		Concentration of aminoglycosides (μg/g) Free	Total	Free/Total	Degree of histological lesions
1 Dose	Amikacin	10±2	16±4	0.64±0.03	0
	Tobramycin	30±1[b]	57±16 ns	0.60±0.13	0
	Gentamycin	65±4[c,e]	105±8[b] NS	0.63±0.08	0
5 Doses	Amikacin	49±2	75±10	0.68±0.10	0
	Tobramycin	124±12[b]	195±30[a]	0.65±0.07	0
	Gentamycin	187±22[b] NS	387±18[c,d]	0.49±0.08	0.3±0.3
12 Doses	Amikacin	74±5	148±9	0.50±0.04	0
	Tobramycin	174±45 ns	288±45[a]	0.59±0.08	0
	Gentamycin	269±40[b] NS	532±101[a] NS	0.53±0.07	1.7±0.3

The concentrations and ratios of free and total aminoglycosides and the degree of histological lesions in the rat kidney (mean ± SE, n = 3). Significance of difference from amikacin is shown by ns = not significant, [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$. Significance of difference between tobramycin and gentamycin, is shown by NS = not significant, [d] $p < 0.01$, [e] $p < 0.001$

animals were killed 24 h after the last drug injection. 1 kidney was rapidly removed and as similar as possible latitudinal slices were cut from the middle part. The slices were homogenized in phosphate buffer (0.1 M, pH 8) with a Potter-Elvehjem homogenizer. 2 equal homogenates were prepared from each kidney, one for the determination of free, another for the determination of total aminoglycosides. The aminoglycosides were determined microbiologically using *Bacillus subtilis* as the marker organism, free and total separately as described by Kornguth and Kunin.

Kidney samples were taken from each kidney for histological examination. They were fixed in buffered 10% formalin, embedded in paraffin, sectioned at 7 μm and stained with hematoxylin eosin. The degree of histological lesions was estimated as follows. 0 = no changes; 1 = small eosinophilic granules in the cytoplasm of the epithelial cells of proximal convoluted tubules, no changes in the nuclei and basement membranes; 2 = necrotic changes in some of the proximal convoluted tubules. Statistical analyses were performed by Student's t-test.

Results and discussion. The results are presented in the table. The concentrations of free and total aminoglycosides in the renal tissue followed the order amikacin < tobramycin < gentamycin, when administered in equal weight by weight doses (mg/kg/day). The ratio between free and total aminoglycosides was of the same order independent on the lenght of the administration and on the aminoglycoside used (about 0.6).

Histological lesions in the kidney were observed only in the groups given gentamycin 5 and 12 doses. In these groups the mean concentration of aminoglycoside was also the highest. It is not known with certainty whether or not the renal concentrations of aminoglycosides correlate with the degree of nephrotoxicity[4]. The present work as well as our recent observations indicate that there is a correlation within certain limits of concentration. No histological tissue damage was observed with low concentrations (roughly < 200 μg/g free aminoglycoside in the whole kidney). On the other hand, the extensive tissue damage occurring with high dose levels sets the limit to the amount of aminoglycoside taken up by the kidney. Thus, no correlation between dose and tissue damage is observed here.

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A triple test for screening biological activity of prostacyclin analogues

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Summary. 6,9-Thiaprostacyclin was used as a representative of prostacyclin analogues with the aim to design the most convenient procedure for evaluation of vasodilator, anti-platelet and other biological properties within this new group of potential anti-thrombotic agents.

Recently, a new vasodilator and anti-thrombotic hormone was discovered[1,2], synthetized[3,4] and named prostacyclin (PGI_2). Prostacyclin is supposed to protect blood vessels against thrombosis[2] and against atherosclerosis[5,6]. Prostacyclin molecule has been thrust into the forefront of chemical prostaglandin research[3,4,7]. The main goal is to obtain a stable analogue of the natural unstable hormone.

Any PGI_2 analogue may mimic biological activity of the parent hormone, but it also may have biological activity of prostaglandins (PGs) or thromboxane A_2 (TXA_2) which are the other members of arachidonic acid cascade[8]. A method here proposed differentiates between these activities on the basis of reaction of vascular smooth muscle, inhibition of blood platelet aggregation in vitro and reversal of platelet aggregation in vivo.

