ability of bacterial endotoxins, in turn, to elicit the lesions characteristic of shock, was reviewed. 2) Although the entire quantity of toxic polysaccharide circulating in the irreversibly shocked animal is innocuous to a normal animal, only a fraction of the total is sufficient to inflict lethal injury to an animal with its defenses already depleted by reversible shock. The mounting evidence for the presence of endotoxemia in shock provides a logical basis for incriminating the loss of functional integrity of the R.E. system in the development of irreversibility to transfusion in hemorrhagic shock, and for an understanding of the phenomenon of variation in resistance to shock under different conditions. The development of irreversibility to transfusion in hemorrhagic shock in the germ-free animal is discussed in the light of these considerations.

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Silicic Acid Chromatography of Lipids of Whole Human Blood.* (24373)

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We examined constituent lipids of 2 samples of human blood by chromatography on columns of silicic acid. One sample (A) was provided by a normal donor (male, age 44 years) and the other by a xanthomatous patient (male, age 11 years). The experiments on both samples were conducted simultaneously, under conditions as nearly identical as possible.

Methods. Blood was collected in evacuated bottles containing citrate anticoagulant solution (62.5 ml), and within 5 min. was poured into a shaken flask containing *n*-propanol (800 ml) at −78°. After 2 hr at −20° the resulting mixture was filtered at 0°; the residue was then extracted successively with eight 100 ml portions of propanol at room temp. The total extract was evaporated in a rotary evaporator at <55°, the residue extracted with ligroin (b.p.<60°), and the lipid weighed after evaporation of this solution. It was then chromatographed on silicic acid (50 g, column dia. 28 mm). The eluate was collected (200 hr) in fractions with a drop-counting collector (originally set to deliver 8.5 ml, but the size of fractions varied with lipid and methanol concentrations). The fractions were evaporated in a vacuum oven at <55°, the residues weighed, and the elution curves A and B (Fig. 1) then plotted. The fractions were grouped for chemical study. Seven paper chromatograms of each group were made, using as solvent propanol: 0.25 N aqueous ammonia: acetone (4:1:1, v/v), which rapidly separates lipids from more polar materials (1). The results of tests applied to these chromatograms are shown in Table I, together with findings after hydrolysis(1). Scherer (inositol) and Pettenkofer (bile acid) tests

* Phospholipids containing amino acids other than serine. Part II (Part I, ref. 1). This work was supported in part by grant from Nat. Sci. Fn.

TABLE I

Peak No.		Cholesterol test(3)	Choline	Dragendorff reagent	Ethanolamine	Inositol	NH test of lipid(4)	Ninhydrin test of lipid	Pettenkofer test	Phosphorus	Plasmalogen	Reducing sugar in lipid	Reducing sugar (free)	Sphingosine	Ninhydrin-positive compounds of low R_f detected before hydrolysis[1]	Amino acids liberated by hydrolysis
1	A	+	—	—	—	—	—	—		—	+[h]	—	—	—	—	—
	B															
2	A	+	—	—	—	—	—	—		—	+[h]	—	—	—	—	—
	B															
3	A	+	—	—	—	—	—	—		—	—	—	—	—	—	Ala,Glu,Gly,Leu,Pro,Ser,Val (all w)
	B															—
4	A	w[a]	—	—	—	—	w	—	+	—	—	—	—	+	—	Gly,Leu,Ser,Val (all w)
	B															Ala(w), Gly(w)
5	A	w[a]	—	w	—	—	w[c]	—	—	—	—	—	—	—	—	Val(w)
	B															Ala(w), Gly(w)
6	A	w[a]	—	—	—	—	w[c]	—	—	—	—	—	—	—	—	Ala,Gly,Leu,Val (all w)
	B															
7	A	w[a]	—	—	—	—	w[c]	—	—	—	—	+	—	—	—	Ala,Gly,Leu,Pro,Val (all w)
	B															
8	A	—	—	—	w	w	+	—	—	+	w	—	—	—	—	Ala,Arg,Asp,Glu,Gly,Leu,Pro,Ser,Thr,Val[j]
	B							w								Ala,Glu,Gly,Pro,Ser,Val(w)
9	A	—	—	—	+	w	+	+	+	+	+	—	—	—	—	Ala(w),Glu(w),Gly(w),Ser
	B															Ala(m),Glu(w),Gly(m),Pro(w),Ser,Val(w)

