man. The objections to their use are numerous and include data recorded in animal studies which link water-in-mineral oil adjuvants to autoimmune disease, allergy and cancer. Moreover, there are unresolved questions concerned with the long term effects of retention of mineral oil in tissues. *C. burneti* presents another kind of immunologic adjuvant and is one of rickettsial origin which together with those of bacterial origin (*Brucella abortus*(7), *Bordetella pertussis*(8)) might constitute a family of adjuvants of biologic origin deserving of intensive study to isolate and to characterize the immunologic potentiating factors. In each of the 3 instances mentioned the enhancing organism is itself an agent of disease against which an effective immunity is desirable.

Summary. 1. Two doses of a partially purified, concentrated, formalin-killed epidemic typhus vaccine yielded excellent antibody responses in guinea pigs and in man. Even a single dose of this vaccine elicited an appreciable antibody response in man. 2. The compatibility of the epidemic typhus vaccine with a formalin-killed Q-fever vaccine administered as a mixture has been established in both guinea pigs and in man. 3. The *C. burneti* component of the combined vaccine definitely serves as an immunologic adjuvant for the epidemic typhus antigen in guinea pigs and may enhance the antibody response to the epidemic typhus component to some degree in man.

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Alterations in Erythrocyte Phospholipids Produced by Environmental Change.* (32318)

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Phospholipids undoubtedly play an important structural and functional role in the erythrocyte membrane. The percentage relationships among individual membrane phospholipids, their fatty acid composition and the permeability characteristics of the membranes vary among mammalian species(1). Although changes in the fatty acids of individual membrane phospholipids are readily produced by dietary manipulation in human subjects(2) and in animals(1,3,4), phospholipid composition itself is felt to be characteristic of, and fixed in, each species(1,5). In man, careful study has shown no variation in red cell phospholipid composition with dietary manipulation(2), in malnutrition(6), and in a variety of hematological disorders (4), with the exceptions of a-beta-lipoproteinemia(6) and of four cases of hereditary nonspherocytic hemolytic disease(7). The existence of abnormal plasma phospholipids in a-beta-lipoproteinemia and the ready exchange of P^{32}-labelled phospholipids between

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plasma and red cells(8) make it possible that certain types of red cell membrane phospholipids may be more responsive to changes in plasma phospholipids than is generally held.

In this study, red cell phospholipid composition has been determined in the presence of normal and of altered plasma phospholipid composition to assess the variability of erythrocyte membrane phospholipids in the presence of changes in their environment.

Methods. Heparinized plasma was obtained from hospitalized patients and from normal subjects in the fasting state. Red cells, washed 3 times with .15 M NaCl, were suspended in NaCl to an hematocrit of approximately 50%. Hematocrit was determined in duplicate. Two 5 ml portions of each red cell suspension were then hemolysed by addition of equal volumes of H_2O and the lipids were extracted by MeOH, then $CHCl_3$, then MeOH-$CHCl_3$ 1:1 (V/V) twice more as described by Reed *et al*(9). Seventy-five ml of 0.1 M KCl were added to each 150 ml of extract. The resulting emulsion was broken by centrifugation, and the aqueous phase was removed. Solvent was removed from the $CHCl_3$ phase by a rotary vacuum evaporator at 40°C and lipid was reextracted in $CHCl_3$. After evaporation of $CHCl_3$, lipid was dissolved in isoamyl alcohol-toluene 1:1 (V/V). Duplicate samples containing 10 μg phosphorus (P) were applied to sheets of Whatman SB-81 paper, and individual phospholipids were separated by ascending chromatography for 2½ hours at 4°C in di-isobutyl ketone:acetic acid:water = 40:25:5. Lipids were detected by Rhodamine 6G staining under ultraviolet light. Each lipid spot was eluted 3 times for a total of 3 hours with 0.1 N HCl in redistilled MeOH at 64°C. Phosphorus was determined by a modification of the Bartlett method. Plasma lipids were extracted 3 times with a total of 15 V MeOH: $CHCl_3$ 1:1 (V/V). Seven V H_2O was added to each pooled extract. Plasma samples were prepared for chromatography as described above for red cell lipid samples. Triplicate samples containing 10 μg P were applied in isoamyl alcohol-toluene to Whatman #1 paper impregnated with silicic acid. Individual phospholipids were separated overnight by ascending chromatography in di-isobutyl ketone:acetic acid:water 40:23:4 at room temperature. Analysis was carried out as described for red cell lipids.

