Surgeon General, Dept. of Army. Vitamins were generously donated by Merck Sharp and Dohme, Rahway, N. J., and DL-methionine by Dow Chemical Co., Midland, Mich.

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Synergistic Action of Pneumococci and Leukocytes in Lowering Cerebrospinal Fluid Glucose.* (25527)

ROBERT G. PETERSDORF,† DAVID R. SWARNER, AND MANUEL GARCIA
Dept. of Medicine, Johns Hopkins School of Medicine, Baltimore, Md.

The mechanism responsible for disappearance of cerebrospinal fluid sugar in bacterial meningitis has not been clearly elucidated and the relative importance of cells, bacteria, changes in meningeal permeability and accelerated utilization of glucose by neural tissue in genesis of hypoglycorrhachia remains poorly defined. It had been demonstrated that glycolysis occurs *in vitro* when CSF from dogs with non-bacterial meningitis is incubated at 37°C; the decrease in CSF sugar is proportional to number of leukocytes present (1,2). In contrast, pneumococci incubated in sterile CSF consume glucose at a very slow rate and large numbers of rapidly growing bacteria must be present before a fall in CSF sugar becomes evident. In the intact animal, however, sterile meningitis is not usually associated with a decrease in CSF glucose while bacterial infections in the meninges are almost invariably accompanied by hypoglycorrhachia. These paradoxical observations have led to the suggestion that neither cells nor bacteria can be held entirely accountable for the fall in CSF glucose(2). In our studies, CSF containing leukocytes was inoculated with pneumococci, and the mixture incubated at 37°C. Under these conditions consumption of glucose was greater than when organisms were incubated in cell-free CSF, or when CSF containing leukocytes obtained from dogs with non-bacterial meningitis was tested in absence of bacteria.

Methods. Aseptic meningitis was produced in mongrel dogs by injection of 6 ml of sterile, pyrogen-free, 0.85% NaCl into the cisterna magna. Four hours later CSF, which regularly contained 3000 to 8000 WBC/mm^3 (95-99% polymorphonuclear leukocytes), was removed by cisternal puncture. Fluids from several animals were pooled for each experiment and used immediately. Seed cultures of Type III pneumococci were stored at −70°C. For each experiment a fresh tube was thawed, added to 9 ml of tryptose phosphate broth and incubated at 37°C for 8 hours. Control tubes containing serum and broth but no bacteria were handled in similar fashion. In each experiment 0.1 ml of bacterial inoculum was added to a series of tubes containing 0.9 ml CSF obtained from animals with non-bacterial meningitis. The same number of bacteria added to 0.9 ml of sterile, cell-free CSF and fluid from animals with aseptic meningitis mixed with serum-broth inoculum not containing organisms, served as controls. One tube was immediately removed from each series for baseline bacterial and leukocyte counts and determination of glucose. The remainder were shaken in constant temperature bath at 37°C and were removed at intervals of 30, 60, 120, 180, and 240 minutes after beginning of experiment. The number of bacteria in each tube was determined by colony counts of serial 10-fold dilutions, a single colony being counted as one viable unit. Leu-

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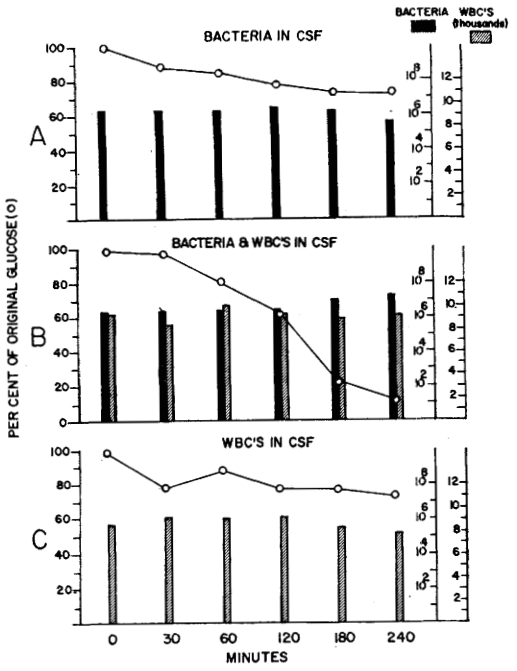
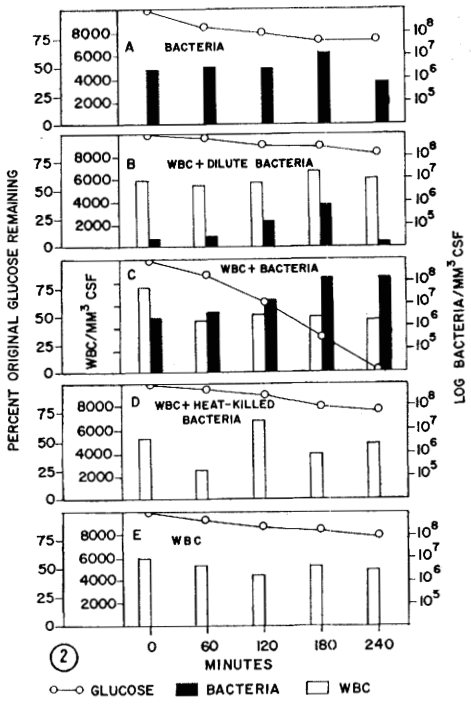


FIG. 1. Effect of pneumococci in sterile CSF, pneumococci in CSF containing leukocytes, and leukocytes in sterile CSF on CSF sugar *in vitro*.

kocytes were counted in standard manner and glucose was determined on cell free supernates by the glucose-oxidase method(3).

