enzymes[2,3]. A general corollary of this idea is that it is the sorting mechanism itself which is disturbed. I wish to: (1) suggest alternatively that this mechanism *per se* is not modified, but that the concentrations of the substrates for sorting change; (2) point out the relevance of this to recent data in parallel systems[4–6]; and (3) sound a note of warning in the interpretation of such experiments with amines.

An alternative hypothesis for the causation of misdirection of ACTH precursor away from the secretory into the constitutive pathway, is that chloroquine inhibits the cleavages necessary for conversion of the 30 K precursor into the 4.5 K and 13 K products, and that consequently entry of substrate into the cleavage locale is feedback inhibited, allowing exaggerated traffic along the constitutive route, as a result of normal sorting.

There are several indications of this possibility in the data and discussion of ref. 1. First, the amount of newly synthesized ACTH and its precursors (that is all forms summed) recovered is enhanced by chloroquine (Table 1 of ref. 1). Second, less of the precursor is converted into the mature forms (Fig. 3 of ref. 1). These two observations indicate that proteolytic cleavage and degradation are retarded. Third, the ratio of the two cleaved mature forms in the cells is reversed by chloroquine (Fig. 3 of ref. 1), also suggesting an effect on cleavage independent of sorting. Fourth, the secretory granule enzyme(s) which can generate native ACTH forms from the precursors have acid *p*H optima, and the vesicles probably have an acidic interior[1]. Finally, while the secretion of 4.5 K ACTH in response to secretagogue or in control conditions is inhibited by chloroquine (Fig. 1 of ref. 1), the secretion of 13 K ACTH product, like that of 30 K precursor, is not substantially elicited by the secretagogue, but is enhanced by chloroquine at least as much as is the secretion of the precursor forms (Fig. 2 of ref. 1): 13 K ACTH has thus presumably entered the constitutive pathway in both control and treated cultures. This suggests that the sorting mechanism is acting on the cleavage products rather than the precursors, and is functional in the chloroquine-treated cells.

We have made two observations which may also be explained by such an interpretation, especially if the relevant proteolytic cleavages include those on the intracellular degradative pathway which seems to be an alternative for many secretory proteins. First, plasminogen activator secretion by mouse macrophages is enhanced by another amine, ammonium chloride[5]. Second, the production by human monocytes of the apoprotein procoagulant thromboplastin, which is mainly transported to the cell surface (and not released), is similarly increased by a wide variety of amines[6]. The latter phenomenon is analogous to the enhancement of output of gp70 by chloroquine (Fig. 2 of ref. 1). gp70 is measured in the medium, but this apparently reflects the amount transported to its normal site on the cell surface, from which it is released during the experimental protocol.

Finally, I stress that the effects of amines are complex, as the authors mention. In their work it is not necessary to postulate any effects of chloroquine on vesicle flux itself, as it may be merely the content of vesicles in the constitutive pathway which is affected (thus, increased 30 K precursor content (Fig. 3 of ref. 1) would result in greater 30 K secretion). But in other systems, inhibition of constitutive endocytosis[7,10,11] and exocytosis by amines may involve changes in vesicle flux *per se*. Furthermore, the effects of a single amine, whether complex (chloroquine) or simple (NH_4Cl), often show biphasic dose–response curves. Thus, a multiplicity of effects may be involved in the present observations.

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MOORE *ET AL.* REPLY—We could not agree more with Dean that experiments that use amines to disrupt membrane traffic must be interpreted with great caution. We believe, however, that they can be of considerable value in dissecting membrane traffic patterns in cells when one pathway is perturbed by the amine while another is left essentially intact. Such was the case here[1] and for the lysosomal experiments[2].

Dean does not refute our conclusion that newly synthesized ACTH does not go to secretory granules in the presence of chloroquine, but is secreted constitutively. He suggests, however, that we are not correct in attributing it to an effect of chloroquine on the "sorting mechanism". The difference may be semantic. To us, a drug that blocks the generation of substrate for the regulated pathway, or its recognition by a specific receptor, or its delivery to the next compartment or the dissociation of substrates from the receptor would still be an inhibitor of sorting. Dean prefers to equate sorting with the recognition step only.

A substantive issue raised by Dean concerns our conjecture that sorting precedes proteolytic cleavage of the ACTH precursor. We concede that the alternative hypothesis raised by Dean that proteolysis precedes sorting cannot be eliminated by currently available experimental data. Our reason for preferring sorting before proteolysis is in part a preference for simplicity. Since it is well established that the major products of the ACTH precursor, the NH_2-terminal fragment, ACTH and β-lipotropin, are secreted in equimolar amounts from AtT-20 cells[3], Dean's hypothesis would require three sorting mechanisms for these three fragments, all working with approximately equivalent efficiency.

Second, if proteolysis precedes sorting there would be no point in packaging protease into secretory granules. In fact, the protease seems to be packaged with the ACTH into secretory granules, because (1) it is found there[4] and (2) β-lipotropin is cleaved to β-endorphin long after packaging[5].

Finally, although Dean's analysis of our results in terms of 13 K ACTH release is ingenious, there is proteolysis even of gp70 in the constitutive pathway and the higher ratio of glycosylated to non-glycosylated forms of ACTH results from a proportional increase in chloroquine in core glycosylation of all forms, perhaps because chloroquine slows passage of precursor ACTH through the Golgi apparatus.

We are also aware that chloroquine may be interfering with the crinophagic pathway and thus altering membrane traffic within the cell. Our data on this point are not, however, of sufficient quality to allow any exact conclusion. All we feel confident in maintaining is that the secretagogue-induced exocytosis is normal and the constitutive pathway is not inhibted at chloroquine concentrations at which there is a block between adding the endoglycosidase-H resistant sugars to ACTH precursor and the packaging into secretory granules. We do not know the nature of the block.

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