At the dosage of 5,000 units given every 8 h after major surgery, heparin is known to prevent deep vein thrombosis and pulmonary embolism[14]. The potentiating effect on Xa inhibitor shown by the heparinoid in the in vitro system was lower than that displayed by sodium and calcium heparin. Hence, the absence of a measurable effect in plasma after its administration in humans at the highest dosage suggested for therapeutic use by the manufactures (18 mg) is not surprizing. On the basis of our in vitro studies, the administered dose should be 8 times higher in order to achieve the same effect of 5,000 units of heparin. These findings indicate that the use of heparinoids can hardly be considered as an alternative to heparin in the prevention and treatment of thromboembolism.

[14] V. V. KAKKAR, T. P. CORRIGAN and D. P. FOSSARD, Lancet *2*, 45 (1975).

Effects of Stress and Adrenocorticotrophin Administration on Plasma Corticosterone Levels at Different Stages of Pregnancy in the Mouse

SUSAN M. BARLOW, A. A. QUYYUMI, D. V. RAJARATNAM and F. M. SULLIVAN

Department of Pharmacology, Guy's Hospital Medical School, London, SE1 9RT (England), 5 April 1976.

Summary. Stress or administration of ACTH to pregnant mice gave rise to much higher plasma corticosterone levels in the second half of pregnancy than in the first half, suggesting that there may be increased adrenal sensitivity to ACTH or decreased metabolism of corticosterone during the second half of pregnancy.

In the mouse, resting plasma corticosterone levels increase markedly during the second half of pregnancy, reaching levels of around 140 μg/100 ml by day 16, that is 60 times the resting level found in the non-pregnant mouse[1]. Acute or chronic stress during this period of pregnancy results in further large increases, individual plasma corticosterone levels ranging from 500 to 900 μg/100 ml 1 h after the start of stress[2]. These large increases in plasma corticosterone levels could be due to the release of more adrenocorticotrophin (ACTH) in response to stress, or an increase in the sensitivity of the adrenal glands to circulating ACTH, or a reduction in the rate of metabolism of corticosterone, during the second half of pregnancy. It is not possible to measure adrenal corticosterone secretion rates accurately in an animal as small as the mouse and so an indirect approach was adopted to investigate the possible cause(s) of the high plasma corticosterone levels following stress during the second half of pregnancy, and to ascertain on which day around mid-pregnancy the corticosterone response to stress begins to increase.

In the first series of experiments mice were subjected to restraint stress by immobilizing them according to the method of RENAUD[3], on one of days 8, 10, 12, 14 or 16 of pregnancy (day of finding of vaginal plug designated day 1). Non-pregnant controls were similarly restrained. After 1 h of restraint stress blood samples were taken from the retro-orbital sinus under brief ether anaesthesia

