Serum Concentrations of Lipids in Rabbits Infected with *Escherichia Coli* and *Staphylococcus aureus*[1] (34463)

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When experimental animals are challenged with certain bacteria or bacterial endotoxins hyperlipemia is known to result (1–4). Recent reports (4, 5) have suggested that different bacteria alter serum lipids differently and that such alterations might be of value in the early diagnosis of infectious diseases.

The following study presents observations on serum lipids of rabbits challenged with viable *Escherichia coli* or *Staphylococcus aureus*.

Materials and Methods. White rabbits weighing 2–3 kg were used in the study. Each rabbit was trained for 1 week prior to the experiment by being placed daily in a restraining cage with a rectal temperature probe inserted. Rectal temperatures were monitored in all rabbits throughout the duration of the experiment by means of rectal probes.

An *E. coli* strain (serotype 0111 B4) and *S. aureus* strain 502A (6) were used for all experiments. Stock cultures were maintained by storing portions of an 18-hr culture in trypticase soy broth at -20°. For each experiment a portion of stock culture was thawed and subcultured into trypticase soy broth. After 18 hr of incubation at 37°, the bacteria were washed twice in trypticase soy broth by centrifuging at 2000*g* in a refrigerated centrifuge (International, Model PR-2, International Equipment Co., Boston) at 4°. The inocula (10^9 viable organisms for *E. coli* and 10^8 viable organisms for *S. aureus* suspended in 1 ml of trypticase soy broth) were injected subcutaneously into the interscapular area of rabbits. Five rabbits were injected with *E. coli,* five with *S. aureus,* and four rabbits received sterile trypticase soy broth subcutaneously as controls. The inocula were examined by Gram stain and culture to assure purity.

Water was supplied to the rabbits throughout the experiment but food was withdrawn 12 hr prior to challenge. The rabbits were bled from the central ear artery, using a 20-gauge needle. Bleedings were performed immediately before inoculation of bacteria, again when the animals developed fever and appeared acutely ill (3 hr for animals challenged with *E. coli* and 6 hr for animals challenged with *S. aureus*) and again 24 hr after challenge. Controls were bled before inoculation of trypticase soy broth and 6 and 24 hr later. All blood samples were examined for the presence of infecting organisms by spreading 0.3 ml of blood on the surfaces of blood agar plates and incubating the plates for 18 hr at 37°.

Blood samples from the rabbits were allowed to clot and then centrifuged in a refrigerated centrifuge at 4° for 30 min at 2000*g*. The serum was separated from the cells and used immediately for lipid analyses.

Direct determinations of unmodified serum lipids were performed using Sperry and Brand's chloroform–methanol extraction procedure (7). After extraction lipids were dried under a nitrogen atmosphere at reduced pressure and then placed over phosphorus pentoxide in an evacuated desiccator previously flushed twice with nitrogen. The total

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serum lipids were weighed after 24 hr of desiccation and then samples were taken immediately for determination of major lipid classes.

The major serum lipid classes were separated using standard thin-layer chromatography procedures (8). Commercially prepared thin-layer plates (20 × 20 cm) were employed that were coated with a layer of neutral absorbent (250-μ thick) containing approximately 85% silicic acid and 15% calcium sulfate (Mallinckrodt Chemical Works, New York). Prior to sample application, plates were activated by heating at 110° for 1 hr. Samples containing 250 μg of serum lipid dissolved in chloroform/methanol (2:1) were applied on the plates and allowed to dry. The solvent was then allowed to migrate 10 cm in a petroleum ether/diethyl ether/acetic acid (90/10/1) solvent system. Fractions so resolved were detected by spraying the developed plates with 50% sulfuric acid followed by heating at 150° for 35 min to char material containing carbon. The following standards were also applied to the plates: oleic acid (Hormel Institute, Austin, Minn.), phosphatidyl choline (Applied Science Laboratories Inc., State College, Pa.), triolein and cholesterol stearate (Nutritional Biochemicals Corporation, Cleveland, Ohio), and cholesterol (Fisher Scientific Co., Springfield, N.J.).

