The influence of the i.m. administration of sex hormones in rats on plasma urate levels and urinary excretion of uric acid and allantoin

Hormones	No. of animals	Plasma urate level (mg/100 ml)		Urinary excretion Uric acid (mg/day)	Allantoin (mg/day)
Androgen (10 mg/kg/day)	16	before	2.10 ± 0.81	1.73 ± 1.93	72.6 ± 12.4
		after	2.24 ± 0.84	1.82 ± 0.55	75.2 ± 14.1
Progesterone (100 mg/kg/day)	13	before	2.43 ± 1.04	1.25 ± 0.21	58.9 ± 19.6
		after	1.53 ± 0.57[a]	1.21 ± 0.26	53.5 ± 16.3
Estrogen (100 mg/kg/day)	13	before	1.85 ± 0.76	1.99 ± 0.24	70.9 ± 11.3
		after	1.87 ± 0.93	1.97 ± 0.61	73.2 ± 11.1

[a] $p < 0.05$. Plasma urate levels were average values ± SD of animals. Urinary excretion were shown by average values ± SD of succession 7 days.

that the renal clearance of urate is increased in pregnancy and the enhanced renal excretion of uric acid and/or hypervolaemia of pregnancy may result in the hypouricemia. However, in early pregnancy they could not show any change in renal excretion of uric acid. Recently NICHOLLS et al.[5] examined the effect of estrogen on plasma and urinary uric acid in trans-sexual men, and showed that stilboestrol increases the renal excretion of uric acid and lowers the plasma urate level. They suggested that uricosuric action of stilboestrol is a possible cause for age and sex differences in plasma uric acid.

In rats, the endproduct of purine metabolism is allantoin and is excreted in urine in large amounts though urinary excretion of uric acid is very little. Therefore, it is difficult to estimate the renal clearance of uric acid exactly.

The present study seemed to indicate that estrogen has no influence on urate production and on the renal clearance of uric acid. However, it cannot be denied that estrogen may influence uric acid excretion. We demonstrated that plasma urate concentration and urinary excretion of uric acid plus allantoin was reduced by progesterone, and therefore it could be assumed that progesterone diminishes purine synthesis and this effect of progesterone would appear to explain the sex difference in urate levels in adults.

ACHESON[6] showed previously a significant positive correlation between serum uric acid and haemoglobin levels in healthy adults and suggested that the sex difference in urate metabolism may be related to the difference in turn-over rate of blood cells in both sexes. MIKKELSEN et al. found that in male subjects the plasma urate levels showed a marked rise at the puberty and speculated that androgen may play an important role in raising the plasma urate levels. However, we could not confirm this hypothesis in the present study, and further experiments will have to be carried out to clarify the effect of androgen on purine metabolism.

Summary. The effects of sex hormones on purine metabolism were investigated in rats. No influence on purine synthesis was shown by the injection of estrogen and androgen. The plasma urate levels were significantly lowered from 2.43 ± 1.04 mg/100 ml to 1.53 ± 0.57 mg/100 ml by the injection of progesterone. Urinary excretion of uric acid plus allantoin was slightly reduced. These results suggested that progesterone may influence age and sex differences in human plasma urate levels.

Y. NISHIDA, I. AKAOKA and T. NISHIZAWA

Department of Medicine and Physical Therapy, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo (Japan 113), 3 June 1975.

[5] A. NICHOLLS, M. L. SNAITH and J. T. SCOTT, Br. med. J. *1*, 449 (1973).
[6] R. M. ACHESON and W. M. O'BRIEN, Lancet *2*, 777 (1966).

The Effect of Hunger on Free Fatty Acid and Corticosterone Plasma Levels in Rats

Fatty acids provide an energy source capable of rapid mobilization for nearly all of an organism tissues. They are stored in the adipose tissue as triglycerides and are set free by lipolysis. When the organism requires more energy than is available from its diet, or from liver or muscle glycogen, mobilization of fatty acids is increased[1,2]. The hormones of the pituitary gland and the adrenal cortex play an important role in the mobilization of fat in fasting animals[1,3-5].

This investigation deals with the changes with time in the plasma concentrations of free fatty acids (FFA) and corticosterone in the case of fasting rats in comparison with normally fed controls. The diurnal cycles of the animals were taken into consideration. Also, the effect of hypoxia on the concentration changes of the FFA was investigated.

