but to what avail? If an excess of "protein" is secreted into the intestine, Rothman *et al.* seem to be proposing that a large percentage of this can be conserved by recycling. The activation processes for zymogens such as chymotrypsinogen and trypsinogen would, however, seem to be too rapid and complete to allow any significant level of zymogen to remain. The digestive capacity is controlled, not by the degree of zymogen activation in the intestine, but by the absolute amount of zymogens released on hormonal stimulus of the pancreas.

Space will not permit critical evaluation or alternative explanation for Rothman *et al.'s* observations. Their experiments have been conducted by following "insensitive" parameters such as protein output by the pancreas and chasing a few radioactive counts around the bloodstream. It would be illuminating to see the experiment done wherein (potential) enzymic activity is followed. Only then would we have to consider the feasibility of an enteropancreatic circulation.

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[1] Götze, H., and Rothman, S. S., *Nature*, **257**, 607 (1975).
[2] Liebow, C., and Rothman, S. S., *Science*, **189**, 472 (1975).

Rothman, Götze and Liebow reply— We are sorry that our observations and ideas have alarmed Kay and Beynon[1]; however, new scientific findings, are appropriately met with interest, not fear. They feel that our observations must be explained in other, although unspecified, ways because in their view the circulation of digestive enzyme is quite impossible. If we understand their position correctly, they argue that all active proteolytic enzyme would be irreversibly bound in blood and that proenzyme entering the intestine would be immediately and completely activated in all circumstances and thus be unavailable for circulation. Since these postulates refer only to proteolytic enzyme and since a large percentage of the pancreatic digestive enzymes are not proteolytic, Kay and Beynon are apparently not questioning the existence of a circulation of digestive enzyme, but more narrowly the circulation of proteolytic digestive enzymes. We have directed our response to this question.

Although the workings and physiological functions of plasma protein inhibitors are not completely understood, certainly the mere presence of various inhibitors in the blood (or acinar cell) does not mean that all

Matters arising

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enzyme is irreversibly bound by these molecules and taken out of circulation. On the contrary, it is the presence of these inhibitors in blood that makes it reasonable to conceive of active proteolytic enzyme being circulated. The point is that to prove whether or not active proteolytic digestive enzyme is circulated is a matter for further investigation, not presupposition. Relative to the potential for proenzyme circulation, Kay and Beynon do not cite the evidence on which they base their contention—that the activation of enzyme in the intestine is "too rapid and complete to allow any significant level of zymogen to remain." We are unaware of such evidence. They should remember that maximal activation does not mean that all the enzyme molecules have been activated, and that the activation kinetics of a few defined components in a test tube does not necessarily reflect what goes on in the intestine in various physiological situations. Kay and Beynon apparently insist on restricting the potential meaning of a biological phenomenon to their view of present knowledge of the chemistry and function of certain molecules. They thereby assume that they understand the chemistry and function of these molecules fully and that there are no unknown elements in the piece. On what basis do they make these assumptions?

Finally, in spite of their preconceived notions, the enteropancreatic circulation of digestive enzyme is a fact documented, not by "chasing a few counts around the bloodstream" as they egregiously suggest, but by demonstrating, with tracer kinetics, that labelled chymotrypsinogen instilled in the intestine reappears in pancreatic secretion collected directly from the duct and exclusively in the chymotrypsinogen band of secreted proteins separated by electrophoresis.

In the recent paper[2] which prompted Kay and Beynon's letter[1], we described experiments which suggest that this circulation may account for the movement of substantial amounts of protein. Two corollary observations further reinforce the interpretation that enzyme circulation is involved: (1) output (the increase in protein secretion) correlates strongly with input (digestive enzyme added to the intestinal lumen or injected into the bloodstream) with a slope of 0.92, or in other words, enhanced protein secretion by the pancreas accounted for 92% of protein input; and (2) these two variables fit the same regression line whether the collected secretion was injected into the bloodstream or instilled into the intestine. While these observations are subject to other interpretations (which were carefully pointed out in our article and which Kay and Beynon do not acknowledge in their letter), the circulation of substantial amounts of digestive enzyme is the most direct, and thus, the best interpretation of the results. It is on this basis that we feel it should be the hypothesis for the moment. If Kay and Beynon really have a more convincing interpretation of these experiments, then why do they hesitate to present it? We would greet it with interest, not alarm.

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[1] Kay, J., and Beynon, R. T., *Nature*, **260**, 78 (1976).
[2] Götze, H., and Rothman, S. S., *Nature*, **257**, 607 (1975).

Doubts about the role of the locus coeruleus in learning and the phosphorylation mechanism engaged in the cerebellum

Gilbert[1] has proposed a model of how the cerebellum, with the assistance of the locus coeruleus, could memorise movements. We have been studying the anatomy and chemistry of these and related systems, and have evidence that bears directly on that hypothesis.

The first point concerns the crucial role of the locus coeruleus in the memory consolidation of "motor signals stored in the cerebellum"[1]. The evidence to support that view comes from one study[2] which observed the effects of locus coeruleus lesions on learning. The results have, however, been brought into question[3]. Using a different approach, our laboratory has been studying the effects of localised brain stimulation on learning and retention performances (see ref. 4). Quite remarkable localisations have been obtained in the medial nucleus of the amygdala[5], substantia nigra, pars compacta[6], medial and sulcal portions