ken cell preparations and ability to inhibit aggregation has previously been noted by Yoshimoto *et al.* [3] and in intact platelets by Needleman *et al.* [5] and Blackwell *et al.* [6].

Several groups have shown that high concentrations of collagen can induce aggregation by a mechanism which does not involve the generation of prostanoids [12, 13] but the low concentration of collagen used in the present experiments (1 μg/ml) and the abolition of aggregation by indomethacin suggests that the aggregation observed both in control plasma and in plasma treated with UK 34787 is caused by a prostanoid aggregating agent. The only candidates are TXA_2 and the endoperoxides. Although it is possible that the residual 25% aggregation produced by 1 μg/ml collagen in the presence of 10^{-4} M UK 34787 is due to the residual 5% of thromboxane produced, inspection of the concentration–response curves for aggregation and thromboxane production suggests this is unlikely. Thus, the abolition of residual aggregation by indomethacin does not appear to be related to the further reduction in thromboxane production from 5 to 2% of control. In our opinion, it is more likely that the residual aggregation seen in the presence of selective inhibition of thromboxane synthetase and abolished by indomethacin is due to accumulation of prostaglandin endoperoxides which may act at the same receptor as TXA_2 [7]. This view is supported by the observation that imidazole-resistant aggregation to exogenous arachidonic acid is due to accumulation of prostaglandin endoperoxides [5].

During the preparation of this paper, Bertele *et al.* [14] showed that another inhibitor of thromboxane synthesis, UK 37,248-01 (4-[2-(1H-imidazol-1-yl)-ethoxy] benzoic acid hydrochloride) at concentrations which abolished thromboxane synthesis failed to inhibit the aggregation of human PRP from one of three subjects in response to exogenous arachidonic acid. The use of arachidonic acid as the aggregating agent provides potential substrate for extensive synthesis of prostaglandin endoperoxides in the absence of thromboxane synthesis. Similarly the use of exogenous endoperoxides [5, 6], may provide unrealistically high concentrations of aggregating agent. With the low concentrations of collagen used in the present study the generation of endogenous arachidonic acid and thus the potential for endoperoxide synthesis and accumulation may more nearly reflect the situation *in vivo* when aggregation is stimulated by vascular damage although PRP contains no source of prostacyclin synthetase which might generate PGI_2.

Whether endoperoxide-mediated aggregation in the presence of an inhibitor of thromboxane synthesis can be a significant thrombotic mechanism *in vivo* remains an open question. Endoperoxides generated by the platelet *in vivo* may, in the presence of an inhibitor of thromboxane synthesis, be diverted to the production of anti-aggregatory prostaglandins PGD_2 and PGI_2 [14, 15]. However, the potential importance of prostaglandin endoperoxides as aggregating agents in their own right should not be underestimated.

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Prednisolone-mediated alterations in ribosomal RNA turnover in rat liver

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Prednisolone (Δ′ hydrocortisone) has found extensive applications in diverse areas of medical therapeutics [1–4]. Unfortunately, prolonged or excessive usage of this drug results in several 'side-effects' including muscle wasting and liver enlargement, to mention but a few. Since the liver is a target organ for prednisolone [5–7] and since all phenotypic response require the generation of RNA [8, 9], it seemed appropriate, in unravelling more clues to the mechanism of action of prednisolone, to investigate the effect of this drug on ribosomal RNA turnover in this organ.

Materials and methods

Male Wistar rats of fasted body wt 140–190g were used in all experiments. 'Test' animals were paired with their 'controls' on the basis of initial fasted body weight. Prednisolone acetate (Boots Pure Drug Company, Nottingham, U.K.) was administered daily to the 'test' animals at a dose of 1.0 mg/100g initial fasted body weight by subcutaneous injections while the 'controls' received an equal volume of 0.9% (w/v) saline. Animals used in RNA turnover studies each received, by intraperitoneal injections, a single dose of 20 μCi [5-^{3}H]orotic acid (sp. act. 19–25 Ci/mmole) two days before the commencement of prednisolone injections.