10	A	—	—	—	+	—	+	+	—	+	+	—	—	—		—	Ala(w),Gly(w),Ser
	B					w											Ala(w),Glu(w),Gly(w),Ser(m)
11	A	—	—	w	w	w[b]	w[d]	w	—	w	—	—	w[b]	—		—	Ala(w)[b], Ser(w)
	B					w		+									Ala(w)
12	A	—	—	+[b]	—	+	+[e,f]	+[e]	—	+[b]	w[b]	—	+	—		—	Ala,Glu[b],Gly[b],Leu[b],Pro[b],Ser,Val[b] (all w)
	B		w[b]				w[e,f]	w[e]		+							Ala(w),Glu[b],Gly(w),Leu(w)[b],Pro(w),Ser(w)
13	A	—	+	+	w	w	—	—	+	+	+	—	+	—	Leu(w),Phe(w)		Glu(w), Gly(w)
	B					—										—	Glu,Gly(w),Ser(w)
14	A	—	+	+	—	—	+	—	+	+	+	—	+	+	(CyS)$_2$(w)[b],Leu,Phe(w),Val[b]		Ala(w)[b],Glu,Gly(w),Ser(w)[b]
	B														(CyS)$_2$(w),Leu,Phe,Tyr(w),Val		Ala(w)[b],Glu(w),Gly(w),Pro(w)[b],Taurine(m)[b]
15	A	—	+	+	—	+	+	—	+	+[g]	—	—	+	+	Leu,Pro(w)[b],Tyr(w),Val,U$_1$[b],U$_2$(w)[b]		Ala,Glu,Gly,Ser (all w)
	B														Ala(w)[b],Pro[b],Tyr(w),Val,U$_2$[b]		Glu,Gly,Leu,Ser,Thr (all w)
16	A	—	+	+	w	+	+	—	+	+[g]	—	—	+	+	Ala,Pro,Thr[b],Val(w),U$_1$(w),U$_2$,U$_3$(w)[b]		Glu,Gly,Leu,Ser (all w)
	B														Ala,Gly(w),Pro,Thr,Val(w),U$_2$(w),U$_3$(w)		Glu(m),Leu(w),Ser(w),Tyr(w)
17	A	—	+	+	w	w	w	—	+	+[g]	w	w	+	—	Ala,Pro,U$_4$(w)		Gly(w),Ser(w)
	B														Ala,Glu(w),Gly(w),Pro,Ser,Val(w),U$_3$(w),U$_4$(w)		Leu,Thr,Tyr (all w)
18	A	—	w	+	—	w	w	—	—	+[g]	w	w	+	—	Ala,Asp,Glu,Gly,Pro,Ser(w),U$_3$(w)		
	B																

Chemical data. Names of amino acids abbreviated according to Brand and Edsall(2): w = weakly positive; m = moderately strongly positive.

Major components of various fractions were found to be: 1. cholesterol esters. 2. triglycerides. 3. cholesterol. 4. aramide. 8. phosphatidic acid. 9 and 10. phosphatidyl- and phosphatidal-ethanolamine plus phosphatidyl-serine. 11. urea. 12. inositol phospholipid plus glucose. 13. phosphatidyl- and phosphatidal-choline plus lysophatidyl-ethanolamine. 14. sphingomyelin. Identifications were aided by determinations of infrared spectra of the fractions. Fractions 1, 2, 3, 11 and 12 contained crystalline products.

