Serum samples in series A had retinol levels, on average, about 7% lower than those in series B, and since a higher proportion of sera from subjects was in series A, this was a source of bias. Our statistical analyses, however, took account of this either by restricting comparisons between subjects and controls within each series or by standardising for series in the same way as would be done for age or sex.

Serum-cholesterol concentration has been shown to be negatively associated with cancer.[13,14] To see whether the negative association we observed between retinol and cancer was independent of cholesterol, we examined the mean cholesterol level in subjects and controls, preserving the original matched design since age is known to influence cholesterol level as well as risk of cancer. (There was no need to examine the cholesterol data according to whether the samples were in series A or B because the measurements were done when the blood was taken.) The mean cholesterol level among subjects in whom there was no suspicion of cancer when blood was taken was similar to that among their matched controls—namely, 260 mg/dl (SE 6) and 258 mg/dl (SE 5) respectively. Differences in cholesterol levels between these subjects and controls could not therefore account for their differences in retinol levels. However, cholesterol levels were lower in subjects in whom there was originally a suspicion of cancer (246 mg/dl, SE 7) than in their controls (262 mg/dl, SE 5). This difference ($p = 0 \cdot 054$) is consistent with the finding of a previous study,[15] that a low cholesterol level may be a metabolic consequence of cancer rather than a precursor.

The results of this study suggest that it would be worth while to investigate what factors influence serum-retinol levels and whether these can be modified, say, by diet. Such intervention may hold some hope of reducing the risk of cancer.

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Requests for reprints should be addressed to N. W.

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PULMONARY FAT ACCUMULATION AFTER INTRALIPID INFUSION IN THE PRETERM INFANT

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Summary Eight preterm infants who died after 'Intralipid' infusion had fat accumulation in the lungs. The rate of infusion in six of the infants was below the recommended maximum for preterm infants and in no case was the plasma lipæmic on regular visual inspection. Histological examination revealed varying degrees of lung involvement. The commonest finding was distension of empty pulmonary capillaries, but specific staining techniques for fat showed that the capillaries were engorged with large lipid globules. Removal of accumulated fat by histiocytes was seen in infants dying some time after cessation of intralipid infusion. Analysis of homogenised lung showed that those who died after intralipid infusion had a significantly greater ($p < 0 \cdot 001$) concentration of linoleic acid, a marker for intralipid, than infants who died without receiving parenteral fat solution. Fat accumulation after intralipid infusion may be common but unrecognised and may seriously exacerbate ventilation/perfusion inequalities.

Introduction

THE importance of maintaining nutrition in very low birth weight infants during intensive care has led to the introduction of techniques such as nasojejunal feeding and total parenteral nutrition. Critical evaluation of these methods of feeding is essential, both to establish their effectiveness and to eliminate untoward hazards. We report here eight cases, seen over two years at Hammersmith Hospital, of intravascular fat accumulation in the lungs of immature infants. We provide evidence that this is due to 'Intralipid' (Kabi Vitrum Ltd.) infusion and believe the problem to be commoner than hitherto realised.

Patients and Methods

Patients

Clinical details of eight infants put on a total parenteral nutrition (TPN) regimen described below are shown in table I. All infants were born at 32 weeks of gestation or less, and all weighed less than 1500 g at birth. Four were small for gestational age. All the infants had severe respiratory difficulties related to immaturity or aspiration pneumonia, and seven were treated with theophylline or aminophylline. Three infants were given oral indomethacin in an attempt to close a patent ductus arteriosus.

All infants were fed on a regimen of 'Vamin' glucose, 5 or 10% dextrose electrolyte solution, and vitamins and intralipid 20%. The intralipid was infused continuously by syringe pump through a "Y" connector simultaneously with the other two solutions. One gram of fat per kilogram bodyweight was given on the first day, increasing to a maximum of 3 g fat/kg body-weight depending on tolerance.[1] Serum, obtained by heel stabs, was examined 6 hourly for lipæmia by naked eye appearance, and no infant in this study had lipæmic serum. Details of intralipid infusion are shown in table II.

