these univalent fragments recombine to form bivalent $F(ab')_2$ molecules, a proportion of which carry both specificities. These reformed $F(ab')_2$ molecules were separated from the remaining monomers by 'Sephadex *G*-100' chromatography, and used for labelling without further purification.

In Fig. 1*a* H–2 alloantigen on a C57Bl ascites leukaemia cell (EL4) is seen labelled with SBMV by the hybrid antibody method. For comparison, Fig. 1*b* shows H–2 on EL4 cells labelled by the same procedure but with ferritin (and the anti-ferritin hybrid antibody) in place of SBMV. SBMV forms a dense monolayer parallel to the surface. Each virion (average diameter: 25 mmicrons) presumably covers several cell surface antigenic determinants. As with ferritin, labelling with SBMV is discontinuous, being restricted to some sectors of the cell surface only. Thus as in previous studies[3,9,10] H–2 antigen is seen to reside in circumscribed areas of the cell surface.

Table 1. SPECIFICITY OF HYBRID-ANTIBODY LABELLING WITH SOUTHERN BEAN MOSAIC VIRUS (SBMV) AND FERRITIN MARKERS

Serum	Hybrid antibody	Marker	Labelling of cells*
$H-2^k$) anti ($H-2^b$) (prepared in an H–2 congenic strain)	aMγG/aSBMV†	SBMV	Positive
,, ,,	aMγG/aFerritin‡	SBMV	Negative
,, ,,	aMγG/aFerritin	Ferritin	Positive
,, ,,	aMγG/aFerritin +	SBMV +	Positive§
,, ,,	aMγG/SBMV (mixture)	Ferritin	
,, ,,	None	SBMV	Negative
,, ,,	None	Ferritin	Negative
($H-2^b$) anti A ($H-2^a$) or normal mouse serum (negative controls)	aMγG/aSBMV	SBMV	Negative

* C57Bl ascites leukaemia EL4 ($H-2^b$).
† aMγG/aSBMV: anti-mouse γG/anti-SBMV hybrid antibody.
‡ aMγG/aFerritin: anti-mouse γG/anti-ferritin hybrid antibody.
§ The markers were interspersed within the same circumscribed areas.

Table 1 summarizes the results with two markers, ferritin and SBMV, including controls to indicate that labelling is specific. We are exploring two other markers, tobacco mosaic virus and T4 bacteriophage, and the combined use of different markers in the mapping of cell surface antigens.

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Arterial Wall Shear and Distribution of Early Atheroma in Man

THE patchy distribution of fatty streaking and early atheroma has been associated with arterial blood mechanics. Mustard *et al.*[1] have noted occurrence of atheroma at sites which are thought to experience particle (platelet) deposition as the result of local rapid flow fluctuations (turbulence) or eddies. Others have proposed platelet deposition in regions of flow separation[2]. Texon[3] invoked damage due to Bernoulli-type suction forces in areas of locally increased blood velocity; but this is considered implausible because the forces are negligible in physiological conditions in comparison with normal variations of mean blood pressure. Mitchell and Schwartz[4] reported the sparing of fatty streaking in localized areas, at which they suggest low wall shear rate (the product of velocity gradient and fluid viscosity) is experienced. Fry[5,6] has shown that acute elevation of shear rate on the aortic wall causes endothelial damage and increased permeability to lipids. These theories assign to fluid mechanics a causative role in atherogenesis.

We consider that fluid mechanics has a controlling and inhibiting (or retarding) effect, rather than a causative one. We show that the observed distribution of fatty streaking and early plaques is coincident with regions in which the shear rate at the arterial wall is locally reduced. Similarly the development of lesions is inhibited in areas where the local wall shear rate is relatively high. These correlations refer to early manifestations, that is, before the appearance of gross changes of the arterial wall.

The localized development of atheroma in large arterial branches can be described in terms of this hypothesis. R. C. S. and Sudlow[7,8] have shown that the important fluid dynamical effects in daughter tubes at large junctions (Fig. 1) are associated with: (1) the establishment of a new (thin) boundary layer on the wall immediately downstream of the flow divider (inner wall); (2) a comparatively thick boundary layer on the opposite (outer) wall; (3) the maintenance of this differential downstream of the junction by secondary motions induced in the fluid.

