TABLE I. Efficiency and Ratio of R_s^2/R_b of Co^{56}, Co^{58} and Co^{60} in NaI Well Scintillation Counter and in Large Plastic Scintillation Counter as a Function of Volume.

	Co^{56}		Co^{58}		Co^{60}	
NaI well	Efficiency (%)	R_s^2/R_b	Efficiency (%)	R_s^2/R_b	Efficiency (%)	R_s^2/R_b
Vial 1 ml	59.6	14.5×10^9	25.7	2.7×10^9	26.3	2.8×10^9
2	56.8	13.4 "	24.0	2.4 "	27.3	3.1 "
4	46.8	8.9 "	20.5	1.7 "	23.2	2.2 "
Beaker 100	7.2	20.8×10^7	3.1	3.7×10^7	3.4	4.7×10^7
200	7.3	21.8 "	3.1	4.0 "	3.6	5.2 "
400	8.2	27.6 "	3.6	5.3 "	4.0	6.7 "
600	8.7	30.4 "	3.8	5.9 "	4.2	7.3 "
800	8.5	29.1 "	3.7	5.6 "	4.1	7.0 "
1000	7.5	24.4 "	3.5	4.9 "	3.8	6.0 "
Plastic well						
1	52.6	3.4×10^9	31.6	1.2×10^9	39.4	1.9×10^9
2	56.6	4.0 "	30.3	1.1 "	39.3	1.9 "
4	49.6	3.1 "	28.6	1.0 "	37.9	1.8 "
10	47.6	2.8 "	28.5	1.0 "	39.2	1.9 "
20	47.4	2.7 "	28.1	1.0 "	38.8	1.9 "
40	46.2	2.6 "	28.4	1.0 "	38.3	1.8 "
60	45.8	2.6 "	27.7	.9 "	37.2	1.7 "
80	45.0	2.5 "	27.5	.9 "	36.8	1.7 "
100	43.4	2.3 "	26.0	.8 "	35.8	1.6 "
125	40.0	1.9 "	24.1	.7 "	32.8	1.3 "
150	35.0	1.5 "	21.2	.6 "	29.2	1.1 "
175	31.4	1.2 "	18.8	.4 "	25.4	.8 "
200	28.0	1.0 "	16.5	.3 "	22.0	.6 "
250	22.6	.6 "	11.6	.2 "	18.3	.4 "

volume from 10-100 ml. Mice or small rats up to about 75 g and tissue samples can be counted in the plastic well without correction for geometry. The efficiency of 250 ml in the plastic scintillation counter is 22.6, 11.6 and 18.3% for Co^{56}, Co^{58}, and Co^{60} respectively and 7.8, 3.5, and 3.8% respectively for 1000 ml using the beaker on the NaI well. The choice of instrument for counting large volumes is discussed.

1. Ellenbogen, L., Williams, W. L., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 617.

2. Hine, G. J., Miller, A., *Nucleonics,* 1956, v14, No. 10, 78.

3. Loveninger, R., Berman, M., *ibid.,* 1951, v9, No. 1, 26.

4. Baskin, R., Demarest, H. L., Sandhaus, S., *ibid.,* 1955, v13, No. 7, 54.

Received September 1, 1959. P.S.E.B.M., 1959, v102.

Mechanism of Hypoglycorrhachia in Experimental Pneumococcal Meningitis.* (25356)

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

It is well known that glucose in cerebrospinal fluid decreases in patients with bacterial meningitis, while non-bacterial meningitides are usually not associated with hypoglycorrhachia(1). The reason for this phenomenon is poorly understood particularly since it has been demonstrated that both leukocytes and bacteria are capable of accelerating consumption of cerebrospinal fluid in glucose *in vitro* (2,3). In our experiments pneumococcal meningitis was produced experimentally in dogs made leukopenic by total body irradiation. In these animals the leukocytic response in the CSF was effectively suppressed, and decrease in CSF glucose was also prevented despite heavy bacterial growth.