At necropsy the infants generally had multiple lesions, including hæmorrhagic and ischæmic lesions of the brain, necrotising enterocolitis, foci of infection, and bile stasis of the liver, and each of a pair of twins had œsophageal atresia and tracheo-œsophageal fistula. All had lipid deposition in the lungs. In the one infant who died within

4 h of commencement of intralipid infusion there was fine droplet lipid visible within the pulmonary vessels. In infants who died during the course of intralipid infusion or within 2 days of its cessation routine paraffin sectioning (fig. 1) showed distended, empty pulmonary capillaries, which often had a refractile "halo" effect. In frozen sections the capillaries were seen to be distended with large fat globules (fig. 2). One case had multiple lung infarcts which contained masses of neutral lipid. In the case in whom intralipid infusion had been discontinued 15 days before death much of the lipid within the capillaries had been broken up into small droplets and there were lipid laden macrophages within the alveoli (fig. 3).

Methods

Lung homogenates from cases 1–4, and 6 and 7, were analysed by gas liquid chromatography (GLC). Frozen lung samples were homogenised in an equivalent weight of water by the use of a 'Polytron PCU-2' tissue homogeniser at setting number six for at least 2 min. Tissue samples were maintained in ice during this procedure. Formalin-fixed tissue was washed with several volumes of water over 24 h before homogenisation.

Lung lipids were extracted from the homogenates in three volumes of chloroform/methanol and dried under nitrogen. The fatty acids were esterified with 5% sulphuric acid in methanol, and extracted into petroleum ether at 60°–80°C. An internal standard of margaric acid (C17:0) was added for quantitation. Separation was achieved in 10% (w/w) 'Silar 10C' on 'Chromosorb W-HP' 100–120 mesh (Field Instruments Ltd, Twickenham, Middlesex) at 180°C by the use of a Pye 104 gas chromotograph.

For all infants except case 1 the lung had been preserved in 10% formol-saline. In case 1 some frozen lung was analysed and the rest immersed in 10% formol-saline for 1 week before examination. The effect of the formalin is to reduce the proportion of double bonds of the fatty acids and hence the proportion of linoleic acid (C18:2);

TABLE I—CLINICAL DETAILS OF INFANTS

Case no.	Sex	Gestation (weeks)	Birth-weight (g)	Day	Clinical progress
1	F	27 AGA	770	0–7	Severe HMD requiring IPPV and CPAP
				7	PDA closed with indomethacin
				12	Pulmonary œdema and cardiac arrest after acute fluid overload (infusion pump malfunction)
				12	NEC
				12	Onset of apnœa treated with CPAP and theophylline
				25	Post-hæmorrhagic hydrocephalus
				26	Conjugated hyperbilirubinæmia
				29	Died
2	M	30 SGA	900		First of twins
				0–2	HMD requiring IPPV
				1	Diagnosis of œsophageal atresia and tracheo-œsophageal fistula
				3	Recurrent apnœa treated with aminophylline
				22	Gastrostomy and jejunostomy
				36	Terminal massive abdominal distension
				42	Died
3	M	30 SGA	1080		Second twin (see case 2)
				1	Oesophageal atresia without apparent fistula
				2	Recurrent apnœa; aminophylline started
				6	IPPV for apnœa and pulmonary hypersecretion
				10	Died
4	M	27 AGA	907	1	Recurrent apnœa treated with CPAP and aminophylline
				6	IPPV for refractory recurrent apnœa
				21	Died
5	M	28 SGA	760	1	IPPV for severe apnœa
				2	Clinical diagnosis of massive intraventricular hæmorrhage
				3	Died
6	M	32 SGA	1230	1	IPPV for severe apnœa; continued intermittently
				6	Cardiac failure and hæmorrhagic pulmonary œdema due to PDA. Indomethacin given
				18	Pulmonary hypersecretion and pneumonia
				30	Generalised convulsions controlled with phenobarbitone
				36	Conjugated hyperbilirubinæmia
				42	Thoracotomy to ligate PDA
				47	Pneumothorax and severe bleeding disorder
				47	Died
7	F	29 AGA	960	1	Recurrent apnœa treated with aminophylline & CPAP
				4	IPPV for apnœa (4 days)
				5	Intermittent ventilatory support for apnœa until death
				30	Convulsions
				31	Death
8	M	27 AGA	950	1–14	Recurrent apnœa treated with CPAP and/or aminophylline
				17	Cardiac failure due to PDA. Frusemide, digoxin and indomethacin given. IPPV started
				32	Palpable left kidney, and *Candida albicans* isolated from urine. Treated with systemic miconazole
				33	NEC
				36	Died