Material and methods. Male Wistar rats (160–200 g) were fed a standard diet (Rat biscuit from the Tagger Co., Graz) for 4 weeks before the start of testing. The feed contained 59.5% carbohydrates, 21.7% protein, 2.6% fats, and was enriched with vitamins and minerals. In all tests, water was available to the animals ad libitum. The same animals were used for all experiments. Once a

[1] G. F. CAHILL JR., M. G. HERRERA, A. P. MORGAN, J. S. SOELDNER, J. STEINKE, P. L. LEVY, G. A. REICHARD JR. and D. M. KIPNIS, J. clin. Invest. *45*, 1751 (1966).
[2] D. S. FREDRICKSON and R. S. GORDON JR., Physiol. Rev. *38*, 585 (1958).
[3] J. N. FAIN, Endocrinology *71*, 633 (1962).
[4] H. M. GOODMAN and E. KNOBIL, Am. J. Physiol. *201*, 1 (1961).
[5] R. O. SCOW and S. S. CHERNICK, Am. J. Physiol. *210*, 1 (1966).

week approximately 1.5 ml blood was taken from the vein of the tongue, while the animal was under light halothane anesthesia[6]. The hypoxia experiments were conducted in a chamber at a pressure of 380 mm Hg, corresponding to half atmospheric pressure[7]. The plasma was deep-frozen at −25 °C and analyzed within 3 days. The modified Duncombe-method according to FALHOLT et al.[8] was used for the determination of FFA. The standard curve was constructed from appropriate dilutions of a 0.2 m*M* palmitic acid solution to give a concentration range of 0.01 to 0.05 μM per sample volume, and was linear in this range. The standard which accompanied the determination had a standard deviation of ± 5%.

The corticosterone content of the plasma was determined radioimmunologically using a test kit from the Sorin Co., Saluggia, Italy, and measured with a Beckman LS 230 fluid scintillation counter.

Results and discussion. It is known that the concentrations of various plasma components are subject to diurnal cycles[9]. Therefore, all tests were started at 06.00 h. When one observes the plasma levels of FFA of fed animals over a 24-hour period (Figure 1 b), one sees that there are no great fluctuations in the curve. Two maxima are seen at 21.00 and 06.00 h and 2 minima at 15.00 and 24.00 h. The curve reflects the relatively large activity connected with the nightly feeding. From Figure 1 a one can see that the values of the plasma levels of the fasting animals all lie far above those for the fed animals. The curve shows 2 peaks, one between 12.00 and 15.00 h, and another around midnight. The midnight peak reaches a value of 4 times that of the starting level. A minimum lies around 18.00 h. Similar results were obtained by others[10, 11].

A simple method to affect a rapid mobilization of fat, in contrast to the gradual fat mobilization by hunger, is the use of hypoxia stress. During hypoxia the curve already reaches its peak value of 4 times the starting level after 2 h (Figure 2). The corticosterone level of the fed animals is also subject to a diurnal rhythm, with a maximum at 18.00 h (Figure 3 b). The fasting curve clearly lies higher than that of the fed animals at all points, and already reaches its maximum at 15.00 h (Figure 3 a).

The many lipolysis-stimulating hormones can be divided into 2 groups, namely into rapidly but short acting hormones, and slowly but long acting ones. It can be regarded as certain that these 2 groups exert their effects over separate mechanisms. While the rapidly acting lipolytic hormones stimulate lipolysis via cyclic adenosine monophosphate[12], the slowly acting ones affect the synthesis of RNA and proteins[13]. Corticosterone belongs in the latter group. A comparison of the plots of the curves (Figures 1 a and 3 a) shows that the corticosterone peak occurs before the maximum plasma concentration of free fatty acids in time. While the increased FFA level after 12 h of fasting can be attributed to an increase in lipolysis due to hormonal influence, the first maximum after 6 h of fasting is probably caused by a decrease in the re-esterification of FFA in the fat cells due to a lack of glycerol-3-phosphate. An increase in lipolysis, therefore, begins only after approximately 12 h,

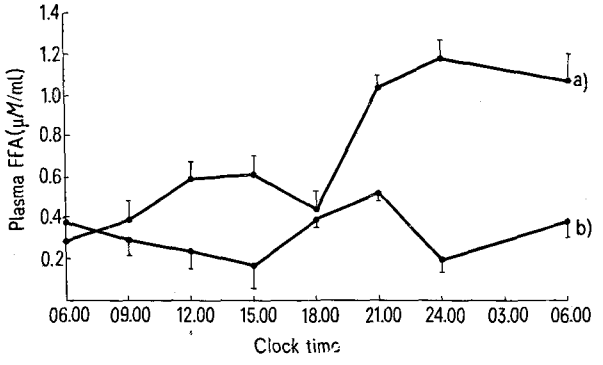

Fig. 1. Concentrations of free fatty acids in the plasma of fed (b) and fasting (a) rats. Each point represents an average value for 5 animals and is plotted together with its standard deviation.

