Comments and Communications

Cytochrome C Labeled With Radioactive Iron

The production and use of tagged hemoglobin has become almost a routine procedure. To our knowledge, no attempt has been made to label related hemoproteins. By supplementing iron-depleted young rats with radioactive iron during the period of rapid growth, we were able to isolate from the tissues of these animals cytochrome C showing an activity of $1,235 \pm 9$ cpm for 1 mg of pure cytochrome C on a molecular-weight basis of 13,000. Radioactive iron of high specific activity (Fe^{55}) is used. Counts are taken by means of an argon-filled Geiger tube with a 1-mm beryllium window. Cytochrome C is isolated from heart and skeletal muscle according to the method of D. Keilin and E. F. Hartree (*Proc. roy. Soc. Lond.*, 1937, **B122**, 298). The catalytic activity of the isolated material in the succinoxidase test is high. Injected intravenously into rats, it is as well tolerated as commercial 85% pure cytochrome C. Special attention was given as to the presence of noncytochrome iron: (1) The spectral purity of our cytochrome C preparation is satisfactory, as the ratio of the extinction value at 550 mμ to that at 535 mμ for reduced cytochrome C and the ratio of the extinction value at 550 mμ for reduced to that of oxidized cytochrome C do not deviate more than 2% from the theoretical ratios; (2) the iron content of the preparation can be fully accounted for by cytochrome C since for 1 mg of pure cytochrome C, as indicated by spectrophotometric analysis, 4 $\mu g \pm 5\%$ of iron was found; and (3) hemoglobin, which is expected to carry the highest radioactivity in the experimental animals, has an activity not considerably higher than that of cytochrome C. Four μg of hemoglobin-iron assayed $1,584 \pm 10$ cpm, so small amounts of admixed hemoglobin, undetectable by spectrophotometric and iron analyses, can therefore not cause any appreciable part of the activity found in the cytochrome preparation.

The radioactivity finally present in the animals certainly reaches the border line of safety, but so far no deleterious effects have been observed. Reproduction and lactation seem unimpaired.

The total dose of radioactive iron injected into the animals used for the cytochrome preparation was about 11 mg. About 80% of the cytochrome-iron and 90% of the hemoglobin-iron are derived from this injected iron. Assuming 14 mg of total cytochrome C, corresponding to 60 μg of iron, for a 250-gm rat (M. W. Crandall and D. L. Drabkin. *J. biol. Chem.*, 1946, **166**, 653), about 0.45% of the injected dose is incorporated into cytochrome C, and assuming 3 gm of hemoglobin, corresponding to 10 mg of iron, about 80% of the injected dose is incorporated into hemoglobin.

Considering the radioactivity of the cytochrome C preparation, 1/500 of the usual 5-mg dose injected into rats should still be detectable. We hope this will enable us to trace the metabolic fate of injected cytochrome C and to attack the question of its actual penetration into tissue cells.

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Comparative Data on Vitamin B_{12} From Liver and From a New Source, *Streptomyces griseus*

The isolation from liver of crystalline vitamin B_{12} (E. L. Rickes, *et al. Science,* April 16, p. 396), a substance active in controlling the hematological (R. West. *Science,* April 16, p. 398) and probably the neurological (L. Berk, *et al. New Eng. J. Med.*, 1948, **239**, 328–330) manifestations of addisonian pernicious anemia, has been reported. The natural vitamins may occur in numerous and diverse animal, plant, and microbiological materials, and it was possible that sources of vitamin B_{12} other than liver could be found. Thus, several materials having growth-promoting activity for *Lactobacillus lactis* have been recorded by Shorb (*Science,* April 16, p. 397). As part of our research program on the distribution of this vitamin, numerous source materials have been investigated, and several have been found to show *L. lactis* activity. These include milk powder, beef extract, and culture broths of strains of *Mycobacterium smegmatis,* of *Lactobacillus arabinosus,* of *Bacillus subtilis,* and of several *Streptomyces* species, such as *S. roseochromogenus, S. griseus,* and *S. antibioticus.* The properties of a red crystalline compound which has been isolated from one of these, a grisein-producing strain of *S. griseus,* have been compared with those of vitamin B_{12}.

When heated on the micro-stage, the crystals lost their red color at about 212° and did not melt up to 320°. Crystalline B_{12} similarly darkened to black at 210–220° and did not melt below 300°. The crystals, after drying, were found to have refractive indices of 1.619 (α), 1.649 (β), and 1.659 (γ), which are in agreement with indices of 1.616 (α), 1.652 (β), and 1.664 (γ) for vitamin B_{12}. Emission spectrographic analysis of the crystals revealed the presence of cobalt and phosphorus, as it did for crystalline B_{12} (E. L. Rickes, *et al. Science,* August 6, p. 134). Solubility tests showed that the crystals and crystalline B_{12} have approximately the same solubility in 80% acetone, and that the addition of crystalline B_{12} to a saturated solution of the crystals in 80% acetone did not lead to a significant change in the concentration of the supernatant solution.

The crystals showed about 11.7×10^6 u/mg for the growth of *L. lactis* as compared with an average of 11 $\times 10^6$ u/mg for crystalline B_{12}. They have shown optimal "animal protein factor" activity for the chick at a level of 30 μg/kg of diet, which is comparable with that found (W. H. Ott, *et al. J. biol. Chem.*, 1948, **174**, 1047) for vitamin B_{12}.

Randolph West has tested these crystals and found (personal communication) that the clinical response in pernicious anemia parallels that shown by vitamin B_{12}.