AGA–appropriate for gestational age
HMD–hyaline membrane disease
CPAP–continuous positive airway pressure
NEC–necrotising enterocolitis
SGA–small for gestational age
IPPV–intermittent positive pressure ventilation
PDA–patent ductus arteriosus

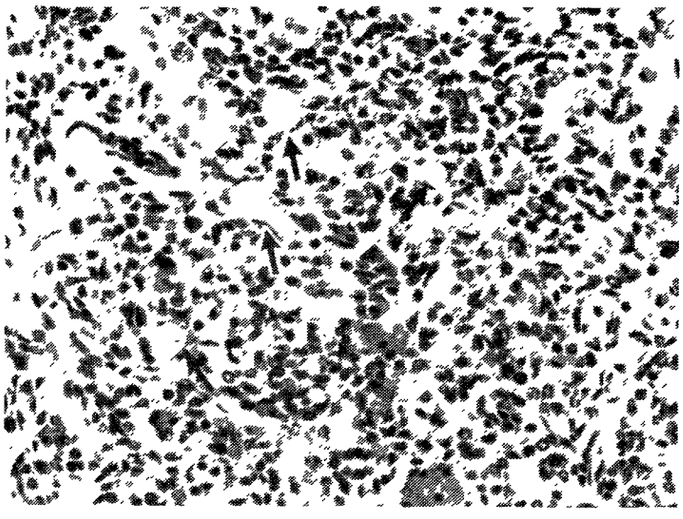

Fig. 1—Case 8. Lung, paraffin section.

Haematoxylin and eosin, reduced by ⅓ from ×400. Distended empty capillaries (arrowed).

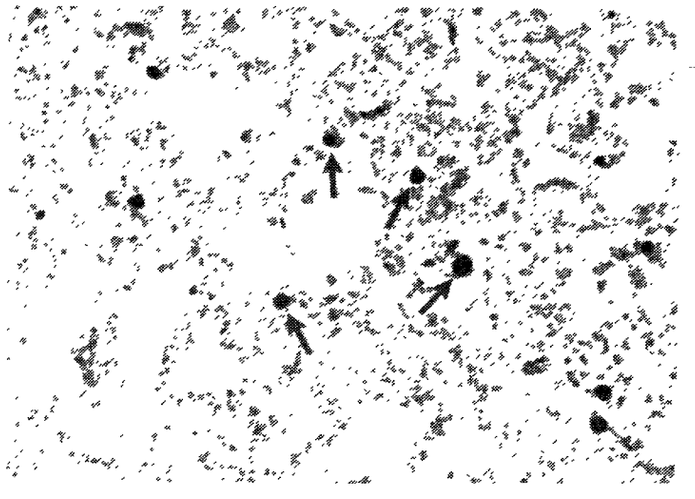

Fig. 2—Case 8. Lung, frozen section.

Oil-red "O", reduced by ⅓ from ×150. Large fat globules (arrow) distending many pulmonary capillaries.

