THE EFFECT OF FASTING AND FOOD ON THE RATE OF PURINE BIOSYNTHESIS DE NOVO IN THE RAT SPLEEN

P. F. SEMPLE, A. R. HENDERSON* AND J. A. BOYLE

Departments of Medicine and Pathological Biochemistry, Glasgow Royal Infirmary, Glasgow

(Received 20 June 1973)

SUMMARY

1. The effect of an 18, 30 or 40 h fast on the rate of purine biosynthesis de novo as measured by the incorporation of [14C]formate into formylglycinamide ribonucleotide during azaserine block has been examined in the rat spleen.

2. There is a reproducible fall in the rate of purine biosynthesis de novo during fasting. Values at 40 h were approximately one-third of the values in the colony-fed non-fasted animals.

3. Re-feeding animals with an amino acid mixture by stomach tube partially reversed the inhibition of purine synthesis induced by a 40 h fast.

Key words: purine synthesis, fasting, gout.

At least two mechanisms appear to be operating either singly or in combination in the production of the hyperuricaemia which characterizes the gouty patient: renal under-excretion of urate and over-production of urate; the latter is consequent on an increased rate of purine biosynthesis de novo (Seegmiller, Grayzel, Laster & Liddle, 1961; Seegmiller, Grayzel, Howell & Plato, 1962). The physiological factors controlling the handling of uric acid by the kidneys are fairly well documented (Steele, 1971), although the nature of the defect(s) causing impaired renal excretion of urate in some gouty patients remains unclear. There is a relative lack of information, however, on the factors concerned in the control of purine biosynthesis de novo although the rate-limiting enzyme in the pathway is known (Wyngaarden & Kelley, 1972; Holmes, McDonald, McCord, Wyngaarden & Kelley, 1973).

Many gouty patients are obese (Boyle, McKiddie, Buchanan, Jasani, Gray, Jackson & Buchanan, 1969; Wiedemann, Rose & Schwartz, 1972) presumably because of excessive caloric intake. In this study we have examined the effect of acute starvation on the rate of purine biosynthesis de novo in a mammalian tissue, rat spleen.

* Present address: University Hospital, Department of Clinical Biochemistry, 339 Windermere Road, London, Ontario N6G 2K3, Canada.

Correspondence: Dr J. A. Boyle, Department of Medicine, Glasgow Royal Infirmary, Glasgow.
MATERIALS AND METHODS

Animals studied and feeding protocol
Male Wistar rats weighing 140–160 g before fasting were used in the experiments. All animals were maintained on Modified Diet 41B (Oxoid Ltd, London) which contains 15.9% crude protein and adequate mineral and vitamin supplements. All animals were handled daily for several weeks preceding the feeding experiments and were housed in a windowless room lit from 08.00 to 20.00 hours. Fasted animals used in the study had food withdrawn 18, 30 or 40 h before the experiments, which were all performed between 09.00 and 11.00 hours. All animals under study were housed in wire-bottomed cages in an attempt to prevent coprophagy and were allowed free access to tap water. The complete amino acid mixture employed by Wunner, Bell & Munro (1966) was used in the feeding experiments. Fasted animals were fed with 1.12 g of amino acids in 3 ml of mixture using a stomach tube 2 or 4 h before injection of [14C]formate.

Measurement of the rate of purine biosynthesis de novo
The rate of purine synthesis de novo was assayed by measurement of the incorporation of [14C]formate (The Radiochemical Centre, Amersham) into formylglycinamide ribonucleotide (FGAR) (Moore & Lepage, 1957) (Fig. 1). The further metabolism of this intermediate is to formylglycinamidine ribonucleotide and this step can be blocked by pretreating the animals with azaserine which irreversibly inactivates the enzyme FGAR amidotransferase (French, Dawid, Day & Buchanan, 1963).

\begin{center}
\includegraphics[width=0.5\textwidth]{purine_biosynthesis.png}
\end{center}

Fig. 1. First steps of the purine biosynthetic pathway are shown. The first step, which is thought to be rate-limiting, involves the conversion of 5-phosphoribosyl-1-pyrophosphate into 5-phosphoribosyl-1-amine. Azaserine irreversibly inactivates the enzyme formylglycinamide ribonucleotide amidotransferase.
The accumulation of [14C]FGAR appears to give a good estimate of the rate of purine synthesis because it has been found that children with the Lesch–Nyhan syndrome (X-linked uricaciduria) display excessive uric acid synthesis in vivo, as judged by measurements of the incorporation of 14C into uric acid in urine, to the same extent as their fibroblasts excessively synthesize [14C]FGAR in tissue culture (Seegmiller, Rosenbloom & Kelley, 1967). This observation would appear to substantiate the concept that the accumulation of FGAR gives a valid measure of the rate of purine biosynthesis.

