these determinations did the serum direct-reacting bilirubin concentration exceed 25 per cent of the total serum bilirubin concentration and usually was less than 10 per cent of the total bilirubin. Hæmoglobin concentrations, reticulocyte counts and serum glutamic–pyruvic and glutamic–oxaloacetic transaminase activities, thymol turbidity, cephalin cholesterol flocculation and the concentration of albumin and globulin remained normal throughout the investigation of each of the five subjects.

This work demonstrates that unconjugated hyperbilirubinæmia can be produced in very young, full-term infants by feeding pregnane-3(α), 20(β)-diol in amounts equivalent to that isolated from inhibitory human milk. Only very young infants became jaundiced after ingestion of pregnane-3(α), 20(β)-diol because the exogenous inhibitor is probably superimposed on the already limited hepatic conjugating capacity of the new-born[2–4]. There appears to be nothing unique about the infants of the mothers whose milk contains the inhibitor.

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Origin of Plasma Cells in Sites of Inflammation

The origin of plasma cells in inflammatory reactions in non-lymphoid tissue is perplexing. They are found in the circulation only in unusual circumstances[1] and are not a usual cellular component of normal connective tissue. A possible source of these cells in areas of injury is suggested from investigations of lymphocytic cells in the vascular spaces and alveolar walls of the lung following the intravenous injection of complete Freund's adjuvant. The changes in some of these lymphocytes are similar to those described for white cells of the peripheral blood grown in tissue culture in the presence of a variety of materials. When phytohæmagglutinin, tuberculin, tetanus toxoid, diphtheria toxoid, smallpox vaccine, hæmophilus pertussis antigen, staphylococcal antigen, leukocyte antiserum, pollen extract and various tissue antigens are added to cultures of white cells from the peripheral blood, the small and medium sized lymphocytes enlarge and mitotic division may occur[2–13]. Tanaka *et al.*[14] have described a developing Golgi apparatus, increased ribosomes and a small amount of endoplasmic reticulum in cultures of lymphocytes from peripheral blood.

In addition to these changes, Bach and Hirschhorn[15] demonstrated that some of the lymphocytes grown in culture in the presence of tuberculin contain gamma globulin and in the presence of phytohæmagglutinin contain 7*S*γ 2 globulin. A few of the lymphocytes in their cultures eventually had the appearance of 'plasma-like cells'.

Albino rabbits, which had been given 1 ml. of complete Freund's adjuvant intravenously, were killed at 15 min, 30 min, 1 h, 12 h and 1 and 3 days after injection. Four rabbits which had not received adjuvant were used as controls. Samples of lung were fixed and embedded for electron microscopy.

Examination of the lungs from normal rabbits showed an occasional lymphocyte in the vascular spaces and none in the extravascular tissue of the alveolar walls. These cells had the ultrastructural features of lymphocytes obtained from the peripheral blood as described by Low and Freeman[16].

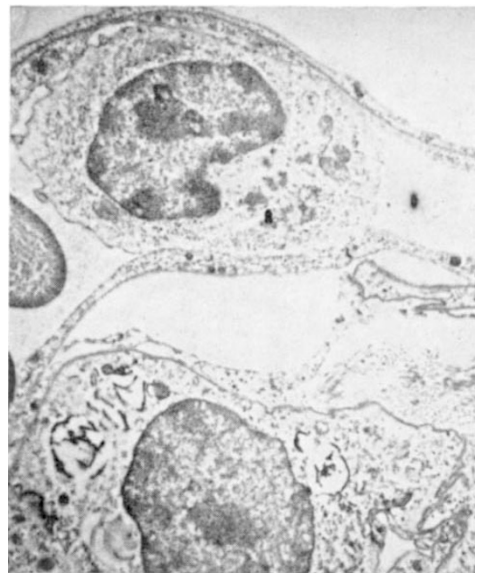

Fig. 1. Lymphocytic cell in the capillary of an alveolar wall from a rabbit killed 15 min after receiving Freund's adjuvant

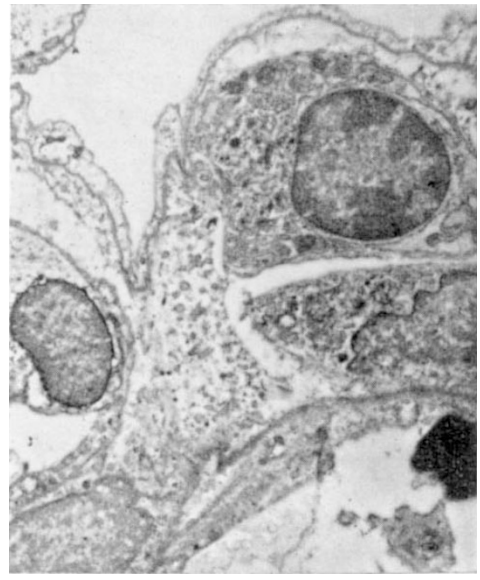

Fig. 2. 'Plasma-like cell' in the alveolar wall from a rabbit killed 30 min after the injection of adjuvant

Fifteen min after the intravenous injection of the adjuvant the number of white blood cells in the vascular spaces of the lungs was markedly increased, and a few of the cells had migrated across the vascular walls into the alveolar septa. Some of the small and medium sized lymphocytic cells, while still in the vascular spaces, showed an increase in ribosomes and a small amount of endoplasmic reticulum and a more prominent Golgi apparatus (Fig. 1). These ultrastructural changes continued after the migration of the cells into and across the walls of the vascular channels (Fig. 2). By 1–3 days after the injection many of the intraluminal lymphocytic cells had acquired a cytoplasmic ultrastructure similar to plasma cells. It is apparent that 'plasma-like cells' can develop from small and medium lymphocytes.