Fatty acid methyl esters of red cell glycerophospholipids were prepared by transesterification with anhydrous sodium methoxide, and were analysed by gas-liquid chromatography on a Perkin Elmer 15D instrument containing a Perkin Elmer P column, at 190°C. Helium carrier gas at 25 psi and a flame detector were used. The area under each peak was used to calculate percentage composition.

Results and discussion. Sphingomyelin (Sph), lecithin (Lec), phosphatidylserine (P S) and phosphatidylethanolamine (P E) were the phospholipids clearly separated in chromatograms of red cell lipids. Lysolecithin, inositol phosphatide, and polyglycerol phosphatide are identifiable in small amounts by other analytical systems(9). Polyglycerol phosphatide was not seen in our system. Lysolecithin and inositol phosphatide were not clearly separated from sphingomyelin, and were partially included in its analysis. Lec, Sph, and lysolecithin were the major components of plasma phospholipids. Small amounts of P E and of inositol phosphatide were seen but were not quantitated. Results are reported as the amount of individual phospholipid phosphorus divided by the total amount of phosphorus recovered from the chromatogram.

Table I presents the phospholipid composition of red cells of 3 normal subjects which showed good agreement with mean data from 4 series of normal subjects in the literature. Three red cell lipid samples were analysed by column chromatography using neutral solvents, as well as by paper. Paper gave equal or higher values for P E + P S, and equal or lower values for Lec than did column, indicating that negligible breakdown of P E or its plasmalogen form occurred during the paper chromatographic analysis.

Table II shows plasma and red cell phospholipid analyses of 12 patients with diseases associated with changes in plasma phospholipids. Abnormalities, when present in plas-

TABLE I. Phospholipid Composition of Red Cells of Normal Subjects.

	Sph %	Lec %	P S %	P E %	μg P/ml packed red cells
W.N.	20.4	33.2	16.2	30.2	115
	21.7	33.4	15.4	29.5	117
S.W.	24.3	33.8	14.2	27.7	132
	24.1	32.0	15.3	28.6	123
E.E.	21.5	33.0	15.1	30.4	135
	24.1	31.7	13.8	30.3	135
11 Subjects (6)	23.8	29.5	15.0	25.7	129*
6 *idem* (11)	22.8	32.9	42.5†		145
20 " (9)	22.0	30.0	14.9	24.6	137
14 " (10)	21.0	36.0	10.0	29.0	119

* Published value is 1.16×10^{-11} mg P per cell. Calculation of 129 μg P/ml packed red cells based on assumption of 45% het and 5×10^6 rbc/mm³ whole blood.
† P S + P E.

ma, consist of a decrease of the percentage of lysolecithin and of Sph and an increase in the percentage of Lec. Red cell changes consist of an increase in Lec percentage usually with a decrease of P E percentage and lesser changes in Sph or P S. Changes in red cell phospholipids appear to correlate positively with changes in plasma phospholipids. Plasma of subjects with liver disease is associated with more change in red cell phospholipids than is lactescent plasma (subject J.O.), due possibly to differences in soluble lipoproteins in these two states. It is noteworthy that subject M.F. who also has lipemia and moderately abnormal plasma phospholipids, has normal red cell phospholipids. The explanation which we favor for the observed abnormalities of red cell phospholipids is that changes in red cell membranes occur during circulation, and are mediated by their interaction with plasma lipoproteins. The marrow is a less likely site for induction of the abnormalities in view of the rapid return to normal of red cell and plasma phospholipids of subject L.Y. after stopping alcohol and eating adequately. Comparison in Table II of the degree of red cell phospholipid abnormality with lipid phosphorus content suggests that the abnormalities of phospholipid composition may at times be due to selective uptake of lecithin by the cells, increasing total phospholipid content to levels above normal, and at other times be due to phospholipid exchange with plasma, resulting in the maintenance of a normal total phospholipid level.

