(Shimazu 165) with a sample monochromator set at 470 nm and a reference monochromator set at 542 nm[3,11]; the formation of Ca-murexide complex was recorded as the difference in absorption by the preparation of the 2 alternating light beams. Optical chopping (200 Hz) was made with a revolving slotted wheel placed between the outputs of the monochromators and a fixed half-silver mirror. The optical changes and the tension were simultaneously recorded on an ink writing oscillograph.

Results. Typical examples of experimental records are shown in figure 1. The calcium transient reached its maximum during the rising phase of isometric tension, and almost returned to the initial level when the tension reached its peak. With increasing stimulus strenght, the height of both the calcium transient and the tension increased (figure 1, A and B). As shown in figure 2, the relation between the peak height of isometric tension and the total area of calcium transient was approximately linear. A similar time course of the calcium transients was observed when the preparation was stimulated with d.c. currents, though the tension decayed very slowly after the termination of d.c. currents[12].

That the above optical changes were a valid measure of the kinetics of calcium-murexide complex was supported by the following results; 1. no appreciable optical changes were produced by stretching the resting preparation or during the contraction of the preparation not treated with murexide; 2. the amount of optical changes during the contraction was dependent on the sample wavelength, decreasing markedly when it was increased from 470 to 500 nm; and 3. the same dependence on the sample wavelength was seen in the absorption changes during in vitro formation of calcium-murexide.

The calcium transients in the ABRM fibres observed in the present study are qualitatively similar to those in striated muscle fibres, except that the time course of both the calcium transients and the mechanical responses is much slower in the ABRM fibres. The slow rates of increase and decay of myoplasmic calcium may result from the slow inward movement of extracellular calcium or the slow release of intracellularly stored calcium[13], and may contribute to the slow mechanical response of the ABRM fibres together with the slow shortening velocity of the contractile mechanism[14].

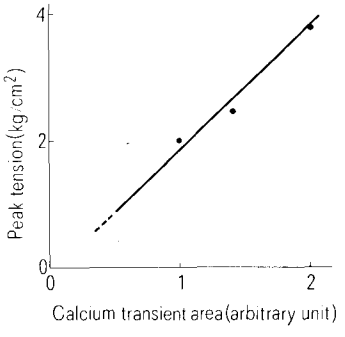

Fig. 2. Relation between the peak isometric tension and the total area of the calcium transient. The preparation was stimulated for 6 sec with a.c. currents of varying intensity.

1 Present address: Faculty of Pharmacoceutical Sciences, University of Tokyo, Tokyo 113 (Japan).
2 S. Ebashi and M. Endo, Prog. Biophys. molec. Biol. *18*, 123 (1968).
3 F.F. Jöbsis and M.J. O'Connor, Biochem. biophys. Res. Commun. *25*, 246 (1966).
4 C.C. Ashley and E.B. Ridgway, Nature, Lond. *219*, 1168 (1968).
5 C.C. Ashley and E.B. Ridgway, J. Physiol. *209*, 105 (1970).
6 R. Rüdel and S.R. Taylor, J. Physiol. *233*, 5P (1973).
7 J.R. Blinks, Eur. J. Cardiol. *1/2*, 135 (1973).
8 S.R. Taylor, R. Rüdel and J.R. Blinks, Fedn. Proc. *34*, 1379 (1975).
9 R. Miledi, I. Parker and G. Schalow, Nature, Lond. *268*, 750 (1977).
10 A. Csapo and D.R. Wilkie, J. Physiol. *134*, 497 (1956).
11 T. Ohnishi and S. Ebashi, J. Biochem., Tokyo *54*, 506 (1963).
12 B.M. Twarog, J. gen. Physiol. *50*, suppl. 157 (1967).
13 H. Sugi and T. Yamaguchi, J. Physiol. *257*, 531 (1976).
14 T. Tameyasu and H. Sugi, J. exp. Biol. *64*, 497 (1976).