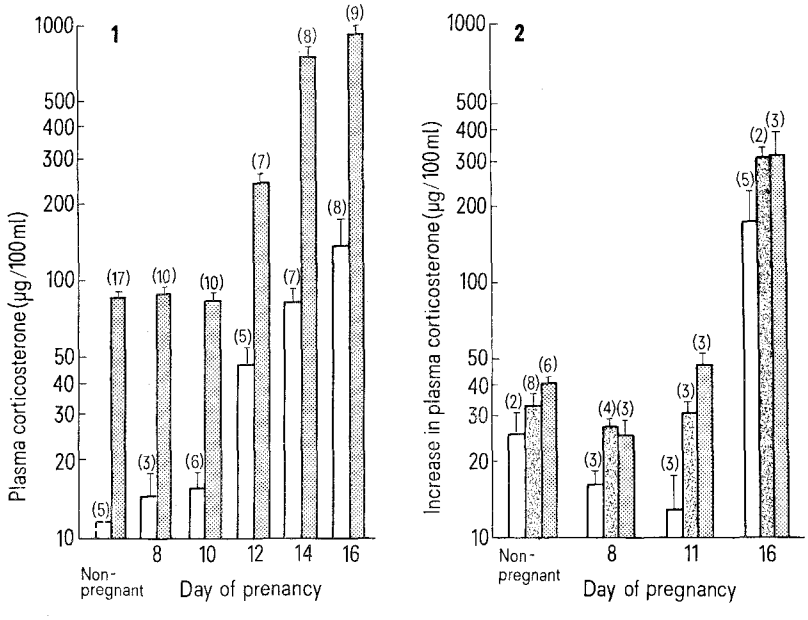

Fig. 1. Plasma corticosterone levels in control (white columns) and stressed (black columns) mice during different stages of pregnancy. The number of mice studied is shown in parentheses. Means ± SEM. The non-pregnant control level was 2.3 μg/ml.

Fig. 2. Increase in plasma corticosterone levels following injection of 40 mIU (white columns), 160 mIU (stippled columns), or 640 mIU (black columns) of ACTH during different stages of pregnancy in mice with endogenous corticosterone levels suppressed to 7–10 μg/100 ml plasma (except day 16, 43 μg/100 ml plasma). The number of mice studied is shown in parentheses. Means ± SEM.

and the plasma assayed for corticosterone content using the microfluorometric method of GLICK et al.[4]. The results are shown in Figure 1. Up to day 10 of pregnancy, the stress response is similar to that of non-pregnant mice. After day 10, there is a progressive increase in plasma corticosterone levels during stress, reaching a 10-fold increase over non-pregnant stress levels by day 16.

In the second series of experiments mice were injected s.c. with 40, 160 or 640 mIU of ACTH (Acthar Corticotrophin, Armour Pharmaceuticals, England) on one of days 8, 11 or 16 of pregnancy after suppression of the pituitary-adrenal axis with dexamethasone (0.4 mg i.p.) 20 h before the injection of ACTH, followed by pentobarbital (80 mg/kg i.p.) plus chlorpromazine (20 mg/kg s.c.) 0.75 h before injection of ACTH[5]. Non-pregnant controls were also treated similarly. A control blood sample was taken from the retro-orbital sinus immediately before the injection of ACTH and a second blood sample taken 1 h later, this having been shown in preliminary experiments to be the time of the peak plasma corticosterone response to ACTH in the pregnant and non-pregnant mouse. The results are shown in Figure 2.

These show that on day 16 of pregnancy, when increases in plasma corticosterone levels in response to stress were much greater than in early pregnancy, there was also a much greater increase in plasma corticosterone levels in response to given doses of ACTH. Thus it is not necessary to propose that there is any increase in the secretion of ACTH in response to stress in the second half of pregnancy compared with the first half of pregnancy. The difference could be accounted for either by an increase in the sensitivity of the adrenal glands to ACTH or by a decreased rate of metabolism of corticosterone. This second possibility would seem to be the most likely mechanism in view of the known increase in protein binding of the hormone during the second half of pregnancy[1,2,6]. The lower maximum response to exogenous ACTH compared with stress observed in the present experiments may be due to a lower sensitivity of the adrenal gland to ACTH, which is known to occur by 24 h following chemical suppression or hypophysectomy[7].

1 SUSAN M. BARLOW, P. J. MORRISON and F. M. SULLIVAN, J. Endocr. *60*, 473 (1974).

2 SUSAN M. BARLOW, P. J. MORRISON and F. M. SULLIVAN, J. Endocr. *66*, 93 (1975).

3 S. RENAUD, J. appl. Physiol. *14*, 868 (1959).

4 D. GLICK, D. VON REDLICH and S. LEVINE, Endocrinology *74*, 653 (1964).

5 D. DE WIED, A. WITTER, D. H. G. VERSTEEG and A. H. MULDER, Endocrinology *85*, 561 (1969).

6 R. R. GALA and U. WESTPHAL, Acta endocr., Copenh. *55*, 47 (1967).

7 F. E. YATES and J. W. MARAN, in *Handbook of Physiology* (Eds. E. KNOBIL and W. H. SAWYER, American Physiology Society, Washington 1974), section 7, vol. 4, part. 2, p. 373.

