to 13 mg/100 ml. The upper limit of normal levels has generally been around 20 mg/100 ml although there have been wide ranges reported.

McCRACKEN[3] reported 2 standard deviations above the means as 44 mg/100 ml; ALFORD[2] found 10% of his values to be greater than 19.5 mg/100 ml; SEVER[11] reported only 0.8% of his values greater than 20 mg/100 ml and MILLER[12] found 4% of his values greater than 16 mg/100 ml and 2% greater than 20 mg/100 ml.

The high IgM mean level in this study may be related to a number of reasons, one of which may be the racial composition of the sample population. Of the 20 patients with IgM levels greater than 2 standard deviations above the mean, 5 were shown to have some perinatal problems, but none were shown to have a definite infection.

All patients, reported by ALFORD[3] with elevated IgM level were followed up with bacterial cultures of the throat, stool, urine, blood and cerebrospinal fluid; viral cultures for rubella and cytomegalovirus; X-ray of the chest, skull, and long bones and examination of the cerebrospinal fluid; and, where indicated, a VDRL, FTA-ABS test for syphilis, Sabin-Feldman dye dilution test for toxoplasmosis and hemagglutination-inhibition (HIA) test for rubella. He found a 34% incidence of infection in the group with elevated IgM levels as compared to 0.8% with infection in the control group. These were primarily infections due to cytomegalovirus, toxoplasma, aseptic meningitis and infections of the urinary tract.

MILLER[12] was able to find only 1 infection out of 37 elevated cord IgM levels in a study of 5006 blood samples. In this study, as in MILLER's, infections were not actively saught in asymptomatic infants.

The contention that elevated IgM in the newborn would indicate asymptomatic infection prior to clinical onset is not warranted by the results reported here. It appears that newborns who later develop infections and elevated IgM levels appear within normal range at birth. Elevated IgM levels are not indications of imminant clinical of infections[5, 8, 13].

Zusammenfassung. Es wird eine Radial-Immun.-Diffusionsmethode verwendet, um durch Vergleich der IgM-Antikörper im Nabelschnurblut zu bestimmen, ob im den homologen mütterlichen Proben eine asymptotische Infektion stattgefunden hat. Es ergab sich, dass das benutzte Verfahren nicht zur Klärung der gestellten Frage geeignet ist.

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The Effect of Lipids from *Listeria monocytogenes* on Immune Response in Mice

The author's previous work has shown that *Listeria monocytogenes* cells, given to mice together with sheep red blood cells (SRBC), cause an acceleration, increase in number and prolongation of time of the multiplication of hemolytic plaque-forming spleen cells (PFC)[1]. The adjuvant effectiveness of listeria organisms was found in primary and secondary responses, but it was more pronounced in the former one. Some authors claim that the adjuvant property of listeria cells depends on the lipid fraction obtained from these bacteria[2].

The present experiments have been designed to observe the effect of lipids from listeria cells on multiplication of hemolysin-producing spleen cells and on the level of these antibodies in the sera of mice immunized with SRBC.

Materials and methods. The experiments were performed on male Porton mice (about 20 g body wt.). The lipid fraction of *Listeria monocytogenes* cells was obtained using a technique described by CARROLL et al.[3]. The lipid fractions extracted with chloroform and methanol were mixed and stored in chloroform. They were given to mice i.p. in emulsion[2]. The number of 19 S hemolysin-producing spleen cells was determined per 10^6 spleen cells, using the method

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Influence of lipids from *Listeria monocytogenes* on the development of plaque-forming cells (PFC) in secondary response

1st challenge	2nd challenge	Days after the 2nd challenge 3 Mean ± S.E.	Adjuvant index	4 Mean ± S.E.	Adjuvant index
SRBC+lipids	SRBC+lipids	1208 ± 320	4.2	2652 ± 563	4.1
SRBC+lipids	SRBC	541 ± 67	1.9	1834 ± 324	2.7
SRBC	SRBC+lipids	668 ± 128	2.3	1436 ± 384	2.1
SRBC	SRBC	286 ± 50	1.0	639 ± 76	1.0

Mice were immunized i.p. with 5×10^7 SRBC. The lipids were given by the same route (50 µg per mice). 40 days after the 1st challenge the animals were repeatedly injected with SRBC only or along with lipids. The figures represent number of PFC per 10^6 spleen cells.

of JERNE with the modification of CUNNINGHAM[4]. The level of hemolysin was measured by single radial hemolysis in gel[5]. The results were expressed in units taken from the standard curve which presented the relationship between the area of lysis and the titer of control serum. In all experiments the animals were sacrificed on the 4th day after i.p. injection of sheep erythrocytes. Each experimental group consisted of 6 or 9 mice. The statistical significance of the difference of the mean values was determined by Student's *t*-test.