Effect of low density lipoprotein and high density lipoprotein on sodium dodecyl sulphate precipitation of very low density lipoprotein from human serum

Ikpendu C. Ononogbu

Department of Biochemistry, University of Nigeria, Nsukka (Nigeria), 2 March 1978

Summary. In the presence of low density lipoprotein, the sodium sodecyl sulphate(SDS)-very low density lipoprotein(VLDL) complex sedimented, while in the presence of high density lipoprotein the complex floated. This SDS-VLDL aggregate floats at serum triglyceride to cholesterol ratio of 0.7–0.9 and sediments at a ratio of 0.2–0.5.

Sodium dodecyl sulphate (SDS) is a useful surfactant in the precipitation of proteins. It is also used in the precipitation of serum lipoproteins in the presence of divalent cations or protamine[1], and without divalent cations or protamine[2]. In precipitation of very low density lipoprotein (VLDL) and chylomicrons by SDS, a serum factor is reported to exist[2]. The effect of other lipoprotein classes, namely low density lipoprotein (LDL) and high density lipoprotein (HDL), on the precipitation of VLDL by SDS has not, however, been investigated previously.

The aim of the present investigation is therefore to find how these lipoprotein classes affect the precipitability of VLDL by SDS.

Materials and methods. Fasting venous blood sample was obtained from a healthy male colleague (J.E.) aged 38 years. Serum was separated at 2000 rpm and 4 °C for 20 min. 4 ml serum was subjected to preparative ultracentrifugation, and VLDL, LDL and HDL were obtained according to the method of Hatch and Lees[3]. SDS was purchased from Sigma chemical company (USA). The following solution preparations were made:

a) 2 ml serum, b) 1 ml serum + 1 ml LDL, c) 1 ml serum + 1 ml VLDL, d) 1 ml serum + 0.5 ml LDL + 0.5 ml VLDL, e) 2 ml VLDL, f) 1 ml LDL + 1 ml VLDL, g) 1 ml serum + 1 ml HDL.

The cholesterol and triglyceride concentrations of VLDL, LDL and HDL used in the assay were: 24 mg/100 ml and 63 mg/100 ml; 153 mg/100 ml and 20 mg/100 ml;

51 mg/100 ml and 12 mg/100 ml respectively. Serum cholesterol and triglyceride concentrations were 234 mg/100 ml and 100 mg/100 ml respectively.
Each of these solutions was mixed with aliquot of 10% SDS in 0.15 M NaCl at final SDS concentration of 0.75%. The SDS mixtures were incubated for 2 h at 35 °C and centrifuged for 10 min at 10,000 rpm and 20 °C.
The assay was also done using sera from normolipidaemic subjects with low serum triglyceride concentrations, hypercholesterolaemic subjects and hypertriglyceridaemic subjects.

Results and discussion. Solutions a), c), d) and g) gave precipitates which floated at the top of the centrifuge tube. Solution b) gave a precipitate which sedimented. The other 2 solutions e) and f) gave no precipitates.
The presence of a serum factor in SDS precipitation of VLDL had been reported[2]. In this precipitation of VLDL by SDS, however, an upper pellicle is usually obtained after centrifugation. The sedimentation of the precipitate in solution b), therefore, appeared unusual.
This unique behaviour, however, could arise from the presence of higher mol.wt LDL, in which case there will be less SDS- lipid complex[4] giving rise to a higher cholesterol to phospholipid ratio. Since unesterified sterols are more associated with peptide than esterified sterols[5], and in type II hyperlipoproteinaemia free cholesterol is raised more than cholesterol ester[6], the increase in free cholesterol can increase critical micellar concentration, thereby inhibiting precipitation of VLDL. All type II hyperlipoproteinaemic sera, however, give precipitates with SDS (Ononogbu, unpublished observation). An abnormal increase in esterified cholesterol, which results in high protein/phospholipid ratio, may also alter the negative sedimentation rate of SDS-aggregated VLDL. Abnormal increase in esterified cholesterol is encountered in Tangier disease[7]. The subject in this study, however, had a normal HDL concentration. There could also be interaction between VLDL, SDS and apo LDL giving rise to particles of higher density.
Results from normolipidaemic subjects with low triglyceride concentration (cholesterol 180–200 mg/100 ml and triglyceride 50–100 mg/100 ml) and from hypercholesterolaemic subjects (cholesterol 280–430 mg/100 ml and triglyceride 60–100 mg/100 ml) showed that the sedimentation of the SDS-aggregated VLDL is due to low concentration of VLDL triglyceride. This low triglyceride concentration is not enough to lower the SDS-VLDL aggregate density so as to float. In normal cord blood sera, where VLDL concentration is low, the SDS-VLDL aggregate was found to sediment instead of floating[8].
Normolipidaemic and hypertriglyceridaemic sera, with serum triglyceride concentrations of 130–150 and 160–270 mg/100 ml respectively, gave precipitates which floated.
Thus, whereas a serum factor is required for precipitation of VLDL by SDS, the triglyceride to cholesterol ratio of the serum determines the behaviour of the precipitated VLDL under centrifugal forces. High serum triglyceride to cholesterol ratio (0.7–0.9) gives floating SDS-VLDL aggregate, while low serum triglyceride to cholesterol ratio (0.2–0.5) gives sedimented SDS-VLDL aggregate.