The total protein, total RNA, and total DNA contents of the rat liver were estimated by previously described methods [10, 11]. Ribosomes were isolated from the organ essentially as described by Hirsch and Hiatt [12], and the RNA contents of these ribosomes were assayed as described by Fleck and Munro [13]. Yeast RNA, treated in the same manner, served as a standard for the purpose of quantitation. The radioactivity of the ribosome suspension in 0.1M KOH was measured by liquid scintillation using the Intertechnique spectrometer (model SL 30).

The nucleotide pool in the rat liver, following the administration of [^{3}H]orotic acid, was assayed as previously described [14].

Student's t-test was used for statistical analyses.

Results and discussion

Both the prednisolone-treated animals and their 'controls' received and consumed the same quantity of food over the experimental period. Thus, our results (Table 1) confirm previous observations [15, 16] of a net loss of body weights of animals receiving glucocorticoids. In contrast, the liver of the prednisolone-treated animals gained between 17% and 24% of the liver weight of the control group of animals. These prednisolone-mediated changes occurred in a constant cell population, as the constancy of DNA levels indicates (Table 1). Thus, the observed changes were intracellular alterations rather than changes in cell number.

Our investigations revealed that the liver enlargement which followed the administration of prednisolone involved an increase in the hepatocellular protein content (Table 1). The simultaneous increases in RNA levels in the organ suggested that the observed changes in protein concentration may be related to alterations in the metabolism of ribonucleic acids in the liver. Since most of liver RNA is ribosomal [11], we proceeded to investigate the turnover of this RNA. A progressive loss of specific radioactivity (cpm/mg RNA) of ribosomal RNA was observed in both 'test' and 'control' animals (Fig. 1). The rate of these losses followed first order kinetics and it was thus possible, as has been demonstrated by several workers [10, 11, 17, 18], to use these data to determine rates of breakdown and synthesis of the RNA. The rate constants of ribosomal RNA degradation in the liver of prednisolone-treated and 'control' animals were calculated, from the slopes of the lines in Fig. 1B, to be 0.1065 and 0.1612, respectively. These constants corresponded to biological half-lives of 6.51 and 4.3 days, respectively. The amount of ribosomal RNA synthesized per day was 8.13 mg in the prednisolone-treated animal and 6.02 mg in the control animal.

Consideration was given to the possibility that prednisolone may have altered the nucleotide pool rather than ribosomal RNA metabolism directly. We found no gross changes in this pool following prednisolone administration. It is recognised, however, that the pathways for ribosomal RNA synthesis do not derive directly from nucleotides and that several complexities are abound in the genetic transcription of this RNA from nuclear DNA.

In summary, the results of our investigations suggest that prednisolone altered ribosomal RNA metabolism in the rat liver by reducing the rate of breakdown and increasing the rate of synthesis of this RNA. These observations may explain the paradoxical increases in the weight and protein content of this organ in the face of a significant loss of the animal's overall body weight.

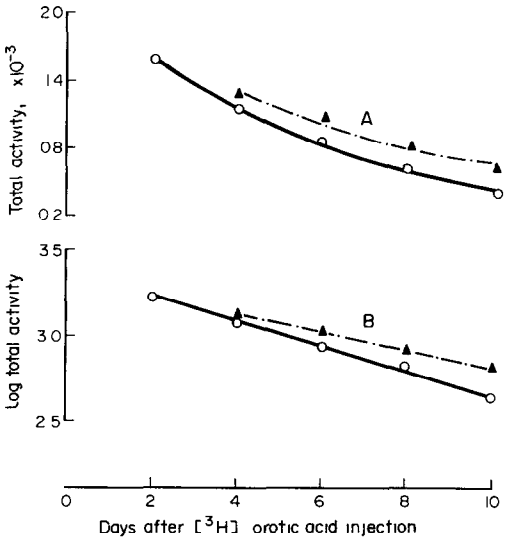

Fig. 1. The alteration in radioactivity of rat liver ribosomal RNA following prednisolone treatment. Each point represents the mean result from 4–6 animals. Triangles indicate results from prednisolone-treated animals and circles represent the 'controls' on graph B, the slope of each line was plotted by the 'least squares' method (total activity = cpm/liver/100g initial body weight).