The major lipid classes were quantitatively analyzed from the thin-layer plates using a Photovolt Model 525 Densitometer (Photovolt Corporation, New York City) equipped with a recorder and an electronic intergrator as described previously (9–11).

Results and Discussion. Rabbits challenged with *E. coli* developed fever 2–3 hr after challenge and were afebrile by 12 hr after inoculation with bacteria (Fig. 1). Animals challenged with *S. aureus* became febrile by 6 hr after challenge and remained febrile for at least 24 hr (Fig. 1). Although the rabbits inoculated with *S. aureus* were febrile for a longer period of time than animals challenged with *E. coli,* the peak fever did not differ significantly in the two groups ($p > .05$).

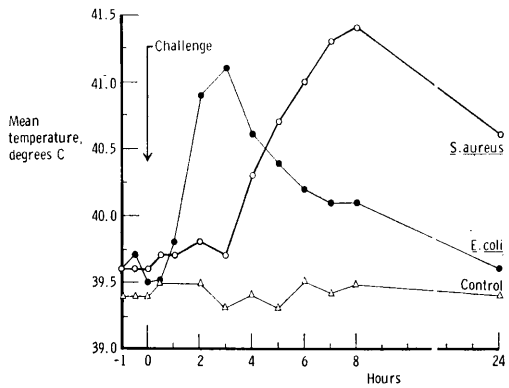

FIG. 1. Mean temperatures of five rabbits after inoculation of *Escherichia coli,* five rabbits after inoculation of *Staphylococcus aureus,* and four rabbits after inoculation of trypticase soy broth.

Blood from rabbits inoculated with *S. aureus* was sterile 6 hr after inoculation, but 24 hr after challenge blood from three of five rabbits contained 60–90 *S. aureus* per ml. Blood from two of the five rabbits inoculated with *E. coli* contained 10–20 *E. coli* per ml 3 hr after inoculation, but all cultures were sterile by 24 hr. All of the rabbits challenged with *S. aureus* had 2- to 3-cm indurated lesions at the site of inoculation 24 hr after challenge; none of the rabbits injected with *E. coli* had local lesions. Two of the rabbits challenged with *S. aureus* died 36 hr after challenge. The other rabbits injected with *S. aureus,* all control rabbits, and all rabbits challenged with *E. coli* survived for at least 72 hr.

The mean total serum lipid concentrations for the different groups of rabbits are shown in Table I. The concentration of total serum lipids in the control rabbits did not differ significantly throughout the study. Three hours after challenge with *E. coli* a moderate hyperlipemia was present with a mean total serum lipid of 527 mg/100 ml. These concentrations were significantly higher ($p < .01$) than controls or than prechallenge levels in the same rabbits. By 24 hr after challenge, the total serum lipid levels in the rabbits injected with *E. coli* had increased to concentrations significantly higher than levels in rabbits 3 hr after injection ($p < .01$).

Six hours after inoculation of *S. aureus* the

TABLE I. Serum Lipids (mg/100 ml).[a]

Inoculation	Time	Total lipids	Phospho-lipids	Free cho-lesterol	Free fatty acids	Tri-glycerides	Cholesterol esters
Trypticase soy broth (controls) four rabbits	Prechallenge	328 ± 15	64 ± 7	65 ± 4	25 ± 7	82 ± 5	71 ± 12
	3 hr after challenge	336 ± 18	86 ± 2	65 ± 9	26 ± 3	84 ± 5	79 ± 9
	24 hr after challenge	333 ± 20	63 ± 7	63 ± 5	28 ± 4	86 ± 8	74 ± 10
E. coli five rabbits	Prechallenge	315 ± 22	64 ± 5	67 ± 8	19 ± 3	59 ± 9	97 ± 10
	3 hr after challenge	527 ± 59	86 ± 9	86 ± 11	137 ± 35	64 ± 12	126 ± 13
	24 hr after challenge	731 ± 69	151 ±69	97 ± 23	44 ± 31	398 ±136	118 ± 76
S. aureus five rabbits	Prechallenge	340 ± 24	70 ± 6	71 ± 15	20 ± 9	72 ± 15	84 ± 11
	6 hr after challenge	373 ± 30	76 ± 9	70 ± 11	22 ± 11	75 ± 24	100 ± 42
	24 hr after challenge	834 ±343	87 ±22	90 ± 26	28 ± 15	433 ±276	173 ± 56