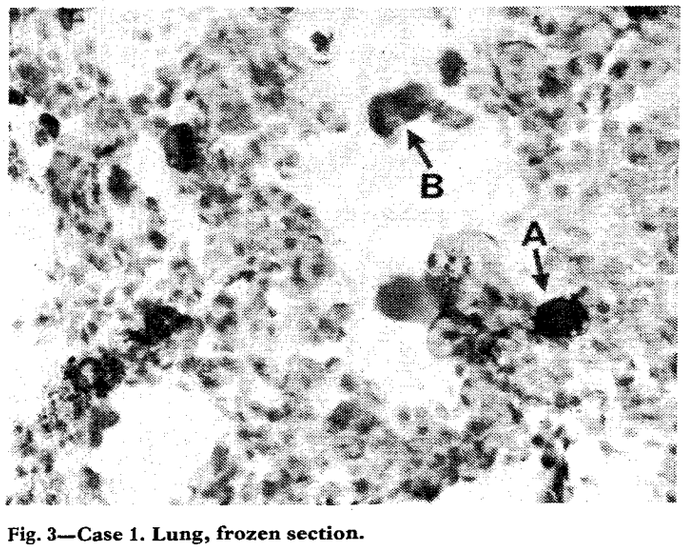

Fig. 3—Case 1. Lung, frozen section.

Oil-red "O", reduced by ⅓ from ×400. Lakes of lipid plugging capillaries (A) are partly broken up into smaller droplets. Lipid filled macrophages (B) are seen in the air spaces.

TABLE II—DETAILS OF INTRALIPID (IL) 20% INFUSIONS

Case	Age at onset of TPN (days)	Duration of IL infusion (days)	Interval between cessation of IL and death (days)	Mean rate of fat admin. (g/kg/h)	Max rate of fat admin. (g/kg/h)	Max fat admin. (g/kg/24 h)
1	2	14	8	0·07	0·26	0·16
2	3	23	10	0·12	0·39	3·30
3	3	6	0	0·09	0·19	2·05
4	8	11	2	0·10	0·44	0·18
5	3	4 h	0	0·36	0·39	0·11
6	5	28	15	0·09	0·32	4·00
7	11	18	0	0·19	0·42	2·40
8	7	27	2	0·11	0·14	2·95

consequently the percentage of this fatty acid will be an underestimate in cases 2 to 7. Lung specimens were not available from cases 5 or 8.

Results

Table III shows the proportion of fatty acids in six infants together with the fatty acid profile of frozen lung homogenates of twelve infants of gestational age from 27 to 40 weeks who died without receiving intralipid. The proportion of fatty acids in intralipid is also shown.

A comparison between group I (formalin specimens only) and group II (frozen specimens only) using the Wilcoxon unpaired test of significance shows a significant difference between linoleic acid ($p<0·001$) and palmitic acid ($p<0·05$) in the two groups. The difference was not significant for the other fatty acids.

Discussion

Intralipid 20% consists of soybean triglyceride (100 g) in water, emulsified with purified egg yolk phospholipids (6 g), and it is made isotonic by the addition of glycerol (12·5%); it

TABLE III—PERCENTAGE OF FATTY ACIDS IN LUNG HOMOGENATES ANALYSED BY GLC

	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Others
Group I						
Case 1 (formalin)	10	21	35	12	1	21
Case 1 (frozen)	10	16	32	20	2	20
Case 2	22	18	37	10	1	12
Case 3	23	13	36	18	2	8
Case 4	38	15	19	13	1	14
Case 5	–	–	–	–	–	–
Case 6	49	12	21	7	0	11
Case 7	23	20	24	15	1	17
Group II						
A	40	15	21	3	2	19
B	23	19	30	7	1	20
C	52	10	22	5	0	11
D	45	12	28	3	0	12
E	54	14	19	3	0	10
F	44	15	23	3	0	15
G	44	11	24	3	0	18
H	40	16	26	5	0	13
I	42	14	23	5	0	16
J	35	17	27	2	0	19
K	38	15	25	4	0	18
L	46	14	22	3	0	15
Intralipid 20%	10	5	25	50	10	0

