COMMENTS
by Readers

The undersigned welcome this opportunity to discuss the questions raised by Dr. Potter (*Science*, October 10, p. 342), which will be taken up under the separate numbers which he has listed:

(1) Whether or not there is a relative unsaturation of cytochrome C within the cell is unknown, since the answer to this question is not accessible to direct experimental proof. We suggested a possible excess of cytochrome oxidase over cytochrome C in the tissues on the basis of the following data:

From the saturation curve of Stotz, Altschul, and Hogness (*J. biol. Chem.*, 1938, **124,** 744) it was calculated how much cytochrome would be required to saturate the amount of oxidase indicated by the oxygen consumption of various tissues under the conditions of the assay method for cytochrome oxidase. These values were compared with the amounts of cytochrome actually found in various tissues by Potter and DuBois (*J. biol. Chem.*, 1942, **142,** 417). Stotz, Altschul, and Hogness stated that in their saturation curve the velocity of cytochrome oxidation and not that of its reduction is the limiting factor. According to the authors, the oxygen uptake at the end of the measurement accounted at each point of the curve for maximally 25 per cent of the cytochrome C. Thus, even assuming a slow re-reduction, at least 75 per cent of cytochrome C was still present in the reduced form at the time of the reading. The assay method for cytochrome oxidase is likewise carried out under conditions under which the rate of cytochrome oxidation is the limiting factor.

It is very likely that the application of such simplified calculations to the conditions of the living tissues ignores important factors such as that of the structure or of the influence of other metabolites. On the other hand, it appears to be of interest to compare the available quantitative data concerning the isolated cytochrome oxidase-cytochrome C complex with the assay figures obtained on various tissues. Actually, our physiologic and clinical experiments with cytochrome C are independent of these theoretical calculations. This should not, however, preclude attempts to interpret the available data in a preliminary manner.

(2) We have been intrigued by the question of whether or not the injected cytochrome C penetrates to the inside of the cells, but we have made no statement to the effect that it does. While we have demonstrated that the cytochrome C contents of some organs are increased following parenteral injection, there is as yet no evidence that cytochrome C, being a protein and hence a fairly large molecule, can penetrate cell membranes and thus take part in intracellular activities. The increase in organ content of cytochrome C is considerably more than can possibly be accounted for by the increased content in the circulating blood which resulted from the injection. If the material does not reach the interior of the cells, it might conceivably accumulate in the tissue spaces or perhaps on the surface of the cells. The fact that it does influence physiologic behavior, however, suggests that the cytochrome C probably does enter the cells. If it were assumed a priori that the cytochrome C molecule is too large to be physiologically active after parenteral injection, then one might with equal logic assume that insulin, which is a much larger molecule, or a host of other substances of large molecular weight, could not be effective. Such effectiveness probably indicates cellular penetration.

(3) In our experiments an attempt was made to control the "dilution factor" by adding sufficient cytochrome C to the control vessel to render the concentration of cytochrome C in the total homogenate equal to that in the undiluted tissue.

(4) We have reported (a) that under the conditions of our experiments (2) anoxia reduces the amounts of the easily hydrolyzable phosphorus of rat organs (hearts and kidneys), and (b) that the previous injection of cytochrome C seems largely to prevent this decrease. While Scheinberg and Michel (*Science*, April 4, pp. 365–366) also found that anoxia would reduce the amounts of easily hydrolyzable phosphorus in rat organs, they were unable to confirm our observations on the cytochrome C effect in overcoming some of this reduction. This led us to repeat our own experiments on rat hearts. Our subsequent results were essentially as originally reported by us. That Scheinberg and Michel did not obtain similar results, although presumably using essentially the same procedure and methods, suggests the desirability of clarification of this question by other workers for, as Dr. Potter states, "this experiment would be decisive if it could be confirmed."

Dr. Potter has suggested that there is some question as to whether our technique of fixing the tissues was sufficiently quick to preserve the phosphorus compounds and implied that we should have used the method of freezing by liquid air. The method which we employed was the immediate homogenization of the quickly excised organ in ice-cold trichloracetic acid. The advantages of freezing in liquid air are at least equivocal for two reasons. First, freezing by air is not instantaneous through any considerable depth of tissue; there is a significant gradient. Second, the immersion of a muscle into liquid air acts to stimulate the muscle to maximal contraction and thus fixes it not in the metabolic stage prior to immersion, but in an extraneously produced physiologic condition. It is very likely that a similar stimulatory effect occurs in other organs.

(5) We feel, as Dr. Potter does, that the question as to whether or not cytochrome C is of any therapeutic value will have to be answered on the basis of the results of investigators in a number of clinics. It is notoriously difficult to evaluate therapeutic effects in many clinical conditions, a good example being angina pectoris, which is one of the conditions we have been studying. The only clinical condition in which we have had any considerable experience with cytochrome C therapy is intermittent claudication. In 26 of 39 such patients there seem to have been significant measurable benefits. It may well be that the mechanism of this benefit is entirely different from that which we have presupposed. Even so, our working hypothesis has not been without value.

We, too, are interested in seeing a sufficient body of facts accumulated by qualified and impartial observers so that the true status of the results of our tentative explorations can be determined. The pharmaceutical houses can be helpful by supplying the material necessary to obtain these facts. (SAMUEL PROGER, G. SCHMIDT, and D. DECANEAS, *Joseph H. Pratt Diagnostic Hospital, Tufts College Medical School, Boston.*)