In this study, we used prostacyclin (PGI_2) and 6,9-thiaprostacyclin (TP), both of which were synthetized by Nicolaou et al.[7]. The stock solutions were prepared in 99% ethanol

and contained 10^{-3} M of PGI_2 or TP and 9×10^{-3} M of NaOEt. Prostaglandin E_2, $F_{2\alpha}$ and 11,9-epoxymethano analogue of PGH_2 (EMA) were kindly offered by Dr J.E. Pike, Upjohn Co., Kalamazoo, USA.

Isolated strips of bovine coronary artery[9], rabbit mesenteric artery[10] and rat colon[11] were superfused with Krebs' solution which was composed of the following in mM: NaCl, 118; KCl, 4,7; $CaCl_2$, 2,5; KH_2PO_4, 1.2; $MgSO_4$, 1.17; $NaHCO_3$, 25.0 and glucose, 5.6, and additionally contained (µg/ml): atropine, 0.1; phenoxybenzamine, 0.1; propranolol, 2.0; mepyramine, 0.2; methysergid 0.2; and indomethacin, 1.0.

Krebs' solution (at 37 °C, pre-gassed with 95% O_2 and 5% CO_2) superfused the assay organs in cascade at a speed of 3 ml/min. Tone of the organs was registered isotonically by Harvard transducers type 386, which were connected to a Watanabe multirecorder.

These 3 detector tissues clearly differentiate between PGI_2 (relaxation of bovine coronary artery, and rabbit mesenteric artery, no effect on rat colon), PGE_2 (contraction of bovine coronary artery and rat colon, relaxation of rabbit mesenteric artery) and TXA_2 (contraction of bovine coronary artery and rabbit mesenteric artery, no effect on rat colon). Figure 1 shows 1 of 6 experiments in which TP had PGI_2-like activity (relaxation of bovine coronary artery) and PGE_2-like activity (contraction of rat colon). Unlike PGI_2 or PGE_2, TP contracted rabbit mesenteric artery in a manner typical for EMA (figure 1), which biological activity resembles that of TXA_2[12]. The contractile action of TP on a cat coronary artery[13] is another example of TXA_2-like activity of this PGI_2 analogue. Thus in a molecule of 6,9-thiaprostacyclin there are combined biological activities of 3 main metabolites of arachidonic acid, i.e. of PGI_2, TXA_2 and PGE_2 (or $PGF_{2\alpha}$).

Platelet-rich plasma (PRP) was obtained from rabbits[6] and aggregated by adenosinediphosphate (ADP) in a Born aggregometer. The anti-aggregatory potencies of PGI_2 and TP were measured by an instillation of either substance (0.5–200 ng/ml) 1 min before ADP was added to PRP. The calculated IC_{50}s for the anti-aggregatory potencies were 2.1±0.3 ng/ml (mean±SE, n=12) for PGI_2 and 56±9 ng/ml (mean±SE, n=6) for TP. Thus in vitro TP was 25 times weaker anti-aggregatory agent than PGI_2.

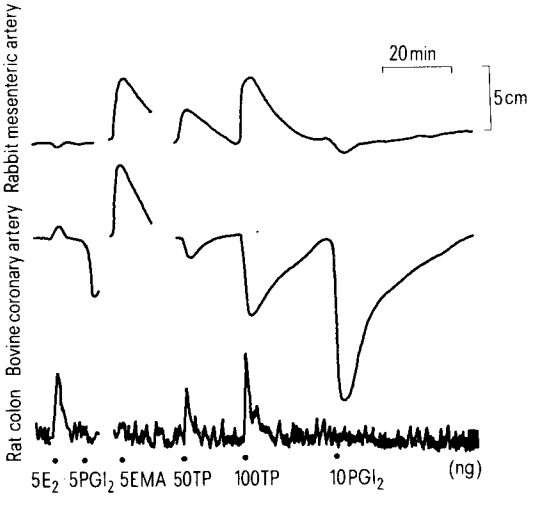

Fig. 1. The effect on the tone of smooth muscle organs of prostaglandin E_2 (E_2), prostacyclin (PGI_2), 11,9-epoxymethano analogue of PGH_2 (EMA) (a compound with thromboxane A_2-like activity) and 6,9-thiaprostacyclin (TP). Figures refer to doses in ng.