* This study supported by Army Chemical Corps, Fort Detrick, Md., Parke-Davis Co., and Upjohn Co.

TABLE I. Mean Values for CSF Leukocyte and Bacterial Counts and Glucose in 12 Irradiated and 13 Normal Dogs with Pneumococcal Meningitis.

Hr after infection	WBC/mm³ CSF		Bacteria/mm³ CSF		Glucose/mm³ CSF, mg %	
	Irrad.	Control	Irrad.	Control	Irrad.	Control
24	490 (12)*	4900 (13)	2×10^3 (12)	9×10^2 (13)	90 (10)	72 (12)
48	530 (6)	4000 (5)	4×10^4 (6)	6×10^4 (5)	82 (6)	50 (5)
72	570 (4)	7300 (4)	8×10^5 (4)	10^6 (4)	82 (4)	52 (4)

* No. of observations in parentheses.

Methods. Mongrel dogs weighing 8 to 12 kg were used. Bacterial meningitis was produced by inoculating a saline suspension of 1 ml of type III pneumococcus (containing 10^3 to 10^5 organisms) into the subarachnoid space through a small midline burr-hole placed equidistant between coronal and sagittal sutures. Cerebrospinal fluid was sampled by cisternal puncture 24, 48, 72 and 96 hours after induction of infection. Number of bacteria 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 glucose was determined by glucose-oxidase method(4). Blood cultures were also made daily in all infected animals. Dogs were rendered leukopenic by administration of 450 r (total body irradiation) and used in experiments when their blood leukocyte counts had fallen to less than 1000/mm³. In a group of control animals aseptic meningitis was produced by intrathecal administration of 1 ml of sterile normal dog serum. Seed cultures of type III pneumococci were stored at −70°C. To prepare a culture for infection of animals, the content of one tube was thawed and added to 10 ml of tryptose phosphate broth. After incubation at 37°C for 8 hours, the broth culture was diluted with 0.85% NaCl solution. Inocula consisted of 1 ml suspension containing 10^3 to 10^5 viable units. In order to minimize differences in virulence of individual cultures, each experiment was performed in leukopenic and normal (control) animals simultaneously.

Results. Pneumococcal meningitis was produced in 12 irradiated dogs; 13 animals served as controls. All irradiated animals had white blood cell counts of less than 1000/mm³. In 21 animals the inoculum consisted of 10^5 pneumococci; in 4, 10^3 organisms were placed into the subarachnoid space. The course of the infection was the same regardless of size of the inoculum. The infection terminated fatally in 24 of 25 dogs; one animal in the control group recovered. Mean survival time of the leukopenic dogs was 60 hours following induction of the infection; control animals lived 55 hours. Bacteremia was present in 10 of 12 leukopenic animals and in 11 of 13 controls.

Average values of CSF leukocytes, bacteria and glucose of all animals are shown in Table I. The decrease in number of observations 48 and 72 hours after onset of infection is due to death in most animals and insufficient quantity of CSF in a few. Irradiated animals were capable of mobilizing only a small number of leukocytes in response to the meningeal infection, while the controls averaged close to 5000/mm³ CSF. Despite this striking difference in CSF pleocytosis, the number of bacteria present in the CSF was approximately equal in both groups. As might be expected, the number of organisms increased on each day of the infection. There was considerable variation in individual dogs, some showing sudden sharp increments in number of bacteria as the infection progressed while in others the number of organisms remained relatively constant.