It has been reported previously that plasma cells accumulate in the tissue of the lung after the intravenous injection of adjuvant, and specific antibody can be demonstrated in their cytoplasm by the immunofluorescent technique 14 days after immunization[17].

The rapidity with which these lymphocytic cells react implies a more rapid functional expression than ordinarily recognized. The significance of these ultrastructural changes and the mechanism for initiating them are not apparent. The long delay between the cytoplasmic changes and the presence of detectable antibody in some of the cells suggest functions, as yet unknown, in addition to antibody formation. These findings do offer an explanation of the origin of plasma cells in sites of injury.

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Localization of Spontaneous Lipid Deposition in the Cerebral Arteries of Sheep

Lipids in plasma and atherosclerotic plaques have been extensively investigated, and the sites of predilection for lipid deposition in hypercholesterolæmic rabbits are the intimal thickenings at bifurcations[1]. However, the precise location of early spontaneous lipid deposition in the arterial wall has not been determined. Therefore, an investigation was made of cerebral arterial forks in the sheep, a herbivorous animal with a low concentration of plasma lipids[2], and serial, rather than random, sections were used to give a much more accurate localization of lipid and intimal thickening.

The cerebral arteries of 45 sheep (28 lambs and 17 sheep 2–2·5 years old) were dissected for examination and, after formalin fixation, serial paraffin sections were prepared of 50 major arterial forks from the circles of Willis. Sections were stained with Verhoeff's elastic stain, azure *A* or Mallory's phosphotungstic acid hæmatoxylin.

The arteries were thin-walled and, though of smaller calibre, were histologically similar to those of man. Intimal thickening was found only at forks and about the orifices of smaller branches. Elsewhere the endothelium appeared to lie almost directly on the prominent internal elastic lamina. The location of the thickenings was similar to that described for the intimal pads or cushions in cerebral arteries of human infants[3]. The pads consisted of fibrillary elastic laminæ, collagen, atypical muscle cells, the nuclei of which were more plump and irregular than those of the media, and in a few forks small cells with scanty but foamy cytoplasm. Interruptions or pale staining of the internal elastic lamina were frequently seen and slight metachromasia was demonstrable in both intimal thickenings and media.

A further 22 brains were obtained from sheep (ranging from lambs to 7-year-old animals). The cerebral arteries were dissected and, after formalin fixation, serial frozen gelatin sections of 51 major arterial forks were prepared and stained with hæmatoxylin and Fett rot to demonstrate fat[1]. Sections from 5 forks were tested histochemically for cholesterol and cholesterol esters by the Schultz technique[4].

Intimal thickenings also occurred at forks and branchings in this material. Lipid was found in 45 of the 51 forks and in all but one of the 22 animals. It occurred even in lambs and was always localized to the zone of intimal thickening (Fig. 1). It was mostly deep in the intima and occurred at large forks and to a lesser extent at smaller branchings included in the sections. Lipid was strongly positive for cholesterol and cholesterol esters in the sections tested, though little birefringence was observed with polarized light.

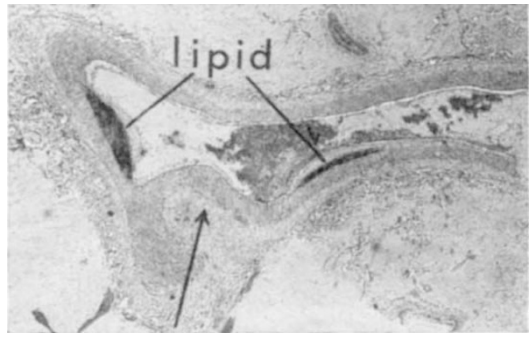

Fig. 1. Lipid deposits are the dark zones indicated in the two intimal pads at this cerebral arterial fork from a sheep. Arrow indicates direction of blood flow. Hæmatoxylin and Fett rot (×14·5)

Lipid in minimal quantities occurred as fine droplets, imparting a faint pink to the deeper half of the intima, but it was not possible to be certain whether or not it was intracellular. In moderate amount, lipid occurred in larger and more densely packed droplets, giving a red appearance to the fatty zones. A few macrophages were found containing densely stained and irregular sized droplets, but the very large foamy lipophages seen in human atherosclerosis and, to a greater extent, in cholesterol atherosclerosis of rabbits were absent. Frequently the lipid was closely applied to the internal elastic lamina outlining its wavy contour. In heavier deposition, red staining was seen in the underlying media as well. In no example was lipid observed in part of the vessel other than at the sites of these intimal thickenings. Lipid could not be demonstrated with certainty in endothelial cells.

These results indicate that intimal proliferation at the forks in these sheep arteries was the elective site for spontaneous lipid deposition.

The assumption has been made that the intimal proliferation seen in the aorta[5,6] and at bifurcations of large distributing arteries in infancy[3,7–10] is physiological and a normal structural component of the vessel wall because it occurs during the period of active growth[10,11]. However, if Duff's contention[12] is true—that lipid deposition under experimental and natural conditions occurs at sites of trauma—the present investigation and that of the cholesterol-fed rabbits[1] indicate that these intimal proliferations may not be physiological components of the arterial wall but rather compensatory thickenings in response to stress. Moreover, the findings support the belief of Wilens[5] and others[6,8,9] that this intimal thickening might be an important precursor of the atherosclerotic lesion.