1 M. Burstein and R. Morfin, Nouv. Rev. fr. Haemat. *II*, 173 (1971).
2 M. Burstein and H.R. Scholnick, Life Sci. *II*, 177 (1972).
3 F.T. Hatch and R.S. Lees, Adv. Lipid Res. *6*, 1 (1968).
4 K. Simons and A. Helenius, FEBS Lett. *7*, 59 (1970).
5 R. Smith and C. Green, Biochem J. *137*, 413 (1974).
6 D.S. Fredrickson and R.I. Levy, in: Metabolic Basis of Inherited Disease, p. 575. Ed. J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson. McGraw-Hill, New York 1972.
7 D.S. Fredrickson, P.H. Altrocchi, L.V. Avoioli, DeW.S. Goodman and H.C. Goodman, Ann. Int. Med. *55*, 1016 (1961).
8 I.C. Ononogbu, Thesis, London University 1976.

Prostacyclin-like activity of endothelium and subendothelium – important for atherosclerosis?

K. Silberbauer, H. Sinzinger, Maya Winter, W. Feigl and F. Ring

2nd Department of Internal Medicine, Department of Physiology, Department of Pathology and 2nd Department of Gastroenterology, University of Vienna Medical School, A-1090 Wien (Austria), 12 February 1978

Summary. Morphological control of Moncada's bioassay for prostacyclin (PG I_2) activity measurement shows that the activity depends not only on endothelium, but in important amounts on subendothelial tissue too. Therefore, it can be concluded that platelet thrombus formation after endothelial cell injury does not depend only on the PG I_2-producing ability of the tissue.

Moncada and his coworkers[1,2] reported that human arteries and veins are able to generate an unstable substance, prostacyclin (PG X, PG I_2), which is the most potent known endogenous inhibitor of platelet aggregation. It has been shown that the main activity of prostacyclin is produced by endothelial cells[1–3]. The very important conclusion drawn from this results was that a mural platelet thrombus can only form itself over areas of destroyed or detached endothelium[4–6], because the subendothelial part of the intima has no (or not enough) ability to produce prostacyclin after interaction with platelets.
We would like to present some evidence that subendothelial tissue of rat and rabbit arteries is able to produce important amounts of prostacyclin too.

Material and methods. 4 male rats (mean b.wt 300 g) and 4 male rabbits (mean b.wt 3 kg) were anaesthesized with Pentothal-Na®, and small rings (18±3 mg wet wt) of the abdominal aorta were immediately removed and kept in tris-HCl buffer (0.05 moles/l, pH 7.5) at 2 °C (pH 7.1). 100 µl supernatant of the buffer in which the arteries were incubated for 3 min at room temperature (22 °C) were added to human and rat[7] platelet rich plasma (PRP). PRP samples were adjusted with platelet poor plasma (PPP) to a definite platelet number of about $250 \times 10^3/\mu l$. Platelet aggregation was induced in 1-ml samples by adding adenosinediphosphate in a final concentration of 2 µmoles/l. Platelet aggregation was measured in a platelet aggregometer (Born) under standardized conditions. At each stage of the experiments, small pieces of arteries free of adventitia were removed with a scalpel under minimal trauma[8] for light and scanning electron microscopy. The material was immediately diffusion-fixed in a buffered (pH 7.4) glutaraldehyde for 48 h.