At zero time each rat was injected intraperitoneally with azaserine (Calbiochem, London) in a dosage of 10 mg/kg body weight in 0·1 ml of normal saline (NaCl; 0·15 mol/l). This dose was found to be optimal by Moore & Lepage (1957) in a study of the distribution of accumulation of [14C]FGAR in various tissues of the mouse after administration of [14C]glycine to azaserine-treated animals, a finding which we have confirmed in the mouse spleen. This was followed 1 h later by the injection of 0·5 ml of a solution containing 500 µg of sodium [14C]-formate (specific radioactivity 5·2 µCi/µmol). The rats were killed 20 min later by decapitation and each spleen was rapidly transferred to a Petri dish containing 5 ml of ice-cold saline. The average time from decapitation to removal of the spleen was 20 s. The spleens from each group of three rats were pooled, rapidly weighed, minced with scissors and homogenized in 10 ml of perchloric acid (0·2 mol/l). The homogenate was then centrifuged at 20 000 g for 30 min at 4°C. Aliquots of the supernatant (200 µl; corresponding to 60–80 mg of spleen weight) were applied to Whatman 3 MM chromatography paper and subjected to descending chromatography for 16 h in butanol–acetic acid–aqueous 5% ammonia–acetone–water (7 : 5 : 3 : 3 : 2, by vol.) (Boyle, Raivio, Becker & Seegmiller, 1972). The position of [14C]FGAR was ascertained (see the Results section) and the areas corresponding to the activity were cut out and placed in 10 ml of toluene-based scintillator [4 g of 2,5-diphenyloxazole and 0·1 g of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene/1 of toluene]. Radioactivity in the samples was measured in a Tri-Carb liquid-scintillation spectrometer (Packard Instrument Co. Inc., La Grange, Ill., U.S.A.). The counting efficiency for 14C on the paper chromatogram was 63%.

RESULTS

Purine synthesis

The synthesis of [14C]FGAR by the rat spleen in vivo was confirmed by the following observations: disappearance of the presumptive FGAR radioactive peak occurred after preincubation of the perchloric acid extract with alkaline phosphatase (Sigma) or 5'-nucleotidase. A new peak of radioactivity was observed, probably representing the dephosphorylated derivative. The administration of adenine (50 mg/kg body weight) by intraperitoneal injection was shown to produce a substantial diminution in the size of the presumed FGAR peak (Fig. 2). Adenine is known to decrease the synthesis of purines de novo (Seegmiller, Klinenberg, Miller & Watts, 1968). In addition the presumed FGAR peak was not seen when the azaserine injection was omitted (Fig. 3) and a new peak of radioactivity then appeared at the origin suggesting that [14C]FGAR may have been further metabolized to nucleoside polyphosphates. The identity of the presumed [14C]FGAR peak was considered to be reasonably established when it was observed to run with an Rf value (0·29) identical to authentic [14C]FGAR (kindly supplied by Dr W. N. Kelley) in the solvent system used in this study. In the experiments
with the spleens of animals treated with azaserine small amounts of radioactivity were occasion-
ally found at the origin and preliminary studies suggest that these counts may reflect the
presence of a polyphosphate of $[^{14}C]$FGAR. Other workers (Rosenbloom, Henderson,
Caldwell, Kelley & Seegmiller, 1968) using a different separation system have also made this
observation. We have not characterized the compound further and the counts at the origin
were not included in our estimation of the rates of purine synthesis. Formate is also utilized

\[ \text{FIG. 2. Effect of injection of animals with adenine in a dose of 50 mg/kg body weight on the dis-
tribution of radioactivity in the paper chromatogram of spleen extract. The animals treated with aden-
ine show a smaller $[^{14}C]$FGAR peak (first peak) than the control animals, but in both groups the}
\]
\[ \text{incorporation of the isotopic label into the peak running in the serine position (the second peak) is}
\]
\[ \text{virtually identical. } O, \text{ + Adenine; } A, \text{ no adenine.}
\]

for the synthesis of serine by the action of L-hydroxymethyltransferase which catalyses the
transfer of a $C_1$ unit from $N_5,N_{10}$-methylenetetrahydrofolinic acid to glycine. The peak of
radioactivity after the $[^{14}C]$FGAR peak was observed to run with an $R_F$ value identical to
that of serine (0-42). Incorporation of isotope into this peak was studied as a control during
the present experiments. Methionine runs with an $R_F$ value of 0-72 in this separation system.
Effect of fasting on purine synthesis de novo