The last test in our procedure was designed to measure in vivo anti-platelet activity of PGI_2 analogues. Mixed-venous blood from right atrium of anaesthetized (40 mg/kg of sodium pentobarbitone, i.p. and i.v.) and heparinized (2500 units/kg, i.v.) cats superfused (3 ml/min) a strip of rabbit tendon of Achilles (RTA). After superfusion, blood was returned by gravity to the venous system of cats. Weight of RTA was continuously recorded by an electronic balance; RTA gained in weight owing to the deposition of platelet clumps on the surface of collagen fibres[14]. The maximal gain in weight (400–600 mg) occurred after 30–40 min of blood superfusion.

PGI_2 or TP were infused into the superfusing blood (figure 2a) or into the left femoral vein (figure 2b), at least 30 min after the experiment started. PGI_2 and TP reversed platelet aggregation i.e. caused platelet de-aggregation, and then the weight of the blood superfused RTA decreased in a dose-dependent manner (figure 2, a and b). A decrease in RTA weight by 50% was obtained after local infusion of PGI_2 or TP at concentrations of 6.5±1.1 ng/ml (mean±SE, n=6) and 122±17 ng/ml (mean±SE, n=4), respectively. The corresponding ID_{50}s for the de-aggregatory action of PGI_2 and TP infused intravenously were 5.4+0.4 µg/kg (mean±SE, n=6) and 62±10 µg/kg (mean±SE, n=3), respectively. The in vivo de-aggregatory potency of TP was 10–20 times weaker than that of PGI_2.

The above 3 tests allow us to conclude that 6,9-thiaprostacyclin has a prostacyclin-like activity on bovine coronary artery and on blood platelets. This activity is approximately 20 times weaker than that of the natural hormone. 6,9-Thiaprostacyclin has also prostaglandin-like activity on gastrointestinal tract (rat colon) and thromboxane A_2-like activity on certain blood vessels (e.g. rabbit mesenteric artery). 6,9-Thiaprostacyclin is active in vivo as an anti-platelet agent; however, the duration of this action is not longer than that of prostacyclin (figure 2b). Since 6,9-thiaprostacyclin is more stable in aqueus solution than prostacyclin[7], one has to assume that in vivo 6,9-thiaprostacyclin is avidly removed from the circulation.

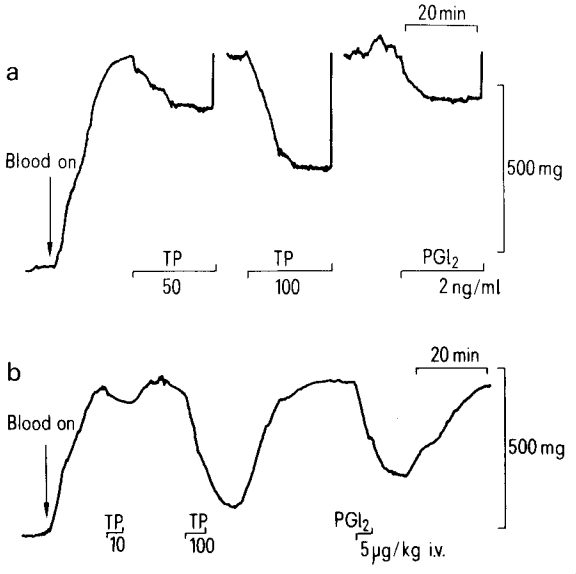

Fig. 2. *a* shows the de-aggregatory effect of 6,9-thiaprostacyclin (TP) and prostacyclin (PGI_2) infused directly into blood which superfused a collagen strip. Concentrations of TP and PGI_2 are in ng/ml. Vertical scale represents the weight of platelet clumps. After the end of each infusion of TP or PGI_2, a 20 min period of recovery was allowed. *b* shows the potency and the duration of de-aggregatory effects of TP and PGI_2 after their intravenous infusion (µg/kg) into an anaesthetized and heparinized cat[14].