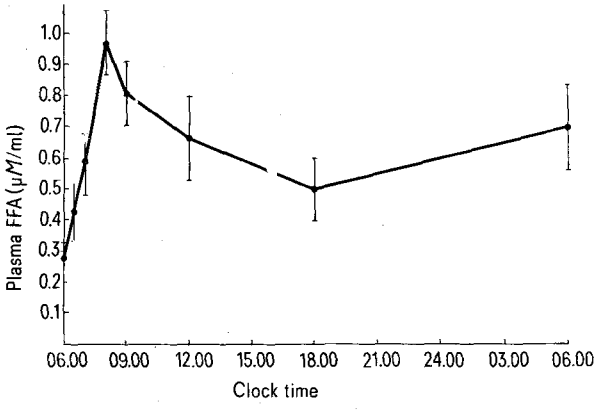

Fig. 2. Concentrations of free fatty acids in the plasma of rats during hypoxia. Each point represents an average value for 5 animals and is plotted together with its standard deviation.

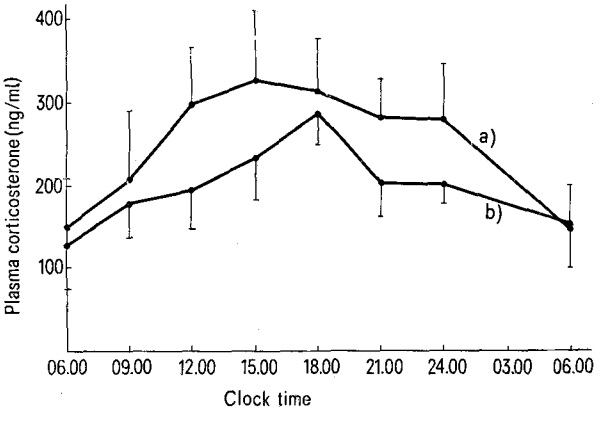

Fig. 3. Concentrations of corticosterone in the plasma of fed (b) and fasting (a) rats. Each point represents an average value for 8–10 animals and is plotted together with its standard deviation.

[6] W. MLEKUSCH, B. PALETTA, P. TOPIC und W. TRUPPE, Experientia *28*, 870 (1972).
[7] B. PALETTA, Z. Biol. *116*, 399 (1971).
[8] K. FALHOLT, B. LUND and W. FALHOLT, Clin. chim. Acta *46*, 105 (1973).
[9] B. E. STATLAND, P. WINKEL and H. BOKELUND, Clin. Chem. *19*, 1374 (1973).
[10] W. M. BORTZ and L. A. STEELE, Biochim. biophys. Acta *306*, 85 (1973).
[11] J. D. MCGARRY, J. M. MEIER and D. W. FOSTER, J. biol. Chem. *248*, 270 (1973).
[12] R. W. BUTCHER and E. W. SUTHERLAND, J. biol. Chem. *237*, 1244 (1962).
[13] J. N. FAIN, V. P. KOVACEV and R. O. SCOW, J. biol. Chem. *240*, 3522 (1965).

when the liver and muscle glycogen supplies have been exhausted.

Summary. The time function of free fatty acids and of corticosterone in plasma in fed and fasted rats was investigated. Also a study of the influence of hypoxia on the concentration of plasma free fatty acids was carried out. Whereas fasting does not seem to stimulate lipolysis markedly before 12 h, 2 h of hypoxia elevate the free fatty acid level to its maximum. In the fasted condition, all values are significantly higher than corticosterone in the fed animal.

W. Mlekusch, W. Truppe, W. Beyer und B. Paletta

Institut für Medizinische Chemie und Pregl-Labor der Universität Graz, Universitätsplatz 2, A-8010 Graz (Austria), 16 April 1975.