Because regions of high wall shear are associated with relatively thin boundary layers, our hypothesis predicts fatty streaking or early atheromatous lesions on areas with relatively thick boundary layers. Thus we expect involvement of outer, rather than inner, walls of daughter arteries. A new boundary layer must, moreover, develop

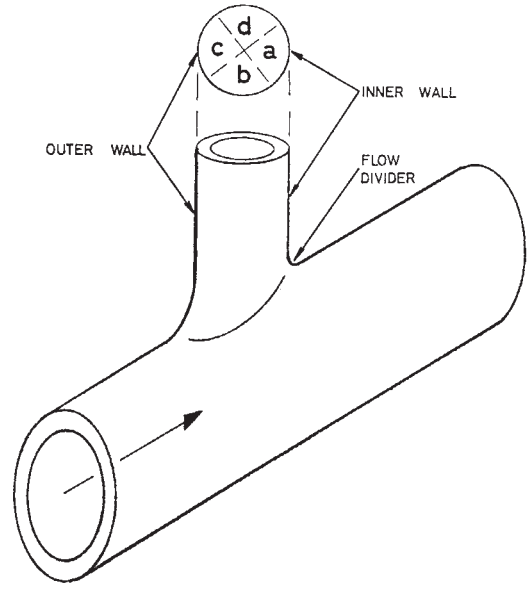

Fig. 1. Schematic geometry of a large branch, showing nomenclature.

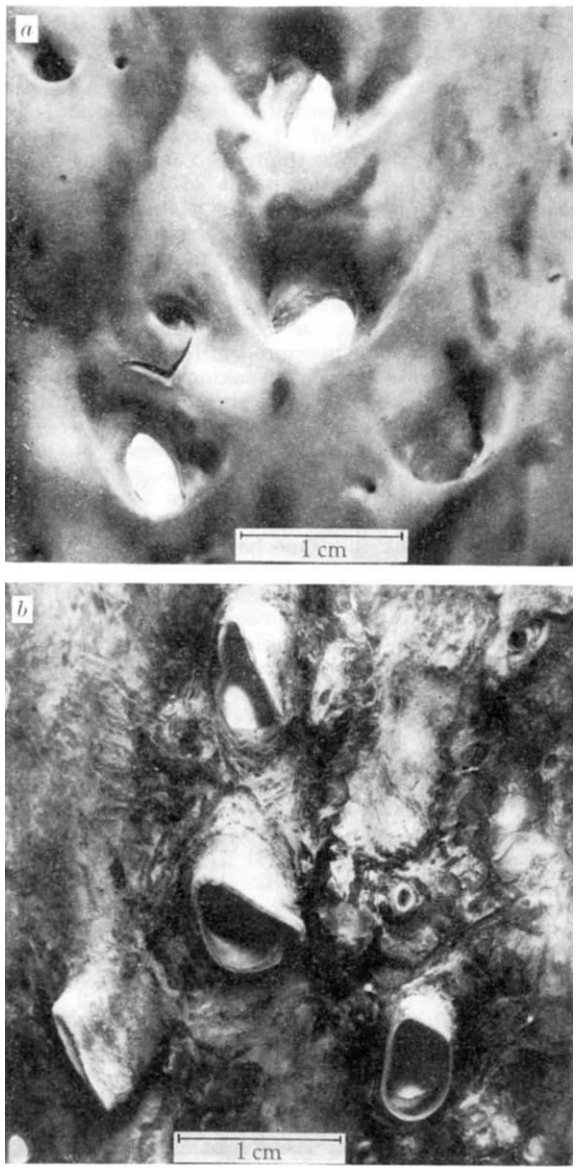

Fig. 2. Human superior mesenteric, coeliac and renal arteries. *a*, From intimal side; *b*, from adventitial side.

in the parent vessel immediately downstream of the flow divider; this would be expected to inhibit development of atheroma.

We studied the distribution of lesions in relation to sites of this type (branches of aortic arch, coeliac, superior mesenteric and renal arteries, and aortic bifurcations). Our findings support the predictions. Twenty-one human aortas, stained with Sudan III, were examined (age range 18 to 67, median 40 yr). Measurements were made on the coeliac, superior mesenteric and renal arteries, an initial segment of each (length approximately equal to diameter) being divided into quadrants (Fig. 1). The degree of involvement in each quadrant was assessed on a discrete four-point scale (0 = negligible, 3 = extensive) based on area affected and intensity of staining. Results of four independent observers were averaged for individual branches and overall (Table 1). The inner wall (quadrant (a)) is much less affected than the other walls, particularly the outer; it is, of course, recognized that measurement technique and statistical interpretation are crude.