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Table 1. Alterations in body weight, liver weight, hepatic protein and nucleic acids of prednisolone-treated rats

Interval	2		4		6		8	
Group	A	B	A	B	A	B	A	B
Initial body weight (g)	157 ± 4.2	153 ± 3.8	150 ± 3.9	151 ± 3.6	150 ± 3.7	148 ± 3.8	152 ± 3.1	154 ± 4.4
Body weight on sacrifice (g)	156 ± 4.5	161 ± 3.6	142 ± 4.1	163 ± 3.1	142 ± 4.5	173 ± 2.9	140 ± 4.8	178 ± 3.3
Percentage loss or gain in body weight	−0.9 ± 2.0	+5.0 ± 2.9	−5.4 ± 3.0	+8.4 ± 2.9	−5.5 ± 4.0	+16.7 ± 3.4	−7.9 ± 3.4	+16.1 ± 4.1
	($P < 0.005$)		($P < 0.005$)		($P < 0.005$)		($P < 0.005$)	
Liver weight (g/100g initial body weight)	5.1 ± 0.4	4.1 ± 0.4	5.7 ± 0.4	4.6 ± 0.3	5.6 ± 0.3	4.8 ± 0.1	5.6 ± 0.2	4.8 ± 0.1
	($P < 0.025$)		($P < 0.025$)		($P < 0.025$)		($P < 0.025$)	
Total liver protein*	722 ± 83	576 ± 78	800 ± 31	620 ± 30	963 ± 67	759 ± 61	979 ± 60	760 ± 47
	($P < 0.05$)		($P < 0.005$)		($P < 0.025$)		($P < 0.025$)	
Total liver RNA*	44.5 ± 5.4	37.6 ± 5.2	47.8 ± 4.7	37.7 ± 4.3	46.2 ± 2.6	38.3 ± 3.8	47.2 ± 2.8	37.6 ± 1.6
	($P < 0.05$)		($P \pm 0.025$)		($P \pm 0.025$)		($P \pm 0.01$)	
Total liver DNA*	12.5 ± 0.35	12.5 ± 0.34	12.6 ± 0.4	12.6 ± 0.45	12.6 ± 0.21	12.9 ± 0.50	12.4 ± 0.51	12.7 ± 0.50
	(N.S.)		(N.S.)		(N.S.)		(N.S.)	

Interval refers to the period, in days, between the commencement of prednisolone treatment and sacrifice of the animal.

Group A indicates results obtained from prednisolone-treated rats and Group B refers to corresponding 'controls'.

* Results are expressed in mg/liver/100g initial body weight of the animal and are the means from 4–6 rats ± S.D.

Figures in parentheses indicate level of statistical significance between prednisolone-treated and 'control' animals using the Student's *t*-test (N.S. = not statistically significant).

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The time course and magnitude of prostacyclin (PGI_2) production by rat aortic rings incubated in human plasma

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The clinical pharmacology of PGI_2 makes it an attractive candidate for a role in protection against vascular disease [1]. In vessel walls it is the intima that has the greatest PGI_2 synthesising activity [2, 3]. In life the intimal endothelium is bathed in plasma and this may contain factors that stimulate [3–5] or inhibit [6–8] prostanoid synthesis. Chopped aortic rings produce PGI_2 when incubated in Krebs' solution or buffer [9] and are a suitable system for the study of PGI_2 synthesis *in vitro*. However, studies on aortic rings incubated in plasma [4] have been hampered by dependence on bioassay, because PGI_2 is rapidly hydrolysed to 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) which is biologically inactive. Consequently PGI_2 concentrations measured sequentially during an incubation reflect the rate of breakdown as well as synthesis. Even though hydrolysis of PGI_2 is a non-enzymic process its rate varies substantially in platelet-poor plasma (PPP) from different subjects. Thus, in one study [10] half-lives in PPP from healthy subjects varied from 8.9 to 23.3 min at 37°, probably because of variable stabilization of PGI_2 by albumin [10–12].