Plasma Fibrinogen Response in the Rat after Thyroid Stimulating Hormone Therapy

J. A. PALMA[1]

Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Santa Rosa 1085, Córdoba-5000 (Argentina), 10 May 1976.

Summary. Thyroid Stimulating Hormone (TSH) increased the levels of plasma fibrinogen in the presence or absence of the thyroid gland. This finding suggests that this hormone produces an elevation of fibrinogen in rats by an extrathyroideal mechanism.

The mechanisms that modify the normal levels of plasma fibrinogen are not well known, although it recently has been demonstrated that both the hypothalamus and the hypophysis, and some of its hormones or products, are involved[2-7]. Besides, the hypothalamus-hypophysis-thyroid axis is very important in the regulation of plasma proteins[8,9]. In a previous study, we have found that the removal of the thyroid gland in rats increments plasma fibrinogen levels (unpublished data). Extirpation of thyroid gland leads to a decrease of the negative feedback exerted by thyroid hormones on the hypophysis and hypothalamus, with a consequent increment in thyroid stimulating hormone (TSH) levels. Thus, we thought it of interest to study the role of TSH on the levels of plasma fibrinogen.

Material and methods. 177 female rats weighing from 170 to 220 g and fed with a balanced diet (20% minimum of proteins) were used. Bovine TSH, NHI-TSH B6 (National Institute of Health, relative mean potency: 2.54 U.S.P. units/mg), highly purified, was used in doses of 12 µg/day. L-thyroxine (T4) and 3'3'5-triiodothyronine (T3) Sigma were employed in doses of 10 µg/day each one. The hormones were dissolved in alkaline saline solution[10] and administrated by daily i.m. injection. Propylthiouracil (PTU) (Gramón) was given in 0.05% solution in drinking water. 10 days after the beginning of the treatment or surgical operation, blood was extracted by decapitation. A mixture of potassium oxalate in 2:1 proportion was used to avoid blood coagulation. The concentration of fibrinogen was determined by RATNOFF et al.[11] method. Values of fibrinogen of 14 healthy intact rats were considered as normal controls. Thyroidectomy was performed through neck incision[12]. Both the thyroid and parathyroid glands were removed at the operation.

1 Acknowledgments. Thanks are given to Dr. MARTA LACUARA for her help in preparing the manuscript.

2 R. H. LANDABURU, C. A. LO PRESTI, M. A. HILLAR and D. E. CASTELLANOS, Acta physiol. latinoam. *21*, 74 (1971).

3 K. N. JEEJEEBHOY, A. BRUCE ROBERTSON, U. SODTKE and M. FOLEY, Biochem J. *119*, 243 (1970).

4 A. C. ATENCIO, P. Y. CHAO, A. Y. CHEN and E. B. REEVE, Am. J. Physiol. *216*, 773 (1969).

5 A. C. ATENCIO and L. LORAND, Am. J. Physiol. *219*, 1161 (1970).

6 A. Y. CHEN and E. B. REEVE, Am. J. Physiol. *227*, 940 (*1974*).

7 V. SELIGSONH, S. I. RAPAPORT and P. R. KUEFLER. Am. J. Physiol. *224*, 1172 (1973).

8 J. A. PALMA, Tesis. Facultad de Ciencias Médicas. Universidad Nacional de Córdoba (1968).

9 J. A. PALMA, 17th Scientific Meeting. Sociedad Argentina de Investigación Clínica, Mar del Plata, Argentina (1972).

10 F. E. YATES, J. URQUHART and A. L. HERBST, Proc. Soc. exp. Biol. Med. *195*, 373 (1958).

11 O. D. RATNOFF and C. MENZIE, J. Lab. clin. Med. *37*, 316 (1951).

12 F. E. D'AMOUR and F. R. BLOOD, *Mammalian Physiology* (The University of Chicago Press, Chicago, Illinois 1948).