# 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 $11.7 \times 10^6$ 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 $B_{12}$.

These comparative data are evidence that the crystals from the microbiological source and vitamin $B_{12}$ are identical.

We wish to thank Dr. Charles Rosenblum for the determination of refractive indices and spectrographic analyses, and Mr. Frederick Bacher for the solubility measurements. We are indebted to Miss Muriel Caswell and her colleagues for the microbiological assays and to Dr. W. H. Ott for the determination of "animal protein factor" activity. We are indebted to Dr. H. B. Woodruff, Mr. David Hendlin, and Miss Myrle Ruger for collaboration on the extension of the research on the microbiological production of vitamin $B_{12}$.

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## Cultivation Is Necessary

The tremendous interest in the use of the various growth-regulating chemicals has brought forth imposing problems regarding unfavorable soil conditions in crop production. Inspection of a number of carrot fields in eastern Pennsylvania in 1947 revealed a very compact and unsatisfactory soil condition resulting from, among other things, a lack of satisfactory cultivation. This was due primarily to the lack of the necessity for cultivation to control weeds because of the use of certain weed-control substances. The yields were phenomenally low. It was believed that a part of this low yield was due to a lack of aeration and unsatisfactory soil conditions.

Experiments with carrots were designed in 1948 to study the influence of cultivation on the growth of the crop. These experiments were located on a Woodstown sandy loam at Cinnaminson, New Jersey, and on a Steinsburg silt loam near Newtown, Pennsylvania. One thousand pounds of a 5–10–10 fertilizer/acre was disked in after plowing in each case. Each of these experiments was carefully replicated 5 times and consisted of (1) hand-cultivation and hand-weeding and (2) no cultivation, with the use of oil spray and hand-weeding to control weeds. The following table gives the mean yield of carrots (lbs/acre):

| Treatment | Steinsburg silt loam | Woodstown sandy loam |
|---|---|---|
| Cultivation and hand-weeding | 11,658 | 11,126 |
| Oil and hand-weeding and no cultivation | 1,204 | 7,007 |

The above differences in yield were highly significant and leave no doubt that under the conditions of these experiments cultivation was a deciding factor in crop yield. Each of the uncultivated soils became extremely compact and unfavorable for root growth. It is realized that some cultivation is practiced by most growers, but in 1947 many soils in eastern Pennsylvania where carrots were grown became extremely compact, and the yields were low on these soils. The same was true to a lesser extent in 1948. Because of this fact and in view of the tremendous interest in chemical weed-control methods at this time, the above information is pertinent and warrants careful consideration by all concerned with crop production.

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## "Serology" and "Immunology"

In a recent communication (*Science*, October 8, pp. 377–378) M. S. Marshall discusses the uses of the terms immunology and serology and their interrelations. He pleads for "separation of the concept of immunity and the phenomena of serology." With much of what he says the writer is in agreement, for it long ago became evident that some of the content of "immunology" had nothing directly to do with immunity. In other words, "immunology" became a term of misrepresentation and was, therefore, contraindicated. The solution made was to take the newer term "serology" to refer to that branch of biology which deals with the nature and interactions of antigens and antibodies (A. Boyden. *Sigma Xi Quart.*, 1936, **24**, 154; *Physiol. Zool.*, 1942, **15**, 109). On this basis serology is the broad term referring to *all phases* of the nature and reactions of antigens and antibodies. Immunology should, then, properly be of a different kind of inclusiveness and refer to those matters, serological or otherwise, which relate to problems of immunity in organisms.

On this basis clarity and truthfulness of thought in both fields can be attained, and the human capacity to confuse with words to some extent held within limits. It would, moreover, be a great mistake to belittle serology and to attempt to restrict it to matters of the technique of handling antigens and antibodies, and nothing of this kind was the intent of Dr. Marshall's remarks. There is a distinct need for the broad term serology to cover the growing field of biology in which the nature and reactions of antigens and antibodies play their part. As in all other branches of biology, "observation and reflection" should go together and the term serology would include both. There has already developed a considerable body of fact, theory, and fundamental principle in serology and especially in systematic serology.

I commend Dr. Marshall for his critical analysis of the possible relation or lack of relation between immunity and serological reactions, and I trust that we will not sacrifice the gains already made in establishing serology as a broad term covering the nature and interactions of antigens and antibodies together with all the applications and implications of such knowledge. In this broad sense serology would include some phases of immunology and would overlap many other fields of biology, but so do genetics and evolution, and ecology, and all other biological subdivisions. However, each of these fields is to some extent distinctive in methods and results and entitled to a place in biology.

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