Thus, a quick insight into anti-platelet and vasomotor activities of prostacyclin analogues, searching out their prostaglandin-like and thromboxane-like properties as well as evaluation of their in vivo de-aggregatory potency, are the main features of the proposed triple test.

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Membrane interiorization by phagocytosing macrophages – an ultrastructural morphometric approach[1]

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Summary. Stereological methods have been used to quantify selected membrane compartments of normal and activated rat peritoneal macrophages, before and after phagocytosis of latex beads. Despite being rounder, activated cells are more efficient phagocytes: 30 min after latex challenge they suffer a greater nett depletion of plasma membrane and sequester more and larger phagocytic vacuoles. However, phagocytosis of latex is not the major route of membrane interiorization.

A wide range of substances with different physical and chemical properties is known to induce alterations in the morphological appearance of peritoneal macrophages. The changes reflect a functional 'stimulation' or 'activation' of cells and this includes an enhanced endocytic ability[2]. At the ultrastructural level, activation involves an increase in cell size characterized by differential hypertrophy of certain intracellular compartments, particularly of lysosomes. Alterations in the number and dimensions of cell surface features are also reported[3-6].

Structural differences between normal and activated, resting and endocytosing cells may be estimated by morphometry. In a series of studies undertaken in this laboratory, stereological techniques[7] were used to quantify ultrastructural variations between resident rat peritoneal macrophages and cells stimulated with Freund's adjuvants[5,6]. Amongst other changes, it was revealed that induced peritoneal exudates contained a population of activated macrophages which had substantially less plasma membrane surface (on average, roughly 30% less than normal). In addition, the cells were rounder than normal and this rounding-up was partly due to the sequestration of adjuvant ingredients within membrane-bound vacuoles (phagosomes)[5].

In this report, we present the results of a preliminary stereological investigation designed to assess the effect of adjuvant-induced membrane depletion on the subsequent phagocytic potential of these cells. At the same time, we have attempted to evaluate changes in plasmalemma surface area that accompany phagocytosis. For this purpose, latex beads afforded a useful means of following phagocytic activity.

Materials and methods. Full particulars of all preparative stages will be found in our earlier reports[5,6,8]. Macrophages were harvested from an inbred strain of rats by peritoneal lavage. Normal animals were used, together with rats challenged 5 days previously by an i.p. injection of Freund's complete adjuvant (FCA) emulsified in Hank's solution. These 2 groups were controls. In addition, experimental groups of normal and FCA-induced cells were challenged in vivo by a single i.p. injection of latex beads (EMscope; mean diameter 0.481 ± 0.002 µm) diluted 1:25 in balanced Hank's solution. The dose (0.2 ml for normal animals and 1.4 ml for FCA-challenged rats) was according to predicted macrophage yields obtained from differential cell counts and haemocytometry. Cells were harvested 30 min later.

Following centrifugation, the 4 groups of cells were fixed in 3% buffered glutaraldehyde (pH 7.4) and postfixed in 2% aqueous osmium tetroxide. After dehydration, cell pellets were embedded in araldite. Ultrathin sections were taken for electron microscopy.

A systematic random sample of cell profiles was taken using an AEI-Corinth Electron Microscope. Only cell profiles in which the nucleus was present were recorded[8]. Final print magnification was ×11,230 as determined from micrographs of a grating replica. Morphometric data were recorded with the aid of a simple quadratic test lattice (spacing between lines 1 cm) superposed on to each micrograph. A total of 100 micrographs per group was analyzed and this sample size was larger than estimated minimal requirements using cumulative mean plots[5].

Standard stereological relationships[5-7] were invoked to estimate morphological parameters we considered important. Membrane surface areas (of plasmalemma and of latex-containing vacuoles) were estimated by counting intersections between the sectioned membranes and the test lines on the lattice. Numerical densities of (latex) vacuoles were calculated from the number and size of vacuole profiles. Cell volume-to-surface ratio was determined using the method described by Chalkley et al.[9]. Estimates of mean cell diameter were derived from measured cell profile areas (assuming circularity) and mean cell volume computed for a sphere of equivalent diameter. Finally, a measure of how much more surface area the average cell had than an equivalent sphere was made by comparing cell volume-to-surface ratio with that of a sphere of equivalent diameter. This surface amplification factor provides a useful indication of cell roundness.