[a] Green-brown color, *cf* cholesterol, green-blue. [b] In later fractions. [c] Weak spot also at R_F 0.30. [d] Weak spot also at R_F 0.20. [e] Reaction chiefly at R_F ~0.8 instead of ~1.0. [f] Very weak spots of low R_F in later fractions. [g] Spots (at low R_F) besides that of lipid. [h] Could be due to aldehydes cleaved from choline or ethanolamine plasmalogen, to ketosteroids, or to "ubiquinone" ("272 mμ substance")(5). Ultraviolet spectrum of peak No. 2A was not definitive: $\lambda_{max}^{isooctane}$ 230 mμ (diene), λ_{inf} 266 mμ (triene?). [i] Four, unidentified, are not peptides since they were not destroyed by hydrolysis: U$_1$ (mauve spot, faded in *ca* 1 hr, R_F .50 in "I" system, .32 in "O" system(6)), U$_2$ (mauve, .47, .40, possibly α-aminobutyric acid), U$_3$ (grey, .24, .29), U$_4$ (purple, .10, .16). [j] In roughly equimolecular quantities. [k] Insoluble in chloroform, soluble in methanol and in water; its ultraviolet spectrum showed weak end absorption; its infrared spectrum (paraffin mull) was indistinguishable from that of urea. [l] Insoluble in chloroform, moderately soluble in methanol, soluble in water, these crystals were the reducing sugar revealed by paper chromatography. In 2 solvent systems the sugar was inseparable from glucose. It showed no ultraviolet absorption above 220 mμ, and its infrared spectrum was indistinguishable from that of D-glucose.

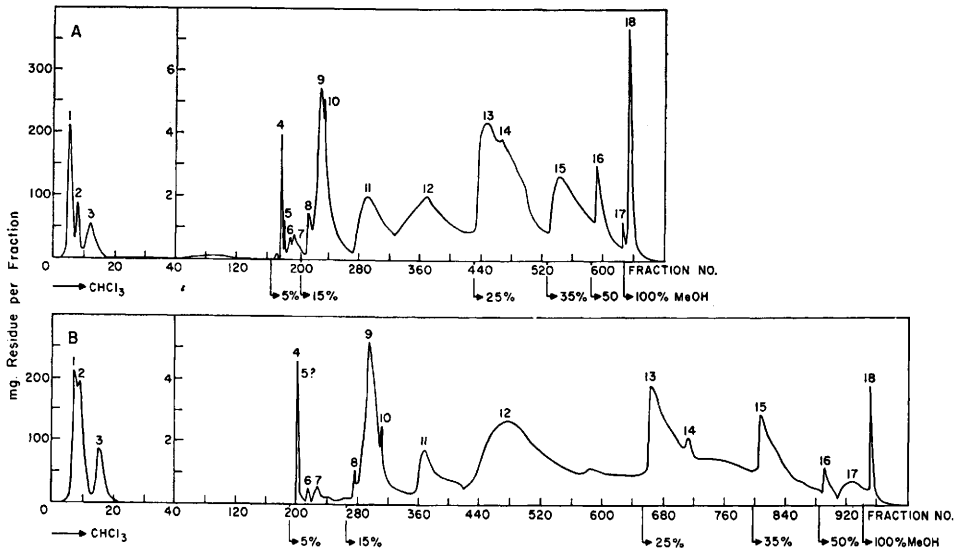

FIG. 1. Elution curves. The eluant was chloroform, followed by increasing percentages (v/v) of methanol in chloroform, applied as shown.

were applied directly to water-soluble hydrolysates.

Results. Elution curves show 18 peaks, none of which, except possibly No. 16, can be accounted for by the effect of a solvent change on the "tail" of its predecessor. Peak No. 11 is due to urea, and succeeding peaks obviously contain varying quantities of glucose and free amino acids, all of which had been rendered soluble in ligroin solution by lipid. The chemical data indicate that some peaks contain more than one type of lipid, and it can be deduced that whole blood contains about 20 types.