The normal mean for CSF glucose in this laboratory is 90 mg%, and the lower limit of normal 62 mg%. These results closely approximate those of Baltch and Osborne obtained by another method(3). The amount of glucose in the serum of dogs is usually slightly lower than that in CSF. Serum sugars were determined along with CSF sugars in all experiments, but since they showed no appreciable variation from normal are not detailed. The CSF of normal dogs with pneumococcal meningitis contained considerably less glucose

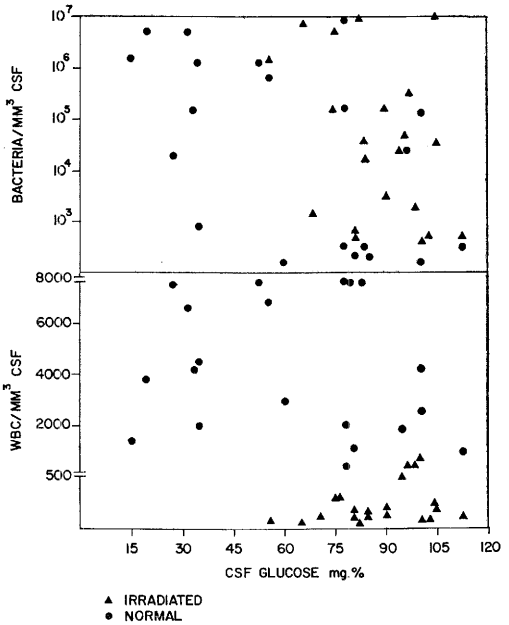

FIG. 1. Relationship between glucose, bacteria and leukocytes in spinal fluid. Glucose values in animals with low leukocyte counts were normal while 50% of animals with significant pleocytosis demonstrated hypoglycorrachia.

than that of irradiated animals (Table I). This difference became more apparent as infection progressed. On the other hand, no hypoglycorrhachia occurred in leukopenic dogs despite the presence of a large number of bacteria in the CSF. The relationship between number of leukocytes and bacteria and glucose in the CSF is illustrated in Fig. 1. Ten of 20 spinal fluids from normal animals with meningitis contained less than the normal amount of glucose; between 1600 and 9000 WBC/mm^3 were found in each of these specimens. In contrast, only 1 of 21 samples of CSF obtained from leukopenic animals, the majority containing less than 500 WBC/mm^3 of CSF, showed a lower than normal glucose level. Failure of glycolysis to occur in half of the infected controls remains unexplained; the infection in these animals did not differ from those in whom hypoglycorrhachia occurred. The inverse relationship between cells and sugar in the CSF was not apparent when the number of bacteria/mm^3 CSF was compared with the glucose content, and the organisms appeared to grow equally well in the CSF of normal and leukopenic animals.

To ascertain whether CSF pleocytosis *per se* may result in a decrease in CSF glucose, nonbacterial meningitis was produced in 8 animals by intrathecal instillation of sterile, normal dog serum. These animals did not experience a fall in CSF glucose, but degree of pleocytosis was not as great as in the dogs with bacterial meningitis, averaging only 1000 WBC/mm^3.

Discussion. These data indicate that suppression of pleocytosis in the CSF which occurs in response to a bacterial infection of the meninges is accompanied by absence of hypoglycorrhachia. This suggests that the leukocytes rather than bacteria are responsible for the decrease in CSF glucose in bacterial meningitis. This is probably not the case since glycolysis has been invariably absent in experimental aseptic meningitis even with cell counts as high as 13000(3). On the other hand, Goldring and Harford demonstrated *in vitro* that sterile cerebrospinal fluid from dogs with aseptic meningitis incubated at 37°C undergoes glycolysis, the decrease in sugar paralleling the number of cells(2). This contrasts sharply with the glycolytic activity of pneumococci which, when incubated with normal CSF, utilize glucose at a very slow rate. Indeed, enormous numbers of bacteria are necessary before the amount of glucose consumed approximates that metabolized by relatively few leukocytes in fluids from dogs with aseptic meningitis. Furthermore, the number of pneumococci necessary to lower CSF glucose *in vitro* in the absence of cells far exceeds that seen in experimental or clinical meningitis. Our studies confirm these observations *in vivo* since they demonstrate that bacteria growing in spinal fluid relatively free of cells do not produce a decrease in the fluid's sugar content. It remains to be demonstrated unequivocally, however, that bacteria growing in the presence of leukocytes utilize glucose better than those incubated in a cell-free medium.