Results. The results of these studies clearly demonstrate that lipids have an adjuvnt effect on immunological response in mice at the cellular and humoral levels (Figure 1). There was a strict correlation between the amount of lipids used and the final results. It should be noted that the highest doses had the adverse effect. Similar results were obtained when the level of antibody was taken into account; however, the differences depending on the doses of lipids and the final effect were less pronounced.

In further experiments the lipids were used in doses of 50 µg per mouse only. The greatest adjuvant effectiveness has been seen when they were given to mice simultaneously with SRBC (Figure 2). These results represent the number of direct PFC in the spleen as well as the hemolysin level. There was a strict correlation between the amount of antigen injected and the number of PFC, the greater antigenic stimulus resulting in a higher number of PFC (Fig-

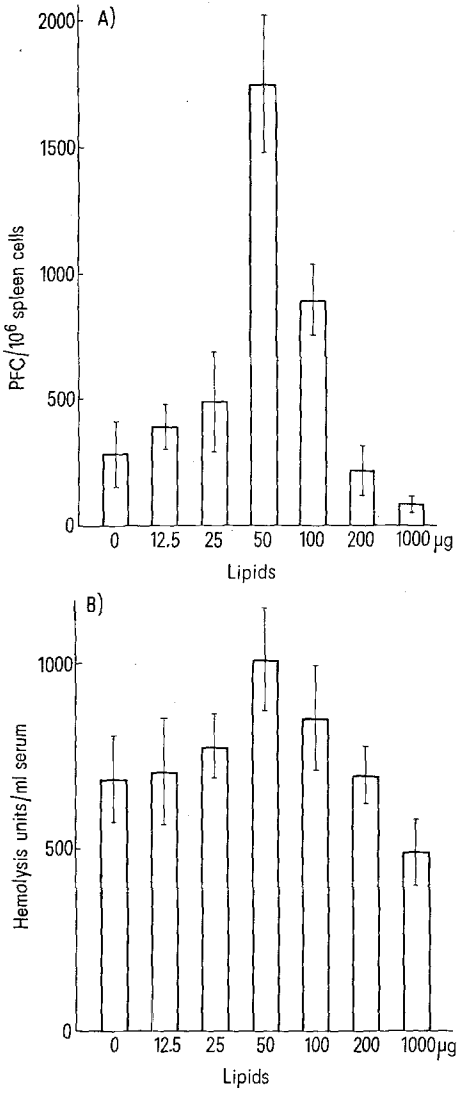

Fig. 1. Influence of different doses of lipids on the number of PFC per 10^6 spleen cells (A) and on the level of hemolysin (B). The mice were given i.p. 5×10^7 SRBC and the lipids.

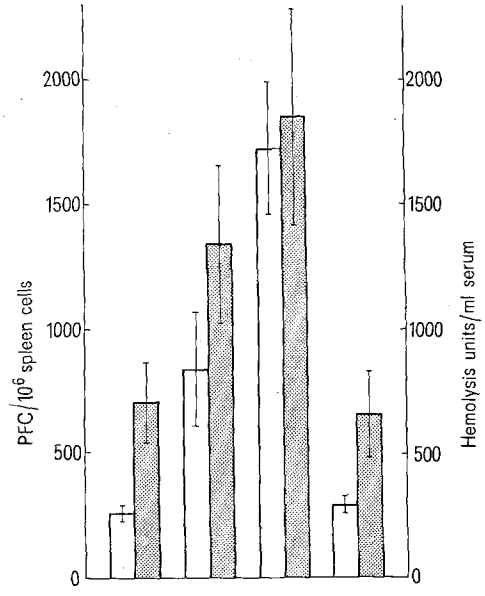

Fig. 2. The number of PFC (white columns) and hemolysin level (dashed columns) after immunization of mice with 5×10^7 SRBC. The lipids were given i.p. to mice 24 h before antigenic stimulus (2nd pair of columns), simultaneously (3rd pair of columns) and 24 h after immunization (last pair of culumns).

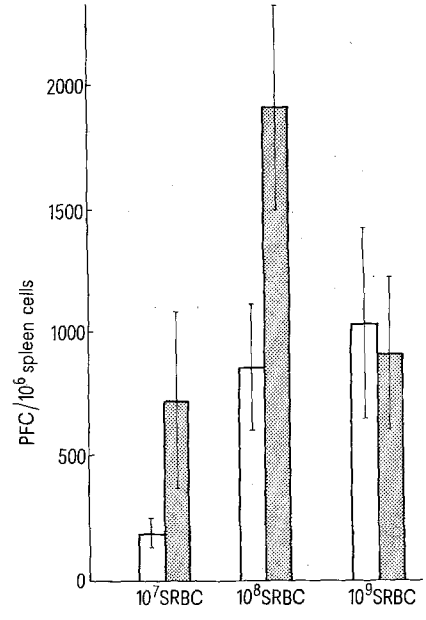

Fig. 3. The number of PFC per 10^6 spleen cells after administration of different doses of SRBC only (white columns) and together with 50 µg of listerial lipids (dashed columns),

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ure 3). However, in case of the highest dose of SRBC, adjuvant effectiveness of lipids was not observed. Further experiments showed that lipids from listeria organisms played an important role not only in primary, but also in secondary response (Table). The lipids administered to mice together with SRBC 40 days before a second challenge with SRBC, resulted in a very high number of PFC.