Assuming additivity of volume of anticoagulant solution, the volume of sample A was 199.5 ml. Propanol extraction yielded 1.817 g ligroin-soluble material, and subsequent Soxhlet extraction (chloroform, 4 hr) only a further 0.015 g, leaving 46.9 g defatted residue. Recovery of weighed lipid after chromatography was 87% (no allowance for losses on walls of collection tubes). The corresponding figures for sample B were: 192.5 ml, 2.338 g, 0.011 g, 37.8 g, 89%. The weights of material accounting for each peak were determined for both samples, and compared as weights/100 ml blood and /100 g defatted residue. Bearing in mind the considerable variations in blood composition known among normal samples(7), we found no striking divergence between these quantitative results for A and B. However, as anticipated, the sample (B) from the xanthomatous patient contained somewhat larger amounts of cholesterol esters and cholesterol than sample A. (Cholesterol esters: A, 932; B, 1394. Cholesterol: A, 431; B, 717 mg/100 g defatted residue.) Since no information is available on the biochemical significance or variability of bound amino acid composition of lipids in general, no comment can be made upon such differences observed between lipid fractions from A and B.

Comprehensive by present standards, these results confirm the usefulness of the methods of extraction and chromatography employed. However, there are shortcomings which deserve consideration, such as column performance; and, more serious, elution with lipids of non-lipid contaminants. These should preferably be removed before chromatography. Our experimental methods will be described in detail later.

Summary. Chromatography on silicic acid is a powerful tool for separation of blood lipids. In one experiment, about 20 different lipids may be detected; some of these remain unidentified.

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Presence of An Active Erythropoietic Factor (Erythropoietin) in Plasma of Rats after Prolonged Cobalt Therapy.* (24374)

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Ability of cobalt to stimulate erythropoiesis has been demonstrated repeatedly(1). The mechanism of its action on red cell formation, however, is not clearly understood. Warren *et al.*(2) have presented evidence that cobalt does not stimulate bone marrow by local anoxia as had been previously believed. Recently Goldwasser *et al.*(3) demonstrated that cobalt would stimulate production of erythropoietin, or similar erythropoietic plasma factor, and suggested that in this manner cobalt exerts its effect on erythropoiesis. These workers demonstrated recently(4) that erythropoietin could be found in plasma in greatest amounts 12 hours after single injection of cobalt and returned to normal 72 hours later. This material had gross properties the same as those of active substances from phenylhydrazine treated rats. Our experiment was conducted to determine whether or not erythropoietin production was still being stimulated after prolonged cobalt therapy, and to compare any effect obtained to the activity of plasma of rats in which erythropoietin production had been stimulated by bleeding.

Methods and materials. Rats used were females of Sprague-Dawley strain. Hematocrit determinations were made using Van Allen tubes (no diluent) and spun 1 hour at 2,000 rpm. Plasma from cobalt treated rats was dialysed to remove excess cobalt as follows. Ten parts plasma plus 1 part buffered NaCN solution (50 ml 0.2M K_2HPO_4 + 260 mg NaCN diluted to 100 ml) was dialysed against a dilute NaCN solution of pH 7.5 (same as above but diluted to 1 l with 0.9% NaCl). Dialysis was carried out in mechanical agitator 4 days at 2°-4°C; the dialysate was changed daily and saved. The plasma mixture was then dialysed against 0.9% NaCl 7 more days to remove cyanide. A portion of normal plasma was similarly treated. To determine effectiveness of dialysis on cobalt removal, chemical analyses‡ were made on samples of dialysed plasma, and dialysates from treated rats, and plasma from normal rats as follows. A 20 ml sample was placed in platinum dish, covered and heated in muffle oven for 16 hours at 500°C. After cooling to room temperature, 3-5 ml of 70% perchloric acid were added dropwise to the charred residue and mixed. This was dried slowly to a grey-white residue which was returned to the partially cooled oven, heated 1 hour at 600°C, then cooled to room temperature. 5 ml of 50% HCl and 2 ml of distilled water were

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‡ The method described is a modification, devised by D. M. Hubbard of Kettering Laboratories, Cincinnati, O., of nitroso-R-salt technic. The authors wish to thank Mr. Hubbard for use of his facilities and his supervision of analyses.