Table III shows the percentage fatty acid composition of the red cell glycerophospholipids (P E, P S and Lec) of 4 subjects. Differences among subjects may be due to variations in previous diet(2). Lack of a consistent pattern of change indicates, as others have shown(2,4), that the relationship between fatty acid patterns and phospholipid composition is not fixed.

Since the initial report of our observations in abstract form(12), an abstract by Ways (13) has reported a similar red cell pattern in a patient with liver disease. The latter shows by means of serial observations, as does our subject L.Y. (Table II), that the abnormalities of red cell phospholipid composition may be reversible.

Summary. In 10 subjects with liver disease and jaundice, and in one out of 2 hyperlipemic subjects, abnormalities in red cell phospholipid composition were found, consisting principally of elevation in percentage of Lec and decrease in percentage of P E, with lesser changes in P S and Sph. Total red cell lipid phosphorus ranged from normal to elevated. Fatty acids of red cell glycerophospholipids showed a variable pattern. Plasma phospholipids were abnormal in 11 out of 12 subjects. In one subject whose disease improved, plasma and red cell phospholipids returned to normal. The phospholipid composition of the erythrocyte membrane has thus been shown to correlate with changes in plasma phospholipids, while the

TABLE II. Phospholipid Composition of Plasma and of Red Cells in Diseases Associated with Altered Plasma Phospholipids.

				Plasma			Red cell				
Subject	Date	Clinical state	μg P/ml plasma	Lyso-lecithin %	Sph %	Lec %	Sph %	Lec %	P S %	P E %	μg P/ml packed red cells
D.L.		biliary cirrhosis, jaundice	127	3.7	9.1	87.2	20.0	41.3	14.2	24.5	139
W.		alcoholic cirrhosis, jaundice	69	6.9	17.8	75.3	22.5 20.6	36.5 38.2	15.6 18.8	25.5 22.4	125 127
L.W.		ca of colon metastatic to liver, jaundice	160	2.7	17.3	80.0	18.6 19.4	48.7 46.6	12.0 13.0	20.7 21.0	148 148
L.Y.	12/30/64	alcoholic cirrhosis, jaundice	86	3.7	17.3	79.0	22.0 22.0	43.8 40.4	15.3 14.9	18.9 22.7	142 156
	1/12/65		77	5.3	20.5	74.2	24.7 23.7	34.9 36.0	16.2 16.0	24.2 24.2	125 124
R.A.	1/26/65	serum hepatitis, jaundice	170	2.0	12.9	85.1	20.0 19.1	42.7 44.4	13.3 12.8	24.0 23.7	170 183
	3/ 5/65		102	6.4	16.8	76.8	21.2 22.8	37.7 35.6	14.3 14.7	26.8 26.8	148 146
C.W.	3/10/65	infectious hepatitis, jaundice	156	5.4	12.4	82.2	16.0 14.8	54.5 54.6	8.8 9.7	20.7 21.0	155 156
	3/20/65		101	5.5	18.2	76.3	19.5 17.7	48.8 48.4	9.1 11.9	22.6 22.0	148 143
J.F.		chronic hepatitis, jaundice	83	4.0	16.8	79.2	21.2 20.9	41.5 42.8	14.5 12.8	22.8 23.5	124
J.G.		ca liver, jaundice	131	3.6	15.8	80.6	15.6	48.1	15.1	21.2	145
E.K.		" " "	59	3.7	22.4	73.9	20.7 22.3	37.7 36.0	15.5 15.4	26.1 26.3	137 137
A.K.		" " "	102	4.5	12.8	82.7	18.2 19.5	45.1 44.6	12.9 13.1	23.8 22.8	138 141
J.O.	6/29/64	diabetes, lipemia	368	6.1	15.9	78.0	25.5	34.6	12.2	27.7	111
	12/14/64		717	2.8	11.0	86.2	16.9 17.2	38.2 40.8	14.8 13.7	30.1 28.4	128 144
M.F.		diabetes, lipemia	140	2.7	18.2	79.1	24.3 22.3	31.1 31.4	15.4 14.8	29.2 31.5	129 132
			Normal values—this laboratory								
9 Subjects			83-125	5.7-9.1	17.2-24.3	66.1-77.1					
3 "							20.4-24.3	31.7-33.8	13.8-16.2	27.7-30.4	115-135

TABLE III. Percentage Fatty Acid Composition of Erythrocyte Glycerophospholipids.