Group I infants had received intralipid 20%; group II controls had not had intralipid. All group I specimens were preserved in formalin, except for case 1 from whom a frozen section was available as well. In all group II cases, frozen specimens were analysed.

has a neutral pH. The intralipid particles are of homogenous size (0·13–0·16 μm diameter) and are of the same order of size as endogenously produced chylomicrons (0·05–0·6 μm diameter). There is evidence that the particle size of intralipid 20% is somewhat larger than that of intralipid 10%.[2]

Intralipid has been widely used as a source of calories and seems to be remarkably free from complications, although fat embolus during infusion with modern fat emulsions have been described, but rarely.[3-6] The only previous report of fat embolism in the neonate describes four infants in whom fat was distending the capillaries of the lungs at necropsy.[6] The similarity of this condition to traumatic fat embolus is commented on in all the reports, and most of the infants described by Barson et al.[7] were extremely ill. These authors question whether the intravascular fat is of endogenous origin.

Our eight patients were all very immature. Their median birth weight was 925 g, and all were being treated for severe respiratory difficulty. Seven of the eight infants had intractable, recurrent apnœa and all received aminophylline. The eighth infant was artificially ventilated for most of his life and had pneumonia due to cytomegalovirus. In no baby did the serum appear lipæmic on regular naked eye examination, and the average rates of infusion in six of the infants were well below the recommended limits of Gustafson et al.[8] (0·15 g fat/kg body-weight in preterm infants if appropriate for gestational age), and Wretlind[9] (0·17 g fat/kg body-weight).

Of the two infants who were over-infused, one (case 5) received intralipid for only the 4 h immediately before death, and the other (case 7) showed no signs of fat overload during life. All infants had a maximum rate of administration well over the recommended limits, but these peak infusion rates never lasted more than an hour, and could not have been avoided given the inaccuracies of the infusion pumps used. Care has been taken to avoid lipæmia of the serum, but visible lactescence correlates very poorly with free fatty acid, cholesterol, and triglyceride levels during intralipid infusion.[10]

To exclude the possibility of the fat being of endogenous origin, lung homogenate was analysed for fatty acid composition. Intralipid triglyceride contains 50–55% linoleic acid (C18:2) and only 8–10% palmitic acid (C16:0). Linoleic acid is an essential fatty acid in humans. Its proportion in the lung is low and that of palmitic acid high.[11] Linoleic acid can be used as a marker of intralipid distribution, and table II shows high concentrations of this fatty acid in lung homogenates of infants who received intralipid compared with non-intralipid controls ($p<0·001$). There is an inverse relation between the two groups of palmitic acid ($p<0·05$).

In no case reported here could death be directly related to intralipid fat accumulation, but the amount of fat in the pulmonary vessels of some infants must cause considerable ventilation/perfusion inequality in those who already have severe respiratory difficulties. In cases 1 and 6 in whom fat infusion had been discontinued for 8 to 15 days before death, many foamy macrophages were seen in the lung parenchyma around accumulations of fat. Breakdown of fat was occurring in these cases, and suggested that fat droplets would be removed eventually in infants who survived. Experimental evidence in rats[12] supports this possibility—intravenous infusion of unstable fat emulsions resulted in large fat globules distending the pulmonary vessels, and this appearance in the lungs was much more severe than in any other organ,[12] but the fat accumulation was not always fatal, and excess fat was removed from the lungs slowly but definitely over a period of weeks. It is reasonable to assume that fat accumulation in the lungs occurs in many ill preterm neonates during intralipid infusion and that fat is removed by the reticuloendothelial system.

