

A PLASMA FACTOR WHICH STIMULATES PROSTACYCLIN FORMATION IS INCREASED IN DIABETES. M. Johnson, A. H. Reece and H. E. Harrison. Department of Bioscience, ICI Pharmaceuticals Division, Macclesfield, England.

Vascular prostacyclin (PGI<sub>2</sub>) generation is decreased in diabetes in experimental animals and in man. In this study, we have investigated the possibility that levels of a plasma factor (s) modifying PGI<sub>2</sub> production are abnormal in diabetes. Aortic rings from diabetic or age-matched control rats were washed in Krebs buffer to reduce endogenous PGI<sub>2</sub> formation. Addition of rat or human cell-free plasma stimulated PGI<sub>2</sub> release by the "exhausted" vascular rings, and this activity was still present after freezing and thawing. The stimulation of PGI<sub>2</sub> synthesis by control tissue was significantly greater ( $p < 0.001$ ) with plasma from diabetic animals ( $0.25 \pm 0.04 \text{ ng/mg}$ ) than from controls ( $0.05 \pm 0.02 \text{ ng/mg}$ ). Similarly, plasma from diabetic volunteers showed increased ( $p < 0.05$ ) PGI<sub>2</sub>-stimulatory activity. Diabetic tissue was less responsive than control tissue to stimulation by diabetic plasma, and the difference between diabetic and control plasmas was not apparent. This suggests that the abnormal vascular PGI<sub>2</sub> synthesis in diabetes may be due to a defect in the vessel wall and not to lack of stimulatory plasma factors.

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PGI<sub>2</sub> PRODUCTION IN HUMAN ENDOTHELIAL CELLS CULTURED IN DIABETIC AND NONDIABETIC SERUM. R.C. Paton, R. Guillot and Ph. Passa. Department of Endocrinology and Metabolism, Hôpital St. Louis and Department of Embryology, UER Biomedical Sts-Pères, Paris, France.

Reduced levels of prostaglandin I<sub>2</sub> (PGI<sub>2</sub>) may contribute to the platelet hyper-reactivity and vascular complications found in diabetes mellitus. This study compared PGI<sub>2</sub> production (PGI<sub>2</sub>-like activity and 6-keto-PGF<sub>1 $\alpha$</sub>  levels) by vascular endothelial cells cultured in the presence of serum from 15 diabetics with proliferative retinopathy (5 treated by surgical hypophysectomy) and 15 sex-matched nondiabetic controls. Endothelial cells from human umbilical veins were cultured in M199 with either 20 % diabetic or control serum. At confluence, cultures were washed and stimulated with 0.1 NIH U/ml bovine thrombin. After 2 min incubation, the supernatant was tested for i) PGI<sub>2</sub>-like activity on ADP-induced platelet aggregation, results expressed as % inhibition and ii) 6-keto-PGF<sub>1 $\alpha$</sub>  by radioimmunoassay, results expressed as nmol/ml. There was a significant correlation between PGI<sub>2</sub>-like activity and 6-keto-PGF<sub>1 $\alpha$</sub>  levels ( $r = 0.78$ ,  $p < 0.001$ ). The liberation of PGI<sub>2</sub> from endothelial cells from different umbilical cords varied, but both PGI<sub>2</sub>-like activity (mean  $\pm$  SEM  $21.9 \pm 4.8$  vs  $28.3 \pm 5.1$   $p < 0.05$ ) and 6-keto-PGF<sub>1 $\alpha$</sub>  ( $3.15 \pm 0.68$  vs  $3.95 \pm 0.91$  nmol/ml,  $p < 0.05$ ) were significantly lower in supernatant from cells cultured in the presence of diabetic compared to control serum. PGI<sub>2</sub> production was not significantly different in cells cultured with serum from hypophysectomized and nonhypophysectomized diabetics.

These results suggest that serum from diabetics with proliferative retinopathy contains factors which impair the release or production of PGI<sub>2</sub> by endothelial cells and that this effect is not mediated by the pituitary.

