# Study on Plasma Cyclic Nucleotide Concentrations in Fasting Rats (37205)

J. TURINSKY
(Introduced by G. S. Kanter)
*Department of Physiology, Albany Medical College of Union University, Albany, New York 12208*

Broadus *et al.* (1) have shown in man that cyclic AMP and cyclic GMP in plasma are in a dynamic steady-state relation with intracellular pools of the nucleotides. While the sources of these extracellular cyclic nucleotides *in vivo* have not been elucidated, a number of investigators have demonstrated that fat tissue or fat cells incubated *in vitro* release cyclic AMP into the medium (2–5) and that the isolated perfused liver from fed rats releases cyclic AMP into the perfusate in response to glucagon or epinephrine stimulation. This release is well correlated with glucose mobilization from the liver (6, 7). Both fat tissue and the liver play an important role in the metabolic adaptations during fasting, as shown by increased mobilization of free fatty acids and increased glycogenolysis and gluconeogenesis. Brodie *et al.* (8) reported a marked increase in adenyl cyclase activity in epididymal fat from 48-hr fasted rats, and Park *et al.* (9) observed a 74% increase in hepatic cyclic AMP concentration after 24 hr of fasting in rats. Since the efflux of cyclic nucleotides from cells can provide a measure of cyclic nucleotide metabolism within an intracellular pool that is in a dynamic steady-state with extracellular cyclic nucleotides (1), we became interested in possible changes in cyclic AMP and cyclic GMP in plasma during fasting in the rat.

*Methods.* Adult male Sprague–Dawley rats (210–230 g) were fed a normal laboratory diet (Wayne Lab-blox, Allied Mills, Inc.) *ad libitum* until the beginning of fasting at 9:00 AM. This time coincided with the beginning of the light phase and is further referred to as zero time of fasting. Groups of animals were sacrificed by stunning and exsanguination from the cut chest after 0, 24, 48, 72, and 120 hr of fasting.

In each animal, blood was collected in a heparinized test tube, mixed rapidly, spun for 1 min, and a plasma aliquot transferred into 10% trichloracetic acid solution for cyclic AMP and cyclic GMP determinations (10). The time interval between taking the rat from the cage and deproteination of the plasma sample was measured and was found to be relatively constant (120–130 sec). After additional spinning, plasma was obtained for the determination of glucose concentration on a Technicon Autoanalyzer using a modification of the ferricyanide-ferrocyanide method (11), and for the determination of free fatty acid concentration by the spectrophotometric method of Novak (12).

*Results.* Plasma glucose concentration fell to about 50% during the first 48 hr of fasting and then did not change significantly, despite the relatively higher values at 120 hr (Table I). The concentration of free fatty acids in plasma almost tripled during the first 24 hr of fasting and remained at high levels throughout the remaining period. There was a significant decrease in plasma free fatty acids 120 hr after food deprivation which coincided with the nonsignificant increase in plasma glucose mentioned above.

The concentration of cyclic AMP at zero hours of fasting was 20.6 pmole/ml of plasma, which is lower than the 37 pmole/ml reported by Broadus *et al.* (13), and the level of cyclic GMP was approximately one-third of the value (24 pmole/ml) reported by the same investigators. The relatively low values of plasma cyclic nucleotides that we found in our rats might reflect the very short half-life of these nucleotides in blood (13, 14).

TABLE I. Effect of Fasting on Plasma Concentrations of Glucose, Free Fatty Acids, Cyclic AMP, and Cyclic GMP.

| Length of fasting (hr) | Plasma glucose | | Plasma free fatty acids | | Plasma cAMP | | Plasma cGMP | |
|---|---|---|---|---|---|---|---|---|
| | mg/100 ml ± SE ($p^a$) | $p^b$ | mEq/liter ± SE ($p^a$) | $p^b$ | pmole/ml ± SE ($p^a$) | $p^b$ | pmole/ml ± SE ($p^a$) | $p^b$ |
| 0 | 152 ± 3.7 | | 0.39 ± 0.02 | | 20.6 ± 0.9 | | 6.9 ± 0.7 | |
| 24 | 92 ± 3.4 (<0.001) | <0.001 | 1.15 ± 0.05 (<0.001) | <0.001 | 17.9 ± 1.7 (NS) | NS | 10.6 ± 1.7 (NS) | NS |
| 48 | 77 ± 5.8 (<0.05) | <0.001 | 1.44 ± 0.17 (NS) | <0.001 | 22.0 ± 1.8 (NS) | NS | 6.9 ± 0.3 (<0.05) | NS |
| 72 | 85 ± 5.4 (NS)[c] | <0.001 | 1.41 ± 0.16 (NS) | <0.001 | 23.9 ± 3.4 (NS) | NS | 5.8 ± 0.7 (NS) | NS |
| 120 | 114 ± 11.6 (NS) | <0.02 | 0.96 ± 0.09 (<0.05) | <0.001 | 24.1 ± 2.9 (NS) | NS | 8.7 ± 0.7 (<0.02) | NS |

Each value is an average of determinations from 5 or 6 rats.
[a] Significance of the difference between the given value and the closest preceding value.
[b] Significance of the difference between the given value and the initial control value at zero time.
[c] NS, not significant.

Nevertheless, in all rats the time interval between taking the animal from the cage and withdrawing the sample of plasma after centrifugation was constant, and thus samples from all groups were affected in the same way. Fasting for up to 120 hr did not change plasma concentration of cyclic AMP. The concentration of cyclic GMP varied during the fasting period, but the changes were not significant when compared with the initial control level at zero time. The increase between 0 and 24 hr of fasting was just below the level of significance ($p = 0.1$–$0.05$).

*Discussion.* In man, fasting is associated with a decrease in plasma insulin and an increase in plasma glucagon levels (15, 16). The change in the insulin/glucagon ratio is believed to be responsible for adaptive metabolic changes observed (17). In the fasting rat, the insulin/glucagon ratio most likely changes in a similar manner as in humans, although reliable data on plasma glucagon concentrations are not yet available. It is interesting in this respect that both insulin and glucagon can affect the release of cyclic AMP from isolated perfused rat livers. It has been shown that doses of glucagon which do not measurably alter hepatic cyclic AMP levels elicited substantial increases in perfusate concentrations of the nucleotide, while insulin lowered the release of cyclic AMP (7). The release of cyclic nucleotides from the adipose tissue in response to glucagon or insulin stimulation was not systematically studied.

The failure to detect changes in peripheral plasma cyclic nucleotide levels in the fasting rat may be explained by the fact that plasma cyclic nucleotides probably represent efflux from a number of tissues and these sources might be masking changes in efflux rate that may be occurring in specific organs, such as the liver and fat tissue. In addition, other organs might be increasing uptake of the cyclic nucleotides from plasma during fasting and hence might be obscuring any increased release of these substances from specific organs.

*Summary.* Fasting in the rat for up to 120 hr does not change the plasma concentrations of cyclic AMP and cyclic GMP, although plasma glucose and free fatty

acid concentrations are very markedly affected.

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