To circumvent these problems we measured prostanoid synthesis by rat aortic rings incubated with PPP at 37°, using a modified radioimmunoassay (RIA) for 6-oxo-$PGF_{1\alpha}$ [13, 14]. Interference in the RIA by plasma protein [14] was avoided by diluting all samples by 20-fold or greater before assay. The diluted samples were temporarily acidified to ensure that all PGI_2 was hydrolysed. 6-oxo-$PGF_{1\alpha}$ measured in this way reflects only the synthesis of PGI_2 and not its breakdown during incubation. In addition, the concentration of PGI_2 present as such was determined by bioassay and by a modification of a 6-oxo-$PGF_{1\alpha}$ RIA.

Materials and methods

Preparation of aortic rings. Male CD rats (Charles River, Margate, U.K.) 250–350 g were stunned and killed by cervical dislocation. The aorta was dissected rapidly from the heart to the aortic bifurcation and rinsed in ice cold balanced salt solution. A 5 cm length immediately distal to the origin of the left subclavian artery was chopped into 1 mm rings with a McIlwain tissue chopper. The fifty rings produced were divided into two groups of 25, alternate rings being allotted to each group. Each group of rings was stored in 5 ml balanced salt solution (Gey's solution, Gibco, Uxbridge, U.K) on ice for less than 30 minutes until the start of the incubation with PPP.

PPP. Venous blood was drawn from healthy male subjects and added to 3.8% trisodium citrate (10 vol. blood: 1 vol. citrate) in plastic vials. It was spun immediately at 1000 *g* at 4° for 20 min and the supernatant plasma separated and stored at −20° until required.

Incubations. PPP was thawed at room temperature and 2 ml added to a polypropylene incubation vial in a shaking water bath at 37°. The pH of the plasma was maintained at pH 7.4–7.6 by gassing with 5% CO_2 in O_2. At zero time a group of 25 rings was added to the plasma. At 4, 8, 15, 30, 45 and 60 min a 100 μl aliquot of incubate was removed and added to 2.5 μl of 1M NaOH on ice to adjust the pH to greater than 10. The samples were then stored at −20° and assayed within 24 hr.

Radioimmunoassay. The 4 and 8 min samples were diluted 20-fold with Tris buffer (50 mM) pH 8.5; the 15–60 min samples were similarly diluted 100-fold. One portion (250 μl) of the diluted sample was then acidified with 5 μl of 2M HCl. After 20 min (allowing conversion of all PGI_2 present to 6-oxo-$PGF_{1\alpha}$) the pH was restored to 8.5 with 5 μl of 2M NaOH. Another portion (250 μl) of the diluted sample was added to 10 μl of 1M NaCl (non hydrolysed sample). This method allows quantitation of both 6-oxo-$PGF_{1\alpha}$ and PGI_2. The total 6-oxo-$PGF_{1\alpha}$ content was that determined in the hydrolysed sample. The PGI_2 concentration was calculated from the difference in 6-oxo-$PGF_{1\alpha}$ content between the hydrolysed and non-hydrolysed samples. The full methodological details and assay validation will be published elsewhere [14].

Bioassay. Platelet-rich plasma (PRP) was prepared from citrated venous blood prepared as above. This was immediately centrifuged at 400 *g* at 20° for 7.5 min. Undiluted incubate (5–20 μl) was added to 250 μl of PRP in the cuvette of a Payton aggregometer at 37° one min before the addition of adenosine diphosphate (Sigma Chemical Co., London, U.K.) sufficient to produce secondary aggregation. Inhibition of aggregation by the incubate was compared with inhibition produced by known amounts of standard PGI_2 (a generous gift from Wellcome Research Laboratories, Beckenham, U.K.). Each sample was bracketed between two standards producing respectively a slightly greater and a slightly smaller effect than the unknown. The concentration of PGI_2-like activity in the incubate was then calculated by interpolation.