DETERMINATION OF MALONDIALDEHYDE IN NORMAL AND DIABETIC PREGNANCIES. I. Rákóczi, Gy. Gerő, J. Demeter and I. Gáti. Department of Obstetrics and Gynecology, Postgraduate Medical School, Budapest, H.

It is known that platelet hyperaggregation observed in diabetic patients is, at least in part, due to an increased activity of the endoperoxide-thromboxane forming metabolic pathway. It was interesting to determine the platelet malondialdehyde (MDA) production in normal and diabetic pregnancies. Following individuals have been studied: /I/ twenty-five healthy non-pregnant volunteers; /II/ thirty women in third trimester of non-complicated pregnancies; /III/ twenty two diabetic pregnant women without retinopathy; /IV/ fifteen diabetic pregnant women with retinopathy. Platelet MDA production following N-ethyl-maleimide induced aggregation was measured according to Stuart et al. The mean value of MDA production was similar in volunteers and normal pregnant women /SDM,  $7.07 \pm 0.73$  nmoles MDA per  $10^3$  platelets;  $7.22 \pm 0.81$ /. The mean MDA production in diabetic women without retinopathy was slightly but non-significantly higher than that in normal pregnant women / $7.57 \pm 1.02$ ;  $p > 0.05$ /. The corresponding value in diabetic women with retinopathy was significantly higher than the values in the other three groups / $8.47 \pm 0.82$ ;  $p < 0.01$ /. These data suggest that the activation of prostaglandin synthetic pathway /measured by MDA/ is significantly increased in diabetic pregnancy complicated by retinopathy. The increase of platelet prostaglandin synthesis in diabetic pregnancy might play an important role in initiating and/or promoting the small-vessel complications of placenta.

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Increased Platelet Aggregation and Decreased Sensitivity of Platelets to Synthesized Prostacyclin in Alloxan-Diabetic Rabbits. K. Yamada, T. Yoda, Y. Goto, T. Hirata and K. Serizawa. The Third Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan

Platelet aggregation and sensitivity of platelets to prostacyclin were examined with a view to clarify platelet function in alloxan-diabetic rabbits. Diabetic rabbits were obtained by intravenous injection of alloxan (100mg/kg body weight) and range of plasma glucose in these diabetic rabbits was 350-500mg/100ml. Blood was collected from the central artery of the ear of normal and alloxan-diabetic rabbits. Platelet aggregation was measured turbidometrically as rate of light transmission at maximal aggregation to light transmission of platelet poor plasma by a NKK aggregometer (Tokyo, Japan). Platelet aggregation was induced by ADP with each final concentration of 0.5, 1.0, 2.0 and 6.0  $\mu\text{M}$ . Sensitivity of platelets to synthesized prostacyclin (Ono Pharma. Co., Tokyo, Japan) was represented as prostacyclin concentration of fifty percent inhibition of ADP-induced platelet aggregation.

Platelet aggregation rate in normal and alloxan-diabetic rabbits was as follows;  $8.4 \pm 1.0$ ,  $22.8 \pm 2.6\%$  (0.5  $\mu\text{M}$ ),  $22.3 \pm 1.5$ ,  $35.2 \pm 3.5\%$  (1.0  $\mu\text{M}$ ),  $37.1 \pm 1.7$ ,  $50.0 \pm 2.6\%$  (2.0  $\mu\text{M}$ ),  $51.0 \pm 2.2$ ,  $66.7 \pm 7.0\%$  (6.0  $\mu\text{M}$  ADP) (Mean  $\pm$  S.E.,  $P < 0.005$ ). Prostacyclin concentration of fifty percent inhibition of ADP-induced platelet aggregation in normal and alloxan-diabetic rabbits was as follows;  $42.37 \pm 1.68$ ,  $48.50 \pm 0.48$  ng/ml (Mean  $\pm$  S.E.,  $P < 0.01$ ).

In conclusion, platelets of alloxan-diabetic rabbits presented a significant decrease in sensitivity to synthesized prostacyclin as compared to platelets from normal rabbits, with a significant increase in ADP-induced platelet aggregation.