were separated by h.p.t.1.c. The t.1.c. plate was developed with hexane/diethyl ether/acetic acid (65:35:1, by vol.) and lipid detected with 3% (w/v) copper acetate in 8% (v/v) phosphoric acid and heating at 130°C for 30 min. The lipid standard used for calibration and identification contained cholesteryl oleate, triolein, oleic acid (sodium salt), cholesterol and cardiolipin.

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The structural feature of the lipids obtained from human aorta samples

The lipids from pooled extracts of hydrated (H) and dehydrated (D) aorta samples after extraction by acetone/butan-1-ol (method A), chloroform/methanol (method B) and acetone/butan-1-ol/chloroform/methanol (method C) were separated by h.p.t.l.c. The t.l.c. plate was developed with hexane/diethyl ether/acetic acid (65:35:1, by vol.) and lipid detected with 3% (w/v) copper acetate in 8% (v/v) phosphoric acid and heating at 130°C for 30 min. The lipid standard (S) contained: cardiolipin (1), cholesterol (2), sodium oleate (3), triolein (4) and cholesteryl oleate (5).

h.p.t.l.c. (Fig. 2). The lipid standard used for calibration and identification contained cholesteryl oleate, triolein, oleic acid (sodium salt), cholesterol and cardiolipin.

The trial has shown that lipids can be quantitatively removed from 0.5 cm bands of human aorta. The structural

ly intact de-lipidated material can be further processed by both biochemical and histochemical techniques.

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The effect of surfactants and aortic lipids on the elastin-elastase reaction

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Mature cross-linked elastin in the form of a macromolecular complex with glycoprotein(s) is a major structural component of the thoracic aorta. Microscopically the elastic material occurs in a series of rings or lamellae (Kagan et al., 1979). Progressive fragmentation of the lamellae is observed with ageing, hypertension and atherosclerosis causing elastic fatigue of the artery (Caro et al., 1978). Accumulation of lipid on the inner surface of the aorta is a common feature of >60-year-old humans (Armstrong & Megan, 1975). Enhanced activation of the elastin–elastase reaction has been found after the pretreatment of elastin with anionic surfactants (Hall & Czerkowski, 1961; Kagan et al., 1972; Jordan et al., 1974). Elastase in vivo could be derived from a number of sources. A pancreatic-like elastase has been detected in plasma and the level of the enzyme is raised in acute pancreatitis (Geokas et al., 1977; Bellon et al., 1978; Toki et al., 1982). Granulocytes, macrophages, platelets and the aortic smooth-muscle cells produce elastases (Bieth, 1978; Jacob et al., 1982; Werb et al., 1982). Elastase activity in blood is controlled by Α2-antitrypsin which binds and inhibits the enzyme and by Α2-macroglobulin which only partially inhibits the enzyme. High levels of Α2-macroglobulin may therefore increase plasma elastase activity by protecting it from Α2-antitrypsin (Hornebeck et al., 1981; Bihari-Varga et al., 1984). This preliminary report describes the action of anionic surfactants and of a human aortic lipid extract on the elastin–elastase reaction (Elliott et al., 1983).

Elastin (Sigma Chemical Company, E-1625) was 14C-labelled by reductive alkylation with [14C]formaldehyde (sp. activity 15mCi/mmol). The elastin was then treated with α-chymotrypsin, to reduce leakage of 14C label (Rice & Means, 1971; Bielefeld et al., 1975; Yu & Yoshida, 1979). The elastin was freeze-dried and stored at −20°C to minimize decomposition.

For the assay 10 mg of elastin was suspended in 2.5 ml of 0.1 M-Tris/HCl buffer, pH 8.8, and aliquots (50 μl) sampled while the suspension was briskly stirred. The aliquots, in 1.5 ml capacity microcentrifuge tubes, were transferred to a shaking water bath at 37°C. The surfactants, sodium lauryl sulphate, sodium laureate, and the bile salts, sodium cholate and deoxycholate, were prepared in the 0.1 M-Tris/HCl buffer at double the concentrations shown in Fig. 1. Fatty plaque material from the intimal surface of human aortas was added to buffer and sonificated for 2 × 30 s periods; the suspension formed was decanted from remaining debris. Fifty microlitres of the selected surfactant, at a range of concentrations, was added to the elastin suspensions and preincubated for 30 min with continuous agitation. Pancreatic elastase (EC 3.4.21.11, at a concentration of 1.25 μg/50 μl of buffer, was added to the elastin suspensions. Incubation at 37°C with agitation was maintained for 45 min. The reaction was stopped by the addition of 2% (w/v) trichloroacetic acid. Tube contents were filtered and
All of the surfactants examined could increase the rate of elastolysis when compared with untreated elastin; however, as shown in Fig. 1, accumulation of unbound surfactant caused enzyme inhibition. In experiments where excess surfactant was removed, by washing the treated elastin with buffer before adding elastase, inhibition effects were greatly reduced. The titration curves of the surfactants (Fig. 1) were similar to results obtained with a pH-stat (Jordan et al., 1974).

The aorta lipid extract was used without any further dilutions and was composed of a complex mixture of lipids (Elliott et al., 1985). Elastin preincubated with human aortic lipid gave c.p.m. values ~20% higher than the untreated elastin controls. One-tailed t-test of the means was significant at the P = 0.05 level. These findings suggest that the presence of lipid plaques in human aortas may contribute to the mechanism causing elastic lamellae fragmentation.

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Solid-phase enzyme-linked immunosorbent assay for the large scale-screening of hybridomas: non-specific effects and their solutions

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The large-scale screening of hybridomas requires a rapid and sensitive assay for the early detection of positive clones.

Abbreviations used: e.l.i.s.a., enzyme-linked immunosorbent assay, LAT cells, Landschiutz ascites tumour cells; PBS, phosphate-buffered saline

Here, we describe a solid-phase e.l.i.s.a. for the detection of monoclonal antibodies against whole fixed cells.

LAT cells were used as a source of antigen. Balb/c mice, immunized with 10⁷ dead LAT cells in PBS, pH 7.3 (Dulbecco A) followed by once weekly boost for 3 weeks, were used as a source of positive control sera. Non-immunized mice acted as sources of negative control sera.

Cell fixation was performed by using modifications of the methods of Stocker & Heusser (1979) and Cobbold & Waldmann (1981). Flat-bottomed, flexible polyvinyl chlo-