TABLE II. Absorption of Agglutinins from Immune Rabbit Serum by Washings of Staphylococci with Various Salt Concentrations.

Absorbing antigen (washing) according to its conc. of NaCl (%)	Max agglutination titer of antiserum after absorption
0 (distilled water)	40
.85	0
1.3	640
3.4	640
Unabsorbed	1280

It appears, therefore, that the change in agglutination demonstrated is related to the release of antigen into the solution since it is demonstrable there.

Summary. Optimal concentration of sodium chloride for quantitative evaluation of staphylococcal agglutinins was 1.3% to 1.7%. This applies to rabbit and human antisera. At this concentration, macroscopic technics are reliable and spontaneous agglutination is inhibited. The quality of the reaction is best at these concentrations for human antisera, while in rabbit antisera, it is best at concentrations of 3.2% sodium chloride. Concentrations below the optimal dissolves the antigen to such extent that macroscopic agglutination is definitely decreased.

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Effects of Storage on Serum Non-Esterified Fatty Acid Concentrations.* (25371)

ALLAN L. FORBES AND JANE A. CAMLIN (Introduced by J. C. Forbes)
Veterans Administration Hospital, Richmond, Va.

Although Dole(1) pointed out that extracts for non-esterified fatty acids (NEFA) should be prepared "as soon as possible after separation of plasma," no studies on possible changes in NEFA concentrations under various storage conditions have been reported. The present study illustrates that such storage, even in the frozen state, is accompanied by steadily increasing NEFA concentrations.

Methods. Fasting blood samples were obtained from 4 randomly selected patients, and from 2 patients with idiopathic hyperlipemia. Hyperlipemic blood samples also were obtained from 2 patients following intravenous infusion of a fat emulsion.† All blood samples were allowed to clot for approximately one hour, and the sera separated. Aliquots were promptly analyzed for NEFA by the method of Dole(1), and for triglyceride by the method of Forbes and Forbes (in preparation for publication). The latter was carried out in order to know the concentration of triglyceride substrate available for possible lipoprotein lipase action. Additional samples of the same sera were promptly separated for storage at −15°C, + 10°C, and room temperature (approximately + 25°C), and were subsequently analyzed for NEFA by the method of Dole (1) on days 1, 3, 8, 15, and 30 (days 1 and 3 only for room temperature stored sera).

Results. The initial (zero day) values for serum triglyceride fatty acids and NEFA for each patient are shown in Table I. Fig. 1 presents the effects of storage at the 3 temperatures on NEFA concentrations. Even in the frozen state, NEFA concentrations consistently rose in the first 24 hours, in one case to +63%. Increasing the storage temperature to +10°C and +25°C augmented these rises. In all of the frozen sera and in 5 of 8 sera stored at +10°C, transient decreases in NEFA following the initial rises were observed between days 3 and 15. In sera containing "artificial" chylomicrons from infused fat emulsion, enormous rises during storage oc-

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† Lipomul, supplied by The Upjohn Co.

TABLE I. Patient Groups and Zero Storage Day Values of Serum Triglyceride and Non-esterified Fatty Acids.

Group	Age	Diagnosis	Serum triglyceride fatty acids (meq/l)	Serum non-esterified fatty acids (meq/l)
No hyperlipemia (randomly selected individuals)	67	Arteriosclerotic heart disease	3.98	.62
	63	Hypertensive " "	5.62	.72
	63	Essential hypertension	4.41	.53
	33	Rheumatic heart disease	2.35	.90
"Physiological" hyperlipemia	47	Idiopathic hyperlipemia	15.43	1.12
	33	*Idem*	13.51	1.22
"Artificial" hyperlipemia (fat emulsion infusion)	37	Arteriosclerotic heart disease	39.53	1.06
	34	Simple obesity	17.56	.99

curred except when the sera were frozen in which case the rises were moderate. Triglyceride from "artificial" chylomicrons therefore appears to be particularly susceptible to lipolysis with resultant release of NEFA.

Since it may be desirable at times to delay analysis, it has been found that refrigerated heptane extracts prepared in the usual manner for NEFA analysis are stable for at least 10 days, within a range of $<\pm 3\%$.

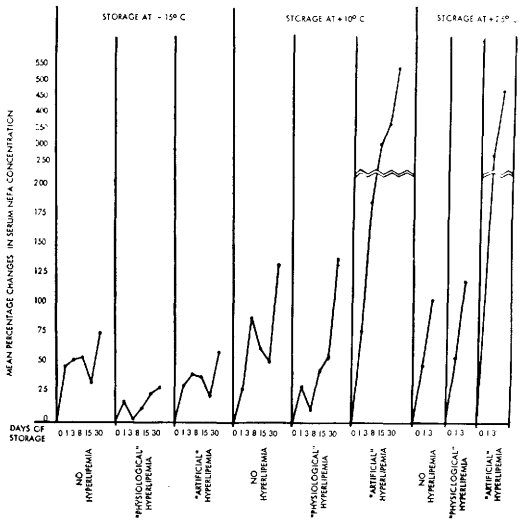

FIG. 1. Effects of storage time and temperature on serum NEFA concentrations, expressed as mean percentage changes from zero day value. Three types of sera at 3 storage temperatures are illustrated. (Note change in vertical scale dimensions for upper portion of Figure.)

Discussion. Extracts for serum NEFA analyses should be prepared promptly to reduce *in vitro* lipolysis to a minimum. The data do not distinguish between lipolysis of enzymatic or of physico-chemical origin; it is reasonable to postulate however that such lipolysis is in part due to serum lipoprotein lipase. The explanation for the transient decrease in NEFA between the 3rd and 15th day observed in some of the sera is not evident. Because of the excellent reproducibility of Dole's NEFA method, we do not feel that the transient decreases are due to methodological error.

Summary. The effects of storage of serum at various temperatures on NEFA concentrations have been studied. Appreciable increases in NEFA occurred within 24 hours even in frozen sera. Refrigerated heptane extracts of sera for NEFA analysis are stable for at least several days. Sera for NEFA analyses should be extracted promptly after collection.

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