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 All type II hyperlipoproteinaemic sera, however, give precipitates with SDS (Ononogbu, unpublished observation).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.

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

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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.

Results and discussion. Arterial rings show prostacyclin-like activity, as demonstrated by inhibition of platelet aggregation. Within 30 min of removal, there is only a slight but not significant decrease in the availability of prostacyclin. However, immediately after removing, the number of endothelial cells shows a continuous decrease in light and scanning microscopy. After 10 min, the number of endothelial cells falls beyond 10% of the starting value. After 15 min, endothelial cells almost completely disappear. As the storing buffer is changed every 3 min, it can be concluded that the nearly complete disappearance of endothelial cells does not alter significantly the availability of prostacyclin-like substance.

Incubation with Ketoprofen, a prostaglandin synthetase inhibitor, prevents prostacyclin production; boiling for 30 sec destroys the platelet active compound as well as incubation of tissue in buffer for 10 min at 37 °C.

Morphological control of Moncada's bioassay[1,2,9,10] for prostacyclin measurement shows that after desquamation of endothelial cells the prostacyclin activity does not alter significantly. This recalls some evidence, as found by Hornstra[11] in a different experiment, that some other than endothelial cells in the subendothelial part of the intima have a similar ability to produce prostacyclin. Though there is an activity in the media[11,12], this is not comparable with the quantity found in the subendothelium. Probably this activity is due to some special differentiated cells[13,14] found in the intima[15]. This view is also supported by the fact that a thrombus during organization has an activity[16], probably derived from the so-called pseudoendothelium[17]. In contrast, removal of endothelium causes an immediate formation of a mural platelet thrombus in vivo[18] and in vitro with rabbit[19] and human[20] arteries. Rubbing of endothelium with sand paper[11] increases prostacyclin activity, whereas mincing has no effect on the renal prostacyclin production[21]. Since it can be accepted that, over an intact endothelium mural platelet, thrombus cannot be found[22], the theory cannot completely be upheld that platelet thrombus is formed after endothelial desquamation, because there is not enough prostacyclin production to prevent the genesis of a mural platelet thrombus.

Beside this fact, we suggest that not only the activity in normal cells[1,12] and tissue[1,21,23-25], but especially the basal[1,26] activity and availability[24,26,27] of altered or transformed cells[13,15], or metabolically influenced tissue, namely in uremia[24,27] myocardial infarction[28] etc, demonstrates the very important, yet uncleared role of prostacyclin in atherosclerosis.

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Chemically mediated cell-to-cell contractile activation in isolated frog atrial cardiac cells[1]

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Summary. A dying single frog atrial cardiac cell liberates an unknown substance which diffuses away from the dying cell and activates contractile activity in other isolated intact single cardiac cells within the vicinity of the dying cell.

Recently we described the successful preparation of isolated intact single frog atrial cells by dispersion of frog atrial tissue with a combination of trypsin and collagenase[3]. We had hoped that it would be possible to record intracellular potentials from these single cells with microelectrodes, but we found that the single cell dies immediately upon penetration of its membrane with a microelectrode. While attempting these microelectrode recordings, we observed the curious phenomenon that neighboring cells not in any obvious direct contact with the impaled cell often gave twitch-like contractions after the death of the impaled cell. The time interval between when a cell was impaled and a neighboring cell contracted increased as the distance between the cells increased suggesting that a substance was