Fig. 4 shows the results from one experiment. The first peak of radioactivity corresponds to \([^{14}C]FGAR\) and the second peak runs with the serine marker. It can be seen that the amount of \([^{14}C]FGAR\) produced in the rat spleens varies with the length of the fast. In this instance the \([^{14}C]FGAR\) peak gave 94.5 \(\times 10^3\) c.p.m./g of tissue, whereas after an 18 and 30 h fast these values were 72 \(\times 10^3\) c.p.m./g of tissue and 50.4 \(\times 10^3\) c.p.m./mg respectively. Fig. 4 also shows a tendency, which was found throughout the experiments, namely that the amount of radioactivity in the peak which ran with the serine marker tended to increase with increasing length of fast. The ‘serine’ peak in the colony-fed animals is less than the peaks at 18 and 30 h. Although the 30 h peak is less high than the 18 h peak it is a broader peak and the actual values in \(10^3\) c.p.m./g of tissue for incorporation of the label into ‘serine’ in this experiment were 50.5, 63.2 and 66.9 for the colony-fed, 18 h and 30 h fasted animals respectively.

Fig. 3. Effect of omission of azaserine on the accumulation of formylglycinamide ribonucleotide. The FGAR peak is abolished in the animals untreated with azaserine. The accumulation of isotope in the peak running with serine is identical in both groups. ●, + Azaserine; ○, no azaserine.

In Fig. 5 the results of a number of experiments are summarized. Each point represents the mean of a number of observations on different animals, the actual numbers of animals involved being given in parenthesis beside each point. Several series of experiments demonstrated that consistent results could be obtained within each set of experiments although the absolute incorporation of \([^{14}C]\)formate into FGAR varied somewhat from experiment to experiment and this variation is reflected in the large range of values and standard error of the mean for the accumulation of FGAR in the colony-fed animals. As can be seen in
Fig. 5 the mean incorporation of $[^{14}\text{C}]$formate fell with increasing duration of fast. The colony-fed controls had a mean value of $91.17$ ($\pm 17.2$ SEM) $\times 10^3$ c.p.m./g of tissue falling to $79.0$ ($\pm 5.8$) $\times 10^3$, $38.7$ ($\pm 7.2$) $\times 10^3$ and $31.0$ ($\pm 1.76$) $\times 10^3$ at 18, 30 and 40 h respectively. The ranges of values at these times were $66.0-94.0$, $33.5-44.1$ and $28.2-33.4 \times 10^3$ c.p.m./g of tissue. The range for colony fast animals was $62.5-164.1 \times 10^3$ c.p.m./g of tissue.

It might be argued that the diminished incorporation of formate into FGAR in the fasted animals reflects merely a change in the pool size of formate during fasting but we think this is an unlikely explanation because if this were so one would expect to find similar changes in the incorporation of formate into the other radioactive peak which runs with serine. That

Fig. 4. Results of a typical experiment are shown. There is less incorporation of isotopic label into FGAR in the animals fasted for 18 and 30 h compared with the control animals. The incorporation of label into the peak running with serine shows no clear-cut tendency in this particular experiment. Feeding regimes: $\bullet$, colony fed; $\Delta$, starved 18 h; $\bigcirc$, starved 30 h.

this was not the case is shown in Fig. 6 where it can be seen that there was, if anything, a tendency in the reverse direction: the incorporation of $[^{14}\text{C}]$formate into serine tended to be greater in the fasted than in the colony-fed animals. Controls had a mean value of $39.3$ ($\pm 5.0$ SEM) $\times 10^3$ c.p.m./g of tissue rising to $48.8$ ($\pm 7.9$) $\times 10^3$ and $61.6$ ($\pm 15.4$) $\times 10^3$ at 18 and 40 h respectively.
Fasting, feeding and purine synthesis

Fig. 5. Mean (± SEM) in c.p.m. incorporated into FGAR/g of tissue in colony-fed animals and in animals fasted for 18, 30 and 40 h. The number of animals used for each point is shown in parentheses. Results were analysed by Student's t-test: colony-fed versus 18 h fasted animals \( t = 2.55, \ P < 0.05 \); colony-fed versus 30 h fasted animals \( t = 10.60, \ P < 0.01 \); colony-fed versus 40 h fasted animals \( t = 13.83, \ P < 0.001 \).

