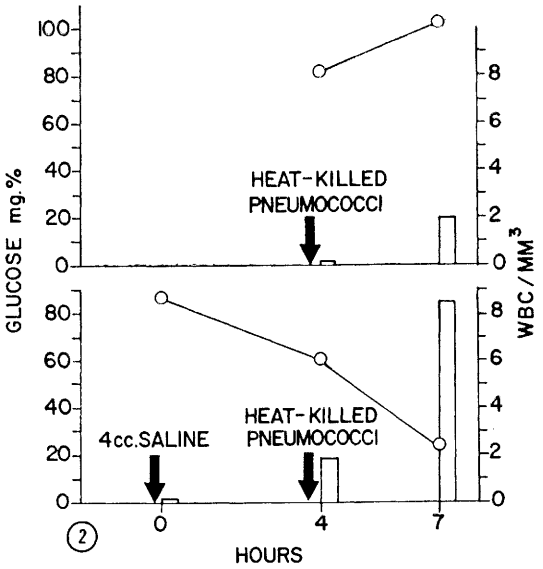


FIG. 1. Cells (open bars), sugar (circles), and bacteria (solid bars) in CSF of dogs with aseptic meningitis (top), aseptic meningitis with superimposed pneumococcal meningitis (center), and pneumococcal meningitis without antecedent aseptic meningitis (bottom).

FIG. 2. Effect of inj. of heat-killed pneumococci on CSF sugar (circles) and cells (bars) in dogs with aseptic meningitis (bottom) and normal controls (top).

ing aseptic meningitis produced by injection of saline alone. Furthermore, production of aseptic meningitis with streptokinasedornase (3) and penicillin or serum(4) has not resulted in hypoglycorrhachia despite appearance of pleocytosis far greater than that observed in our animals. It seems unlikely then that increase in number of leukocytes *per se* is responsible for reduction in glucose.

More attractive is the possibility that in presence of bacteria, leukocytes were stimulated to active phagocytosis. It has been shown that leukocytes engaged in phagocytosis of bacteria and inert particles manifest augmented consumption of glucose and oxygen and produce more lactic acid(5-7). Evidence that phagocytosis of bacteria by leukocytes played an important part in decrease in CSF glucose lies in the finding that administration of India ink particles to dogs with aseptic meningitis resulted in a comparable fall in CSF glucose. Most particles were visible within leukocytes and studies employing quantitative technics are now in progress to correlate degree of phagocytosis with hypoglycorrhachia.

The drop in glucose following intrathecal administration of a purified soluble bacterial endotoxin to dogs with aseptic meningitis is consistent with the hypothesis that hypoglycorrhachia is a consequence of phagocytosis. It has been demonstrated that granulocytes from animals and man given purified bacterial lipopolysaccharides possess an enhanced capacity for phagocytosis of staphylococci(8). In addition, increased glycolysis by granulocytes in contact with endotoxin has been described(9). Whether this is related to increase in cells' capacity for phagocytosis remains to be determined.

Summary. When dogs with aseptic meningitis were infected with pneumococci intrathecally, a profound drop in CSF glucose occurred which was not observed in control animals given pneumococci without antecedent production of aseptic meningitis, or in animals with aseptic meningitis in absence of superimposed bacterial infection. Hypoglycorrhachia also occurred when heat-killed pneumococci, India ink particles and bacterial endotoxin were administered to animals with aseptic meningitis. These data suggest that

the fall in CSF glucose is dependent upon presence of leukocytes engaged in active phagocytosis.

1. Petersdorf, R. G., Swarner, D. R., Garcia, M., PROC. SOC. EXP. BIOL. AND MED., in press.
2. Froesch, E. R., Renold, A. E., *Diabetes*, 1956, v5, 1.
3. Baltch, A., Osborne, W., *J. Lab. Clin. Med.*, 1957, v49, 382.
4. Goldring, S., Harford, C. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 669.
5. Sbarra, A. J., Karnovsky, M. L., *J. Biol. Chem.*, 1959, v234, 1355.
6. Becker, H., Munder, G., Fisher, H., *Hoppe-Seylers Z. Physiol. Chem.*, 1958, v313, 266.
7. Hartman, J. D., Reidenberg, M., *J. Appl. Physiol.*, 1958, v12, 477.
8. Bergmann, H., Burchmann, G., Doering, P., Fritze, E., Wendt, F., *Klin. Wchnschr.*, 1954, v32, 500.
9. Bennett, I. L., Jr., Cluff, L. E., *Pharm. Rev.*, 1957, v9, 427.

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Production of Cardiac Necroses and Nephrocalcinosis by Stress in Adrenalectomized Rats.* (25729)

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In various animal species, including the rat, large doses of certain corticoids and electrolytes produce massive "infarctoid" myocardial necroses, irrespective of presence of adrenals. Furthermore, small doses of corticoids and electrolytes, although by themselves ineffective, can so prepare or "condition" the myocardium that upon subsequent exposure to stressors (*e.g.*, cold, surgical trauma, restraint or forced muscular exercise) it responds with acute development of a usually fatal, infarctoid cardiopathy. In all these circumstances, the cardiac lesions are accompanied by nephrocalcinosis if Na_2HPO_4 is used as conditioning electrolyte(1). In previous studies, soon after it became clear that numerous manifestations of the alarm reaction result from discharge of ACTH and corticoids, it was shown that not all stress-induced changes are relayed through the hypophyseal-adrenal system, since many of them occur even after hypophysectomy(2) and adrenalectomy(3). It seemed of interest to determine, therefore, whether induction by stress of an infarctoid cardiopathy and of nephrocalcinosis in suitably conditioned rats is dependent upon integrity of the adrenals.

Materials and methods. Eighty female Holtzman rats, with mean initial body weight of 100 g (range: 94-110 g), were subdivided into 8 equal groups and treated as indicated in Table I. Adrenalectomy was performed on first day of experiment, under ether anesthesia. Na_2HPO_4 was given at dose of 1 mM in 2 ml of water twice daily by stomach tube. Triamcinolone§ or 9α-fluorocortisol (F-COL)‖ acetate was administered as microcrystal suspension, subcutaneously, at daily dose of 500 μg in 0.2 ml of water, during first 4 days; then, the daily dose of both these steroids was raised to 750 μg. Treatment with various stressors was initiated on sixth day. The animals were exposed to cold (3°C) 19 hours in a refrigerated room. Motor denervation of all 4 extremities was performed under ether anesthesia. Restraint procedure consisted in tying the rats to boards with adhesive tape, in prone position, for 7 hours. A detailed description of all these technics has been given elsewhere (1). Throughout period of observation the animals were fed exclusively on Purina Fox Chow. The experiment was terminated on eighth day by killing all surviving rats with chloroform. Immediately after autopsy, hearts and kidneys were fixed in alcohol-for-

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