Discussion. The data accumulated at the cellular and humoral levels indicate that lipids from *Listeria monocytogenes* used in appriopriate dose may be regarded as a strong adjuvant. The depressive action of high doses of listerial lipids probably depends on the damage of immunocytes or disorder of antigen-processing[6,7]. The results give evidence that the adjuvant action of these lipids depends on interaction in the early stages of immune response, probably when antigen is being processed by macrophages or recognized by antigen-sensitive cells[8,9]; the lipids injected after the antigenic stimulus did not show an adjuvant effect. According to some authors the lipids of *Listeria monocytogenes* influence the phagocytic activity of macrophages[10], cause an elevation of wet spleen weight[1] and increase the number of circulating monocytes[2]. It may be concluded that they act by increasing the number of memory cells during the primary response. Similar action of other adjuvants of bacterial origin (*Bordetella pertussis*, endotoxins from Enterobacteriaceae) was shown by FINGER et al.[11,12].

Résumé. Nos expériences ont démontré que les lipides de *Listeria monocytogenes* ont un fort effet adjuvant aux niveaux cellulaire et humoral. Comme antigène, on a utilisé des èrythrocytes de mouton. On a déterminé le nombre de cellules de la rate produisant des anticorps ainsi que le titre de l'hémolysine d'après la méthode de l'hémolyse radiale en gélose.

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Central Glucoprivation: Some Physiological Effects Induced by the Intraventricular Administration of 2-Deoxy-D-Glucose

2-deoxy-D-glucose (2-DG), an unmetabolizable analogue of glucose, blocks glycolysis and produces a 'metabolic paradox' of intracellular glucopenia associated with hyperglycemia[1]. As a result of glucoprivation, systemic administration of large doses of 2-DG induces feeding behaviour[2], inhibition of insulin secretory response to insulin secretagogues[3] and an influence on growth hormone and corticosteroid release[4]. Systemic administration of 2-DG induces also a striking increase in epinephrine secretion, which is the usual response of the organism to glucose unavailability[5,6]. The fact that systemic administration of 2-DG is coupled with cerebral symptoms characteristic of hypoglycemia (drowsiness, stupor and ataxia, hunger and sweating) despite marked hyperglycemia, suggested that both cerebral and peripheral glucoprivation occurred in animals[2] and humans[5]. We have recently been interested in determining whether 2-DG was capable of exerting physiological effects by an action on the central nervous system (CNS) independent of its inhibitory[3] or stimulatory effects[2,4] at the peripheral level. This report summarizes part of our recent work on the effects of the intraventricular (IVT) administration of 2-DG in the unanesthetized rat.

Material and methods. Intact Sprague-Dawley (SD) female rats, 150–200 g or SD female rats, hypophysectomized at 26 days of age, were used in the experiments. Food was withdrawn at the start of each experiment whereas access to water was permitted throughout. A small polyethylene cannula (PE 10) was implanted into the lateral ventricle of the brain under light pentobarbital anesthesia[7] and the rats were then placed in individual cages. After allowing 2–3 days for recovery, the substance was injected through the implanted cannula without anesthesia in a volume of 20 µl.

Blood glucose. A sample of blood was collected for blood glucose (BG) determinations (glucose oxidase) immediately before and at various time intervals (see Results and discussion) following 2-DG or pyrogen-free saline administration[8].

Plasma insulin. Plasma levels of insulin (I) were determined according to a radioimmunoassay (RIA) method previously described[9].

Food Intake. Rats bearing an IVT cannula were individually housed and for 2 consecutive days their spontaneous food intake was measured accurately every hour, beginning at 10.00 h and for the next 6 h. 2-DG (2,5 mg) was injected IVT on the day of the experiment at 10.00 h. Control rats were injected with saline. Food intake ingested hourly on the experimental day was compared to the mean food intake ingested during the same time on the 2 days prior to the experiment. Tap water was available during the experiment, but water intake was not measured.

Body temperature (BT). These experiments were performed in an ambient temperature of 24 °C at 10.00 h. Animals were injected IVT with 2-DG (2,5 mg) or pyrogen-free saline. Core temperature was measured by means of a

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