One of the explanations advanced for failure to observe hypoglycorrhachia in aseptic meningitis is that the host is able to replenish CSF glucose more rapidly than it is metabolized by the leukocytes. Perhaps the irradiated dogs maintained CSF glucose close to normal by a similar mechanism since the radi-

ation injury may have disrupted the blood-CSF barrier in some unknown fashion. There are no data which bear out this point. It is conceivable that leukopenia influenced the growth characteristics of bacteria in some unknown fashion to prevent utilization of glucose. This idea gains support in the observation that, *in vitro,* rapidly-growing pneumococci consume more glucose than organisms growing at a slow rate(2). Since only the absolute numbers of bacteria in the CSF were measured in our studies no information is available concerning their rate of multiplication.

These experiments do not provide the answer to the problem of hypoglycorrhachia in tuberculous meningitis which is usually characterized by relatively few cells and bacteria or in the occasional patient with pneumococcal meningitis with a large number of bacteria in the absence of significant pleocytosis. Nor do they shed light on the mechanism of transfer of glucose from the blood to the CSF. They do emphasize, however, that the mechanism governing the decrease in CSF glucose in bacterial meningitis is a complex one, and provide support for the argument that both cells and bacteria must be present for hypoglycorrhachia to occur.

Summary. Pneumococcal meningitis was produced in normal and irradiated dogs. The latter became leukopenic and the pleocytosis occurring in the CSF in response to the infection was suppressed. The number of bacteria in the CSF was the same in both groups, but CSF sugars remained consistently normal in the irradiated animals while hypoglycorrhachia occurred in 50% of controls. These findings suggest that both cells and bacteria are necessary to lower the CSF sugar in bacterial meningitis.

1. Merritt, H. H., Fremont-Smith, F., *Cerebrospinal Fluid,* Philadelphia and London, W. B. Saunders Co., 1937.

2. Goldring, S., Harford, C. G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 669.

3. Baltch, A., Osborne, W., *J. Lab. Clin. Med.,* 1957, v49, 382.

4. Froesch, E. R., Renold, A. E., *Diabetes,* 1956, v5, 1.

Received September 3, 1959. P.S.E.B.M., 1959, v102.

Liver Regeneration in Experimental Carbon Tetrachloride Intoxication.* (25357)

CARROLL M. LEEVY,† ROBERT M. HOLLISTER, RUDI SCHMID, RICHARD A. MACDONALD AND CHARLES S. DAVIDSON

Thorndike Memorial Lab., Second and Fourth (Harvard) Medical Services, and Mallory Inst. of Pathology, Boston City Hosp., and Depts. of Medicine and Pathology, Harvard Medical School

Hepatic regeneration which follows carbon tetrachloride induced centrilobular necrosis has been assessed previously by histological (1), cytological(2-3), and chemical(4) means. These methods often yielded conflicting results and provided only limited insight into the process of liver cell proliferation. Availability of tritiated thymidine for detection of cells that are actively synthesizing DNA (5-6) made it possible to investigate carbon tetrachloride induced liver regeneration in more detail. Our purpose was to determine the time at which maximum regeneration occurs following a single dose of carbon tetrachloride; to localize the anatomical site and time sequence of parenchymal cell proliferation; and to attempt demonstration of a humoral factor responsible for hepatic regeneration after toxic liver injury.

Material and methods. Forty-five male Sprague-Dawley rats weighing 190-340 g were housed in individual cages and fed a standard diet of Purina Lab. Chow and water *ad lib.* Thirty-six rats were given 0.2 ml of CCl_4/100 g weight intragastrically *via* a polyethylene catheter; saline was given in the same manner

* Aided by grant from NIAID, USPHS.

† Special Research Fellow, USPHS (1958-59).