Evidence for Purine Biosynthesis in Human Leukocytes

Previous studies have shown that isolated human leukocytes are able to incorporate ^{14}C-labelled formate and adenine into their nucleic acid bases[1]. Diamond et al.[2] observed also an increased ^{14}C-glycine incorporation into adenine and guanine of RNA and DNA of leukocytes from gouty patients. However, adenine and guanine are not metabolic end products of purine metabolism and their concentration cannot give adequate information on the behaviour of purine metabolism; moreover, the glycine incorporation rate determined in leukocytes in vitro does not necessarily reflect the actual importance of de novo purine biosynthesis. Recently, Reem[3] found that Burkitt lymphoma and human spleen cells could synthetize the first and second intermediate compounds of the purine biosynthetic pathway, and Wood and Seegmiller[4] assayed and determined some properties of 5-phosphoribosyl-1-pyrophosphate amidotransferase, the enzyme that catalyzes the first step involved in de novo purine biosynthesis, in human lymphoblasts maintained in tissue culture. The purpose of the present study was to investigate whether human leukocytes and lymphocytes are capable of de novo purine biosynthesis. We assayed the activity of 5-phosphoribosyl-1-pyrophosphate amidotransferase in leukocytes and lymphocytes isolated by the usual procedures from 11 healthy volunteer subjects according to the method of Wyngaarden and Ashton[5]. The assays were performed on leukocytes and lymphocytes extracts dialyzed 24 h against distilled water in order to remove heparin and other molecules interfering or inhibiting the enzyme. Enzyme activity was expressed as units/10^6 cells, where one unit of enzyme activity is the amount that produces an increase of 0.001 absorbance unit/min at 363 nm at pH 8 and room temperature (25 °C). Enzyme activity has been detected in both kinds of cells examined. The mean values resulted 1.34 $\pm$ 0.58 units/10^6 cells in leukocytes and 0.86 $\pm$ 0.66 units/10^6 cells in lymphocytes (Table).

Preformed purines converted by cellular metabolism to ribonucleotides have been considered the only source of purine nucleotides for many human cells[3,6]. Most of the conclusions on this dependence are, however, based on indirect or negative evidence. Study of the early steps of de novo purine biosynthesis in human cells, such as fibroblasts, leukocytes and platelets, has been limited to evaluating the accumulation of formylglycinamide ribonucleotide [3,6] or observing the failure to incorporate glycine or formate into purine nucleotides in vitro[6], while the presence and the properties of 5-phosphoribosyl-1-pyrophosphate amidotransferase have not been extensively examined[4]. However, the interpretation of isotope-incorporation studies involving purine synthesis reactions requires some care concerning the concentration of precursors and intermediate compounds[1,6], and the possibility that some substrate necessary for de novo synthesis may been lacking[6]. Our results provide evidende that human leukocytes and lymphocytes possess the enzyme activity that catalyzes the formation of the first intermediate compound of the purine biosynthetic pathway. The finding of the presence of 5-phosphoribosyl-1-pyrophosphate amidotransferase in human leukocytes and lymphocytes suggests, therefore, that these cells have the capacity for de novo purine biosynthesis and are not solely dependent on the liver for their supply of purines. De novo purine synthesis may therefore be more widespread than previously reported, and human leukocytes and lymphocytes may be an important extrahepatic site for de nove purine biosynthesis in man. The study of the purine biosynthetic pathway in human leukocytes and lymphocytes seems of particular interest since it could provide a valuable experimental model to investigate the factors involved in the regulation of de novo purine biosynthesis and clarify purine metabolism alterations in patients with hyperuricemia, gout, or leukemia and other myeloproliferative diseases.

5-Phosphoribosyl-1-pyrophosphate amidotransferase activity in leukocytes and lymphocytes of healthy human subjects

Cell type	Enzyme activity (units/10^6 cells)
Leukocytes	1.34 $\pm$ 0.58
Lymphocytes	0.86 $\pm$ 0.66

Summary. Human leukocytes and lymphocytes have shown to be equipped with 5-phosphoribosyl-1-pyrophosphate amidotransrease, the enzyme which catalyzes the synthesis of the first intermediate of the purine pathway, thus providing evidence that these cells have the capacity for de novo purine biosynthesis.

R. Marcolongo, G. Pompucci and V. Micheli

Cattedra di Reumatologia and Institute of Biochemistry, University of Siena, Via Ponizetti 7, Siena (Italy), 26 May 1975.

[1] J. L. Scott, J. clin. Invest. *41*, 67 (1962).
[2] H. S. Diamond, M. Friedland, D. Holberstam and D. Kaplan, Ann. rheum. Dis. *28*, 275 (1969).
[3] G. H. Reem, J. clin. Invest. *51*, 1058 (1972).
[4] A. W. Wood and J. E. Seegmiller, J. biol. Chem. *248*, 138 (1973).
[5] J. B. Wyngaarden and D. M. Ashton, J. biol. Chem. *234*, 1492 (1959).
[6] A. W. Murray, Ann. Rev. Biochem. *40*, 811 (1971).