The Response of Serum and Pancreas Tryptophan to Starving and Re-feeding in the Rat

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Fernstrom & Wurtman (1971) showed that re-feeding starved rats with a protein-free meal led to a rise in brain tryptophan and 5-hydroxytryptamine concentrations, and an increase in the total serum tryptophan. The rate-limiting factor in brain 5-hydroxytryptamine synthesis is considered to be the availability of tryptophan in the brain; this in turn is regulated, not by the total serum tryptophan, but by that small fraction which is diffusible, the majority being bound to serum albumin. Knott & Curzon (1972) showed that starvation caused a rise in the diffusible fraction without affecting the total serum tryptophan concentration.

Madras et al. (1973) have shown that at 1 and 2h after administration of glucose to starved animals there is a significant rise in total serum tryptophan, accompanied by a fall in the diffusible fraction. In the present study, the short-term (10-20min) responses of rats to glucose administration were studied, after food deprivation for one normal feeding period (24h). Courtauld Institute male Wistar rats (150-200g) were killed by decapitation, and the blood was collected and allowed to clot. Serum total tryptophan was measured by the method of Denckla & Dewey (1967), non-esterified fatty acids as described by Mikač-Devic et al. (1973), and the ratio of diffusible to bound tryptophan by equilibrium dialysis at 4°C, by using tracer amounts of high-specific-radioactivity [14C]tryptophan. The small-scale equilibrium dialysis method used here gave results in good agreement with those reported by Knott & Curzon (1972): diffusible tryptophan

Fig. 1. Response of serum non-esterified fatty acid and diffusible tryptophan to glucose administration in starved rats

○, Fatty acid; ◦, diffusible tryptophan. Values are means ± S.D.
after starvation, 14.4% of total, after feeding, 9.8%. This is in sharp distinction to the results obtained by Madras et al. (1973), who showed a diffusible tryptophan after starvation of 34% of total.

There was no change in the total serum tryptophan in response to starvation, and a small, but non-significant, rise 10–15 min after administration of glucose (100mg in 1 ml, intraperitoneally). The diffusible tryptophan concentration was somewhat higher in starved animals than in fed control rats, and rose significantly 10 min after glucose administration, as can be seen in Fig. 1. Serum non-esterified fatty acid concentrations were increased in the starved animals, as would be expected, but had fallen to only slightly above the amounts in fed rats 10 min after glucose. The ratio of diffusible/albumin-bound tryptophan was significantly correlated with the serum non-esterified fatty acid concentration ($r = 0.46, P = 0.005, 36 \text{ df}$), thus providing further evidence for the hypothesis advanced by Curzon et al. (1973), that the changes in serum tryptophan binding in response to stress can be explained by changes in fatty acid concentrations.

There is some evidence that 5-hydroxytryptamine affects the release of insulin by pancreas in response to glucose in vitro (Telib et al., 1968). In view of the changes of diffusible serum tryptophan noted above, the pancreatic amounts of tryptophan, 5-hydroxytryptamine and its principal metabolite, 5-hydroxyindol-3-ylacetic acid, were measured. Pancreases were removed from the rats immediately after death, frozen in liquid $N_2$, and stored at $-20^\circ \text{C}$ until required. Pancreatic 5-hydroxytryptamine was found to be relatively constant (2.6–3.6 nmol/g), with no change with the fed or starved status of the animals. The origin of the pancreatic 5-hydroxytryptamine is not clear, since there was no detectable conversion of radioactive tryptophan into 5-hydroxytryptamine by pancreas homogenates in vitro, although they would decarboxylate 5-hydroxytryptophan to 5-hydroxytryptamine. It therefore appears that pancreas can accumulate exogenous 5-hydroxytryptamine or 5-hydroxytryptophan. Although pancreas homogenates in vitro were capable of oxidizing 5-hydroxytryptamine to 5-hydroxyindol-3-ylacetic acid, there was no detectable 5-hydroxyindol-3-ylacetic acid in any of the samples examined, suggesting that the acid is rapidly removed from the tissue after formation.

In animals fed ad libitum the pancreas contained $3.7 \pm 0.9 \text{ nmol/g}$ of tryptophan wet wt.

![Graph](image-url)

Fig. 2. Response of pancreatic tryptophan to glucose administration in starved rats

For details see the text. Values are means ± S.D.

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In starved animals this had increased to 12.8 ± 4.3 nmol/g. Fig. 2 shows that the pancreatic tryptophan of starved animals declined towards the amount in fed rats in response to glucose administration, suggesting that the rise in serum tryptophan in response to glucose administration may be due to release from the pancreas, possibly in association with insulin. Injection of saline had no effect on the pancreatic tryptophan, or any other parameters measured, at intervals of 10 min or longer after injection.

It is suggested that the accumulation of tryptophan in the pancreas during food deprivation may represent an attempt to conserve this essential amino acid during gluconeogenesis from muscle protein.

Fernstrom, J. D. & Wurtman, R. J. (1971) Science 174, 1023-1025

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Nitrite Oxidation Rate and the Energized State in Nitrobacter winogradskyi

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Autotrophs of the genus Nitrobacter obtain all their energy from the oxidation of the weak reductant NO\textsuperscript{2-} with O\textsubscript{2}. It has been reported that substituted phenols which uncouple oxidative phosphorylation inhibit the oxidation of NO\textsuperscript{2-} both by Nitrobacter whole cells and in cell-free particles (Simpson, 1955; Aleem & Alexander, 1958; Butt & Lees, 1960). Aleem & Nason (1960) have demonstrated that NO\textsuperscript{2-} oxidation can be coupled to the phosphorylation of ADP, and have concluded that, because uncouplers inhibit both phosphorylation and NO\textsuperscript{2-} oxidation true uncoupling cannot be said to occur. The oxidation of NADH in cell-free particles can be efficiently coupled to phosphorylation (Kiesow, 1964). In view of these observations we have studied rates of NO\textsuperscript{2-} and NADH oxidation under phosphorylating and non-phosphorylating conditions, and in the presence of the uncoupler carbonyl cyanide phenylhydrazone (Heytler & Pritchard, 1962).

Cells were grown continuously at 28°C in a 20-litre aspirator (16-litre working volume) and in a medium basically similar to that described by Van Droogenbroeck & Laudelout (1967). The concentration of NaN\textsubscript{3}O\textsubscript{2} entering the growth vessel was 70 mM. The dilution rate and the air-flow rate were respectively 0.02 h\textsuperscript{-1} and 15 litres·min\textsuperscript{-1}. Cell-free particles were prepared basically as described by Kiesow (1964) except that cells were broken in a Braun MSK vibration mill (4000 rev./min for 1 min). A full description of the growth conditions and of the preparation of cell-free particles is given elsewhere (Cobley, 1973).

At saturating amounts of carbonyl cyanide phenylhydrazone (250 μM), and under the conditions described in Fig. 2 the oxidation of NaNO\textsubscript{2} (3 mM) was inhibited by 65% and the oxidation of NADH stimulated by 90%. Both half-maximum inhibition of NO\textsuperscript{2-} oxidation and half-maximum stimulation of NADH oxidation were observed in the presence of 60 μM-carbonyl cyanide phenylhydrazone suggesting that the mechanism whereby the uncoupler stimulated NADH oxidation was also responsible for the inhibition of NO\textsuperscript{2-} oxidation. Fig. 1 shows the inhibition of NO\textsuperscript{2-} oxidation under phosphorylating conditions. The fact that the effect is insignificant above pH 7.8 might explain why this inhibition has not been previously reported. The stimulation of NADH oxidation under phosphorylating conditions (not illustrated) is also more pronounced.