[a] Mean ± SD.

levels of total serum lipids did not significantly differ from preinoculation concentrations ($p > .05$). However, seum collected 24 hr after inoculation with *S. aureus* was hyperlipemic with a mean total serum lipid of 834 mg/100 ml; this was significantly higher than the preinoculation values or concentrations in the control rabbits ($p < .01$). The 24-hr values for rabbits injected with *S. aureus* were not significantly different from the 24-hr

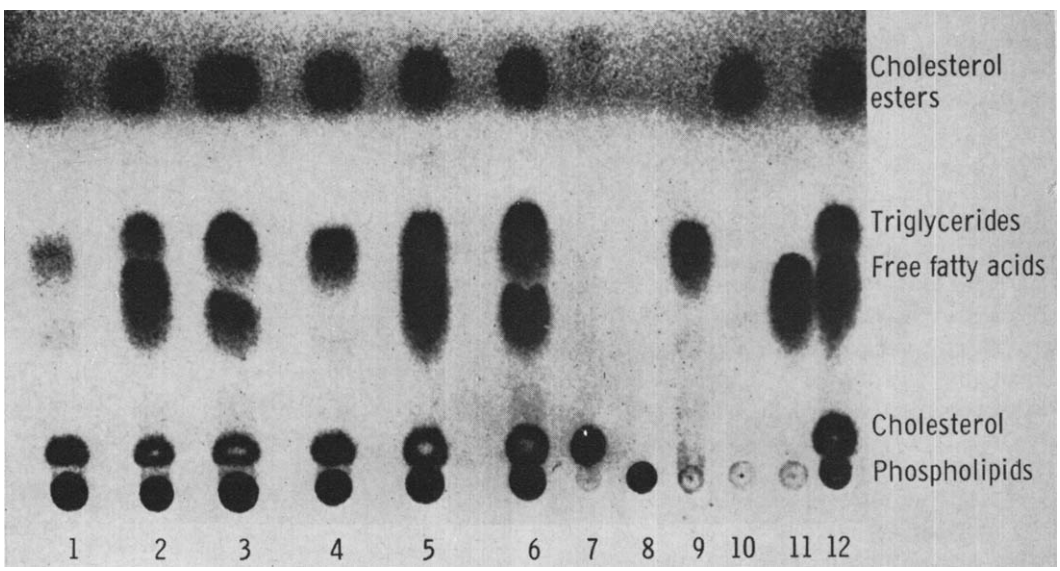

FIG. 2. Thin-layer chromatography of serum total lipids of rabbits challenged with *Escherichia coli* and of reference standards: two animals challenged with *Escherichia coli* prechallenge (1 and 4), 3 hr after challenge (2 and 5), and 24 hr after challenge (3 and 6); free cholesterol (7); phosphatidyl choline (8); triolein (9); cholesterol stearate (10); oleic acid (11); and mixture of 7–11 (12).