In man intralipid appears to be handled in the same way as endogenous chylomicrons of dietary origin.[13] In infancy, however, the efficiency of metabolism is less well established. Forget[14] showed that children differed widely in their tolerance to intralipid, and some became hyperlipæmic whilst receiving low quantities of fat. Premature[15] and small for gestational age[8] infants have poor clearance of intralipid and an increased free fatty acid level after infusion with fat emulsion.

There are several possible reasons for the impaired ability of immature neonates to handle intralipid. Lipoprotein lipase (LPL) is present on lung capillary endothelium in adults,[16] but the amount available in neonatal lung is unknown. The LPL content of rat lung is seven times higher in the adult than it is in the fetus only 2 days before birth.[17] It appears reasonable to assume that LPL levels in the immature human lung are also very low. The neonate also has a limited capacity for fatty acid oxidation,[18] and long-chain fatty-acid metabolism is highly dependent on the presence of carnitine, but the level of this substance in the premature infant is very low.[19] Consequently the immature neonate seems poorly equipped to handle the fat emulsion.

Intravascular fat accumulation in the lungs of critically ill preterm infants has been seen in every one of the last eight infants who have died after receiving intralipid at this hospital. The condition may be missed at necropsy if not specifically looked for, and we believe it to occur in infants who survive. Intralipid 20% has been used exclusively over the past 2 years at Hammersmith Hospital. This solution may be inherently less stable than 10% intralipid. Monitoring of intralipid infusion and detection of overdosage require sophisticated techniques and free fatty acid and triglyceride levels must be measured frequently since intralipid levels bear poor correlation with overload.[10] However, until close biochemical monitoring is shown to reduce the risk of intralipid pulmonary fat accumulation the place of intralipid in total parenteral nutrition for the ill premature infant must be questioned. We are currently carrying out a prospective controlled study on the safety and benefit of intralipid 10% in the neonate in an effort to resolve this problem.

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Requests for reprints should be addressed to M. I. L.

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DIMINISHED BACTERIAL DEFENCES WITH INTRALIPID

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Summary 'Intralipid', a lipid emulsion used in parenteral nutrition, impaired bacterial clearance and enhanced bacterial virulence in mice. In addition it inhibited the chemotaxis of human neutrophils in vitro. Intralipid may enhance the risk of bacterial sepsis in certain patients.

Introduction

'Intralipid', an emulsion of soya bean oil, egg lecithin, and glycerol, has become a standard component of parenteral nutrition regimens in many hospitals.[1,2] Many of the patients are immunodeficient because of chronic debilitating illness, malnutrition, malignant disease, or prematurity, and are at increased risk of infection.[3-5] Theoretically, intralipid might be expected further to lessen resistance to infection: lipid substances such as oleic acid are used in laboratory animals to produce reticuloendothelial blockade and to increase susceptibility to infection;[6] and lipid accumulation has been reported in the reticuloendothelial system (RES) of human beings receiving intralipid infusions.[7] We have evaluated the effect of intralipid on the susceptibility of mice to bacterial infections and on the chemotaxis of human neutrophils.

Materials and Methods

Bacteria

Group B streptococcus (GBS) strain D136C, a standard type III strain, was kindly supplied by Dr Rebecca Lancefield, Rockefeller University. This organism has been studied in our laboratory in mice and is a relatively avirulent strain. Group B streptococcal strain IIINor is a type III strain isolated from the cerebrospinal fluid of an infant with meningitis. This organism has high virulence in the AKR mouse. The bacteria were grown to mid-log phase in Todd Hewitt broth (THB) and stored at −70°C until used.

Mouse Lethality and Bacteræmia

To evaluate the in-vivo effect of lipid emulsion, 6-week-old AKR/J mice were given intraperitoneal injections of intralipid (Cutter Laboratories) 2·5 g/kg per dose, by the following schedule: 1 dose on day −2, 2 doses on day −1, and 1 dose on the day of infection. The bacteria were cultured on blood agar plates overnight and then grown to mid-log phase in THB and adjusted to the appropriate dilution by optical density analysis. 2-3 h after the last dose of intralipid, 0·5 ml of either 1×10^8 GBS type III (strain D136C) or 1×10^6 GBS type III (strain IIINor) was given by intraperitoneal injection. We have previously studied these bacteria in the AKR mouse and these challenge doses were found to be below 1 LD_{50}.