Fig. 6. Incorporation of \(^{14}\text{C}\)formate into the radioactive peak which runs in the position of serine in colony-fed and fasted animals. The duration of fast in hours is shown on the abscissa. Results are expressed as mean (± SEM)/g of tissue. The number of animals used for each point is shown in parentheses. Differences between colony-fed animals and animals fasted for 18 and 40 h are not significant using Student's t-test.
Kinetics of incorporation of formate into \([^{14}C]\)FGAR in colony-fed and fasted animals

The kinetics of incorporation of \([^{14}C]\)formate into FGAR were studied to exclude the possibility that in the fasted animals there was for some reason a very rapid incorporation of \([^{14}C]\)formate into FGAR with subsequent rapid breakdown of the radioactive product thereby producing spuriously low values. Fig. 7 shows results of a kinetic experiment undertaken with colony-fed animals and animals fasted for a period of 30 h. As can be seen there was a low incorporation of the label into FGAR when animals were killed at each of the time-intervals studied and even as early as 10 min the difference between the colony-fed and fasted animals is clearly evident and in keeping with our previous observations.

![Graph showing kinetics of net incorporation of \([^{14}C]\)formate into formylglycinamide ribonucleotide (FGAR) in two fasted (30 h) and two colony-fed animals. Feeding regimes: ●, colony-fed; ○, fasted. Individual readings are shown as △.](image)

Effect of re-feeding on purine biosynthesis de novo

The effect of re-feeding was examined as described in the Materials and Methods section. The results of this experiment are shown in Fig. 8. The incorporation of formate into FGAR in the colony-fed animals is represented as 100%. In the animals fasted for 40 h and killed the value for FGAR formation was 20% of the control value in this particular experiment. The values in the animals which were re-fed 2 and 4 h before the formate injection were 55% and 45% of the control value. These 2 and 4 h values do not appear significantly different
FIG. 8. Effect of re-feeding on incorporation of radioactivity into FGAR. The results are expressed as percentages of FGAR peak in the colony-fed animals.

from each other but we think they are significantly higher than the values in the fasted animals. We have not encountered rates of purine synthesis de novo as high as this in animals fasted for 40 h and we feel that the data show that re-feeding partially reverses the inhibition of purine synthesis induced by fasting.

**DISCUSSION**

The spleen was chosen as the tissue for study in this series of investigations because it is relatively homogeneous tissue, easily obtained, and in our hands it has been found to synthesize sufficient quantities of FGAR. It might be argued that the liver which is the main purine-forming organ would have been a better tissue to study, but we and others (Moore & Lepage, 1957) have failed to isolate FGAR from liver using [14C]formate and azaserine block.

Our results appear to show that fasting causes a diminution in the rate of purine synthesis de novo in the rat spleen. We feel that we have excluded the possibility that fasting effects a sufficient change in the formate pool size to explain our results because of the findings with regard to the incorporation of the [14C]formate into the radioactive peak running with serine. We also feel that the data exclude the possibility that the kinetics of 14C incorporation into FGAR are substantially different in fasted and colony-fed animals. The data show that it is possible to reverse partially the effects of fasting by feeding an amino acid mixture and this finding would support our contention that diet does have an effect on the rate of purine synthesis de novo.

One can only speculate on the mechanism whereby purine synthesis is inhibited by fasting. It has been shown that rat liver RNA is degraded during protein depletion and fasting (Munro, Naismith & Wikramanayake, 1953; Munro, 1968), and there is a possibility that the expanded nucleotide pool (Munro, 1968) consequent on RNA catabolism exerts feedback inhibition on the first and rate-limiting enzyme reaction of purine synthesis (Wyngaarden & Ashton, 1959;
Holmes et al., 1973), producing the decrease in the rate of purine synthesis that we have found. Depletion of 5-phosphoribosyl-1-pyrophosphate, glutamine, glycine or tetrahydrofolate may also be important. Nonetheless, we have no information at the present time as to the detailed mechanisms involved in the inhibition of purine synthesis in fasted animals and this point is the subject of further study.

It is unwise to extrapolate too firmly from findings in animals to the situation in man but if there is a parallel then our data would suggest that a restricted food intake might be of value in the treatment of the obese patient with hyperuricaemia and gout. In connection with this speculation the results of the study by Nicholls & Scott (1972) are interesting. These workers have shown that in the obese gouty patient the restriction of caloric intake to a degree to cause weight loss without causing ketosis is associated with a significant fall in the serum urate levels which is not accompanied by an increased excretion of urate in urine. The explanation which these authors have proposed to account for their findings is that there is a decrease in the rate of purine synthesis de novo in their obese patients consequent on restricted food intake. The findings of the present study show this relationship directly in animal tissue during complete starvation and suggest that this explanation might perhaps be true in man during decreased caloric intake.

REFERENCES


Fasting, feeding and purine synthesis