Results. Incubation of 10^6 pneumococci in sterile cell-free CSF resulted in very slow decrease in glucose (Fig. 1A). Similarly in CSF containing approximately 7000 polymorphonuclear leukocytes/mm^3 CSF, in absence of bacteria, glycolysis was very slow (Fig. 1C). In contrast, addition of 10^6 pneumococci to CSF containing 7000 WBC/mm^3 was followed by marked decrease in glucose which began one hour after onset of incubation; 3 hours after beginning of experiment glucose had almost entirely disappeared from the CSF. The number of leukocytes and bacteria remained relatively constant throughout but some multiplication of bacteria occurred during the last 2 hours.

The results were reproducible regularly provided a sufficient number of bacteria are present. For example, a marked decrease in glucose occurred when 5 x 10^6 bacteria were incubated with 5000 WBC/mm^3 (Fig. 2C). However, when the inoculum was diluted to contain only 5 x 10^4 organisms, glycolysis did



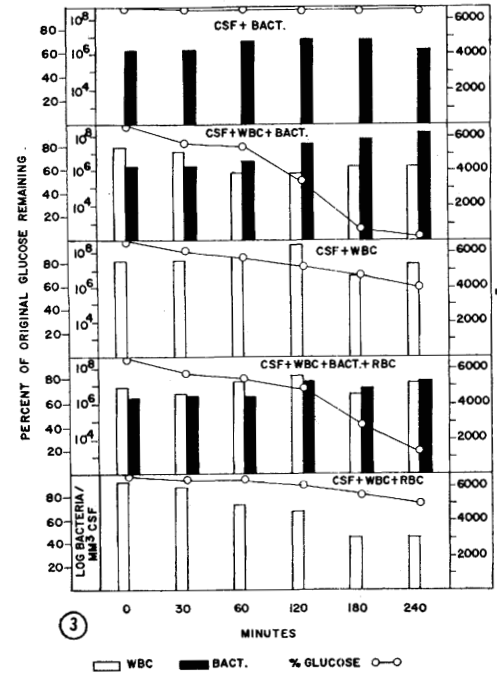


FIG. 2. Failure of dilute inoculum of pneumococci and heat-killed pneumococci to depress sugar in CSF from dogs with aseptic meningitis.

FIG. 3. Hypoglycorrhachia *in vitro* produced by combination of pneumococci and leukocytes. Erythrocytes in CSF did not influence the reaction.

not occur (Fig. 2B). In another experiment in which only 1200 leukocytes/mm^3 of CSF were present the sugar also failed to decrease despite addition of 10^7 pneumococci. This observation indicates that a sufficient number of leukocytes as well as bacteria was necessary for production of hypoglycorrhachia. These *in vitro* studies also illustrate that viable pneumococci were necessary to produce a fall in CSF sugar; addition of heat-killed bacteria to CSF containing leukocytes was not associated with a fall in glucose (Fig. 2D).

The contribution of erythrocytes or serum to the decrease in CSF glucose was evaluated in an experiment in which 2 pools of CSF each containing 5000 WBC/mm^3 were employed. In one of these 2 samples, 30,000 RBC/mm^3 were also present. The organism was grown in the absence of serum prior to inoculation into CSF. The results (Fig. 3) indicate that degree of glycolysis was not affected by presence of red blood cells.

Discussion. These studies indicate that a fall in CSF glucose can be produced *in vitro* by a combination of leukocytes and pneumococci when neither alone is capable of producing glycolysis. They also support previous observations which demonstrated that leukopenic dogs, which did not respond to pneumococcal meningitis with pleocytosis, did not manifest a decrease in CSF glucose, while normal controls showed a marked fall in sugar(4). Whether synergism between cells and bacteria occurs *in vivo* is being investigated.

The reason for the fall in glucose is not readily apparent. In some instances, at least, multiplication of organisms occurred during third and fourth hours of incubation, suggesting that the cells enhanced the nutritive value of the medium. However, a fall in glucose was noted at least one hour prior to demonstrable multiplication of bacteria, and in some experiments the bacterial count remained constant.

An alternative explanation is that in the presence of bacteria, leukocytes became actively phagocytic. It has been shown that during phagocytosis of bacteria or inert particles, leukocytes manifest increased consumption of oxygen and glucose, as well as increased production of lactic acid(5-7). It is conceivable that actively phagocyting leukocytes rather than bacteria were responsible for hypoglycorrhachia. The failure of CSF glucose to decrease when heat-killed pneumococci were added to the mixture, mitigates against this possibility but the poor environment for phagocytosis provided by a glass surface may have sharply restricted activity of the cells. As has been demonstrated in experimental pneumococcal pneumonia, phagocytosis is much more active in the intact animal than in the test tube(8). Studies are in progress to determine whether leukocytes stimulated to phagocytosis in the subarachnoid space produce hypoglycorrhachia, particularly in absence of viable bacteria.

Summary. Cerebrospinal fluid containing leukocytes was inoculated with a known quantity of pneumococci and incubated at 37°C. Under these conditions the fall in glucose was greater than when bacteria were incubated with cell-free CSF or when leukocytes were incubated in absence of bacteria. The data indicate that cells and bacteria act synergistically in producing the decrease in CSF sugar in bacterial meningitis.

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