Fig. 2*a* shows the more extensive involvement of the outer walls of branches of the abdominal aorta compared with the inner walls (Fig. 2*b*) (dark area on inner wall of superior mesenteric is shadow; inner wall of right renal also unaffected). Sparing is seen on the aorta, immediately downstream of the coeliac flow divider, but as the aortic wall merges with the outer wall of the superior mesenteric, involvement again occurs. The distribution of lesions in branches of the aortic arch also conforms to the patterns described; for example, a plaque on the outer wall of the innominate artery, and sparing of the inner wall. Conceivably, structural or functional peculiarities of flow dividers could explain their relative sparing. It is of particular interest, however, that there may be no recognizable aortic wall segment between the innominate and left common carotid arteries: the flow divider for the innominate also forms the outer wall of the left common carotid. The inner wall is spared; the outer wall—while structurally part of the flow divider—is affected.

Table 1. DISTRIBUTION OF LESIONS

Arterial site	Quadrants *a*	*b*	*c*	*d*
Coeliac	0·44	1·30	1·79	1·50
Superior mesenteric	0·82	1·47	1·78	1·57
Left renal	0·39	0·71	1·65	1·06
Right renal	0·46	0·93	1·62	1·30
Overall	0·54	1·12	1·72	1·36

Relative sparing has been reported within the aortic posterior fatty streak, immediately downstream of intercostal orifices[4]. Our observations on similar vessels are in agreement. It is suggested[4] that this sparing results from local low shear. In fact such areas experience relatively high wall shear. Blood will enter the intercostals from the boundary layer on the wall of the aorta, so that a new boundary layer must develop downstream of the orifice, and this region is therefore exposed to increased shear. These predictions have been confirmed in model experiments, using the technique of evaporation of a volatile indicator in a straight 'Perspex' pipe with small side branches; flow in these was a small fraction of the parent flow (parent Reynolds number, *Re*, approximately 10^3).

The theory also relates to other documented areas of early involvement, now seen to be low shear regions, for example inner wall of bends in coronary arteries, and outer walls of iliac bifurcation[1].

We now contrast our theory with previous interpretations of arterial fluid mechanics. It is unlikely that damage could occur to the arterial wall in regions of flow separation[9] (*Re* much lower than required for damage by turbulent pressure fluctuations transmitted through a separation bubble). Fox and Hugh[2] associate particle deposition with relative stasis in separation regions. Separation is unlikely to occur[7,8], however, with the gentle curvature of outer walls normally observed at junctions (for example, arterial casts produced by Tompsett). Nevertheless it is interesting that a Duguid-type mechanism[1] is not incompatible with our theory—if in fact particle (platelet) deposition does occur preferentially in low shear regions. While our theory predicts sparing in regions of increased shear, it is reasonable to expect an upper limit of vessel tolerance; if this is exceeded, the damage mechanism proposed by Fry[5,6] may well be important.

Having established this correlation between the wall shear and occurrence of atheroma, we suggest a mechanism involving mass transfer for the development of lesions. Shear dependent transfer rates have been extensively studied in other situations (for example, heat[10]). The arterial wall can synthesize lipid[11], apparently from precursors supplied from the lumen[12], and there is *in vitro* evidence of cholesterol exchange between the wall and incubating fluid[13]. We propose a process of simultaneous ingress and egress, not necessarily solely of cholesterol, with the gradual accumulation of materials in the arterial wall. This process would depend on many factors, such as diffusion, reaction kinetics and chemical equilibria. Local variations of wall shear, affecting local supply and re-

moval rates, may control the rate of accumulation of material constituting atheromatous lesions. It is interesting that this theory predicts that physical exercise involving increase of cardiac output, and hence increased shear rate, might retard the development of atheroma. An overall reduction of shear rate, for example a normal volume flow rate through a dilated artery, will tend to favour the development of atheroma. This is in contrast to other predictions[1,3–6,9].

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Specific Inhibition of Immunocompetence

In intact animals there is a period of depressed direct haemolytic plaque-forming cell (DPFC) response, below that of the primary response, in primed animals challenged 1 to 9 weeks after the initial immunization[1]. This depressed DPFC response, during a period when the total haemagglutinin levels are maximum, is difficult to interpret in terms of antibody-mediated immune suppression[2,3]. During this interval of the primary response to sheep erythrocytes in mice, the haemagglutinin which persists is primarily IgG, which has been demonstrated to exert an inhibitory effect on IgM (DPFC) production. Byers and Sercarz[4] have suggested that this depression represents an "exhaustion" of both the immune progenitor (X cells) and the sensitized (Y cells) cell compartments and that the IgM memory is short-lived.

