## Brain tryptophan in rats on a high fat diet

INASMUCH as the article by Hutson et al.1 constitutes a serious criticism of the data reported from our laboratories over the past three years2-6, we would like to make the following reply:

- In 1974, some of us examined the validity of the various methods then available for measuring free tryptophan in blood. We described fully that the equilibrium dialysis method, performed at 37 or at 0 °C, gave identical results for serum-free tryptophan and for serum NEFA levels. The values for NEFA were the same as those measured before dialysis; this test was done with sera containing high  $(1 \text{ meq } 1^{-1}) \text{ and low } (0.2 \text{ meq } 1^{-1})$ amounts of NEFA. We thus ruled out the potential problem of lipolysis during dialysis over two years ago, as Hutson et al. admit only parenthetically (page 143).
- In this same paper, we tested the method of Knott, Curzon, and their colleagues, which involves separation of free tryptophan using Amicon filter cones centrifuged at 800g. In this method, no attempt is made to control  $pCO_2$ , and thus serum or plasma pH, during dialysis. Tryptophan binding is extremely sensitive to pH changes; using this method, the pH can run as high as 8.0 or more, as observed by Madras et al.4. We note that in the article by Hutson et al.1, on page 143, pH values of 7.75, 7.78, and higher were recorded, which affirms the noncontrol of pH in this method, and confirms the findings of Madras et al.4.
- Hutson et al.1 claim that they obtain similar findings when they assay either plasma or serum. But they fail to provide data on free tryptophan values in serum; instead they give data for serum UFA—and assume that free tryptophan behaves similarly. This assumption must be proved in each new experimental situation.

• Finally, we would like to note the following: (1) Hutson et al.1 provide little information about the diets consumed by their animals. What were the protein and fat contents of these diets? How can the authors call the standard laboratory chow diet a "control diet?" (2) How much food was consumed by each group? (3) Why was plasma total tryptophan lower in the group eating the high fat diet? It seems to us that this "diet" experiment is uninterpretable as it is presented.

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HUTSON, KNOTT AND CURZON REPLY-We fail to see that the essential point of our letter is seriously affected by any of the issues raised by Fernstrom. Munro and Wurtman. They refer to the work of Madras et al.2 in which rats on a high-fat diet apparently had high serum free tryptophan not associated with high brain tryptophan. Our own results lead us to question the physiological relevance of these findings as large amounts of unesterified fatty acids (UFA) can be formed by plasma or serum of fat-fed rats in vitro and this may lead to the liberation of tryptophan from albumin after blood collection.

Taking the points raised in their letter in order:

- In quoting the finding of Madras et al.2 that UFA did not increase on dialysing serum at 37 °C our use of the grammatical convenience of parentheses was not intended in any way as a criticism of the validity of this result. However our own findings1 suggest that UFA may have already increased during the preparation of serum from fat-fed rats.
- As already explained the small pH differences between plasmas of control and fat-fed rats would have had negligible effects on free tryptophan concentration.
- It would be most surprising if UFA changes did not cause free tryptophan changes in rat serum as in plasma. We find a significant positive correlation (P < 0.01, n = 16) between percentage free tryptophan and UFA of human
- We did not provide detailed information on the food presented to the rats or on the amount they ate, because this had little relevance to our main conclusion—that changes occur in vitro in rat plasma and serum taken from fat-fed rats.

In the circumstances, and considering the many findings consistent with tryptophan influencing brain tryptophan (refs 3-8 are to papers published in 1976) the results of Madras et al.2 can hardly be interpreted with confidence.

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