Subject	Date	16:0	18:0	18:1	18:2	20:4
R.A.	1/26/65	28.5	14.7	19.0	16.6	21.2
L.W.		36.3	12.4	20.4	16.8	14.1
L.Y.	12/30/64	33.6	15.8	29.7	12.2	8.7
	1/12/65	42.3	18.2	18.2	18.0	3.2
J.G.		36.5	14.1	20.0	12.9	16.5

fatty acids of the erythrocyte phospholipids show variability in composition.

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Reaction of Heterozygous Swiss Mice to Implantation of C_3H Myeloma X5563.* (32319)

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Myeloma X5563, which originated spontaneously in the cecum of a C_3H/HE mouse, is readily transplanted to mice of this strain (1) with practically 100% takes and uniformly progressive growth. This report deals with the reaction of Swiss mice to allogeneic implantation of this tumor.

Materials. Tumor-bearing C_3H mice were kindly furnished by Dr. George D. Sorenson. C_3H mice were obtained from the Jackson Memorial Laboratories, and random-bred white Swiss mice from a local dealer. All mice were males, 6-7 weeks old when tumors were implanted. They were caged in groups of 5-7, and given Purina laboratory chow and water *ad lib.*

Methods. Implantation. Viable appearing tumor tissue was minced in phosphate buffered saline, and selected fragments with an estimated volume of about 0.1 ml were placed subcutaneously in the right mid-dorsal area, using a 10 gauge trocar. (Trypsin-dispersed cells gave less uniform results.) Fragments from a particular tumor were usually transferred to two genetically different groups of mice: for example, a C_3H tumor to 6 C_3H and 6 Swiss; a tumor from a Swiss mouse to 7 Swiss and 6 C_3H, etc., until significant data for each of the 4 permutations became available (Table I). For implantation, tumors with a volume of 1.5 to 2.0 ml were used; these tumors were excluded from the data since their ultimate fate was unknown.

Progressively enlarging tumors were left undisturbed until their hosts became moribund or died with large (8-15 g) tumors. When rejection occurred, mice were maintained for 20 weeks unless death occurred sooner.

Histology. Selected tumors in C_3H and Swiss mice, and the rejection sites in Swiss mice, were studied by routine formalin fixation, paraffin embedding, and staining by hematoxylin-eosin and by the Giemsa method.

Electrophoretic studies of sera obtained by snipping the tails were carried out in normal, tumor-bearing, and tumor-rejecting Swiss mice, using the cellulose acetate strip method, as well as a Canalco disc electrophoresis apparatus (Model 200 power plant).

Results. From Table I it is seen that implants from C_3H to Swiss and from Swiss to Swiss mice, resulted in 47% and 44% takes, respectively, in contrast to 99+% takes with syngeneic implantation. About 40% of the tumors developing in Swiss mice were rejected after reaching an estimated volume of 0.5 to 4.5 ml. When returned from Swiss to C_3H mice, progressive tumor growth was noted in 100% of 74 mice. The latent period of tumor development was prolonged in the Swiss mice.

Rejection was most often manifested by blackening and extrusion of dried necrotic tumor tissue (Fig. 1) followed by rapid repair by granulation. Less often (with deeper tumors) internal resolution occurred, with unbroken skin. Small secondary tumors sometimes appeared adjacent to or near the rejection sites, but these in turn were always rapidly rejected. Tumors in Swiss mice larger than about 4.5 ml were not rejected, but grew progressively until death occurred.

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