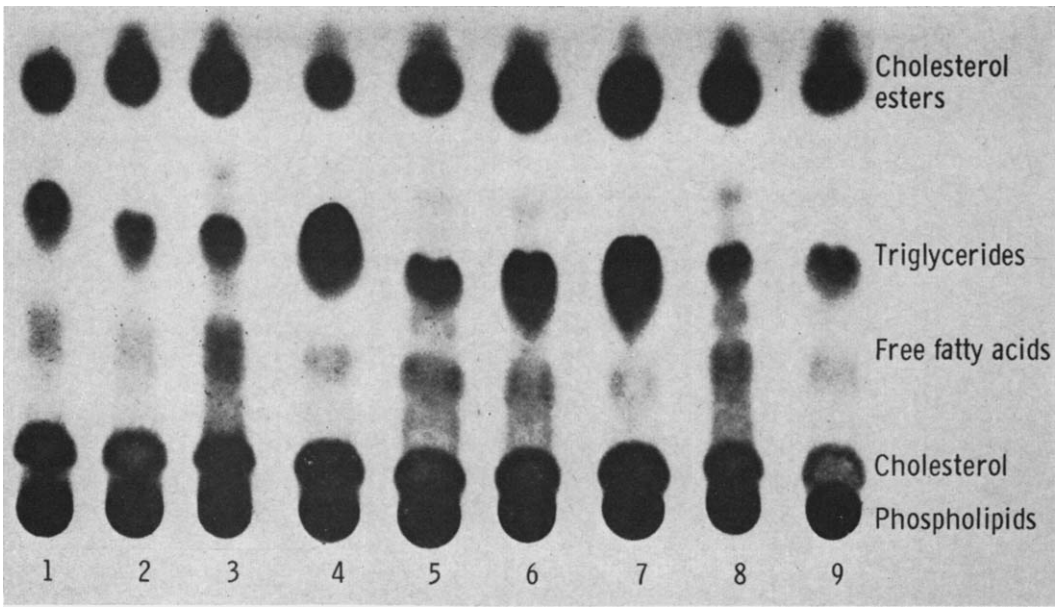

FIG. 3. Thin-layer chromatography of serum total lipids of rabbits challenged with trypticase soy broth or *Staphylococcus aureus*. A rabbit challenged with trypticase soy broth prechallenge (1), 6 hr after challenge (2) and 24 hr after challenge (3); two rabbits challenged with *Staphylococcus aureus* prechallenge (6 and 9), 6 hr after challenge (5 and 8), and 24 hr after challenge (4 and 7).

values for rabbits challenged with *E. coli* ($p > .05$).

These results were consistent with previous reports in which rabbits challenged intravenously with endotoxin derived from *E. coli* developed hyperlipemia within 2 hr after challenge (2) and rabbits injected with two gram-positive bacteria (*Bacillus anthracis* and *Diplococcus pneumoniae*) were observed to have hyperlipemia 30 hr after subcutaneous challenge (4).

Figures 2 and 3 demonstrate results of thin-layer chromatography of serum of selected control rabbits and rabbits challenged with *E. coli* and *S. aureus*. These figures also demonstrate chromatography of standards for each major lipid class (15 μg of each standard was used). As demonstrated in Figs. 2 and 3 and in Table I the hyperlipemia in rabbits inoculated with *E. coli* was primarily the result of an increase in free fatty acid levels early after inoculation and of triglycerides 24 hr after challenge ($p < .01$ in comparison with prechallenge concentrations and levels in controls). Lesser elevations in phospholipids, free cholesterol, and cholesterol esters also contributed to the hyperlipemia after challenge with *E. coli*. The hyperlipemia in rabbits 24 hr after challenge with *S. aureus* was primarily the result of increased serum concentrations of triglycerides which were significantly elevated over the prechallenge values and over values for control animals ($p < .05$ for each comparison) but were not significantly different from the concentrations in animals studied 24 hr after injection with *E. coli* ($p > .05$). Lesser elevations in phospholipids, free cholesterol and cholesterol esters also contributed to the hyperlipemia after challenge with *S. aureus*.

These observations are consistent with the findings of Hirsch, McKay, Travers, and Skraly (2) who observed that the serum free fatty acids of rabbits rise early after challenge with endotoxin and that the serum triglycerides rise later. The findings with gram-positive organisms are similar to those reported by Farshtchi and Lewis (4) who demonstrated hypertriglyceridemia after infection with *Bacillus anthracis* and *Diplococcus pneumoniae*.

Work in this laboratory (5) has shown

that similar to the findings in rabbits, humans with severe infection caused by gram-negative bacilli also developed a hyperlipemia with increased free fatty acids early and elevated triglycerides later in the course of the infection. However, in contrast to rabbits, humans infected with gram-positive bacteria fail to develop hyperlipemia.

Summary. Rabbits infected subcutaneously with *E. coli* developed hyperlipemia related to increased serum levels of free fatty acids early in the infection and later to increased levels of triglycerides. Rabbits challenged subcutaneously with *S. aureus* had normal levels of serum lipids during the acute febrile period but after 24 hr developed hyperlipemia related to hypertriglyceridemia.

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