Blood cultures were obtained from 22 mice to evaluate the effect of lipid emulsion on bacteræmia. 1 μl samples of blood from the tail vein were drawn into sterile micropipettes 24, 48, and 72 h after infection and cultured in 1·0 ml of THB. After 24 h of incubation at 37°C, all broth cultures were subcultured on blood agar for positive GBS identification.

Chemotaxis

Citrated peripheral venous blood from healthy adult volunteers was collected and centrifuged on 'Ficoll-Hypaque' gradients followed by dextran sedimentation.[8] Erythrocytes were lysed with hypotonic saline and the resulting leucocyte suspensions were washed twice in Hanks' balanced salt solution (Microbiological Associates Inc.) without Ca^{++} and Mg^{++}. Greater than 95% viable neutrophils were obtained by these techniques.

Zymosan-activated serum was prepared by a modification of the method of Vallota.[9] 10 ml of activated human serum was fractionated by gel filtration through 'Sephadex G-100' as previously described.[10] Fractions corresponding to the molecular weight range of 10 000 to 25 000 daltons were pooled. The partly purified fragment of the fifth component of complement, referred to hereafter as C5a, was tested for chemotactic activity at a 1:7·5 dilution of the original serum volume. Before assay for chemotactic response the C5a was further diluted to 1:2 with veronal (barbitone) buffer.

A modification of the neutrophil chemotaxis radioassay described by Gallin[11] was used to measure chemotactic response of adult neutrophil populations. After isolation the cells were adjusted to a concentration of $2·0 \times 10^7$ neutrophils/ml and incubated for 30 min with various concentrations of intralipid. After washing to remove the intralipid, 1 μCi of $Na_2{}^{51}CrO_4$ in 0·9% saline was added per 10^6 neutrophils, then the cells were incubated at 37°C for 60 min with gentle agitation. The cells were washed twice with Ca^{++} and Mg^{++} free Hanks' balanced salt solution and resuspended to $3·0 \times 10^6$ neutrophils/ml in Gey's balanced salt solution containing 2% human serum albumin. A 0·5 ml volume was added to the upper compartment of a two-compartment chemotaxis chamber; the lower compartment contained buffer, casein, zymosan-activated serum, or C5a. The two compartments were separated by two cellulose nitrate

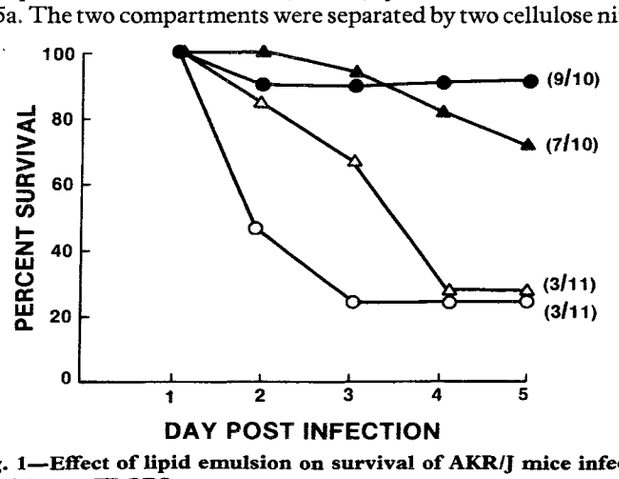

Fig. 1—Effect of lipid emulsion on survival of AKR/J mice infected with type III GBS.

Strain D136C alone (●), or D136C + lipid (○); strain IIINor alone (▲), or IIINor + lipid (△).

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