Using the *in vivo* transfer method we have shown a similar depression of the DPFC capacity at 2 and 4 weeks in primed mice[5]. Spleen cells from donors primed for 2 weeks with sheep erythrocytes, when transferred with optimum antigen dose into irradiated recipients, elicited a DPFC response which was significantly below that achieved with an equivalent number of nonprimed cells. Because of the lack of interference from high levels of pre-existing antibody in this transfer system, this depressed DPFC capacity at 2 weeks after priming reflects not only the lack of sensitized cells but also of the progenitor cell compartment. It is unclear, however, whether in this system we are dealing with a general depression of progenitor cells or a specific suppression for the priming antigen. The following experiments were designed to determine whether a normal primary level of DPFCs to a different antigen could be obtained during this period of reduced capacity.

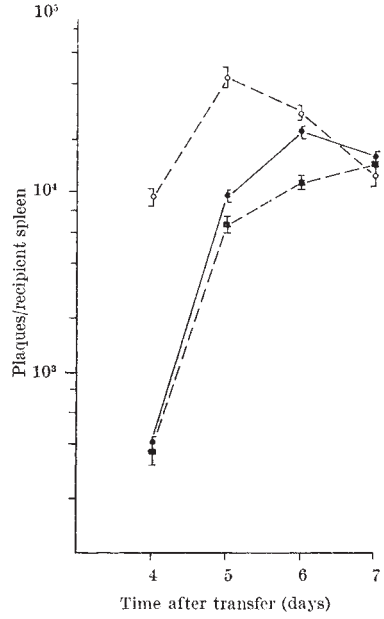

Fig. 1. Mean total DPFCs per recipient spleen after transfers of SRBC-primed or nonprimed spleen cells with SRBC test antigen. Each point represents the mean of twenty to sixty mice ± one standard error of the mean. ●—●, Nonprimed spleen cells; ○ - - - ○, spleen cells transferred 7 days after priming; ■ - - - ■, spleen cells transferred 14 days after priming.

Male [(C57Bl6 ♂ × C3H/An ♀)F_1 CUM] specific-pathogen-free mice 12 to 14 weeks of age were used together with the haemolytic plaque-forming technique[6]. For assaying haemolytic plaque-forming cells against rat erythrocytes this method was modified by using a 1 : 6 dilution of frozen rabbit serum as a source of complement[7].

The transfer system has been described previously[5]. Donors were injected intraperitoneally with 1 ml. of 1·0 per cent sheep erythrocytes at 0 time, or were uninjected. Recipients were given 800 r. of X-radiation and then injected intravenously with 25×10^6 spleen cells from either primed or nonprimed donors mixed with 25×10^7 of sheep, rat or horse erythrocytes. The plaque-forming assay was performed 4, 5 and 6, and sometimes 7 days after transfer. The results are pooled data taken from two to eight experiments.

The number of DPFCs on day 4 after transfer of nonprimed spleen cells (primary control) is approximately 4×10^2, and the measured peak is achieved on day 6 at 2×10^4 cells (Fig. 1). When the secondary immune capacity of cells transferred at 1 week after priming of donor mice are compared with nonprimed cells, the priming effect is detectable as an elevated DPFC compartment size at 4 days (approximately 20-fold increase), and the peak is measured at 5 days ($4{\cdot}4 \times 10^4$ cells). Thus the peak DPFC potential of spleen cells primed for 1 week is elevated by two-fold and is achieved 24 h earlier when compared with nonprimed spleen cells. In spleen cells primed for 2 weeks, the DPFC response reaches peak level between 5 and 7 days after transfer, but the detected peak secondary DPFC response is depressed below that of an equivalent number of nonprimed spleen cells.

The primary DPFC response to rat and horse erythrocytes, measured at 1 and 2 weeks in mice primed initially with an equivalent dose of sheep erythrocytes, is shown in Figs. 2*a* and 2*b*. This response is compared with the normal DPFC capacity of 25×10^6 nonprimed spleen cells against rat and horse erythrocytes. The measurements made at days 4, 5 or 6 after transfer show no real difference