Properties of Cytochrome *c*

A STRONG solution of pure cytochrome *c* obtained by the method which we have previously described[1] shows in the oxidized state an absorption spectrum of the parahæmatin type. In a very much higher concentration and in a thick layer this compound reveals the two additional feeble absorption bands at about 695 mμ and 625 mμ discovered by Yakushiji[2]. The absorption spectrum of oxidized cytochrome *c* changes with the hydrogen ion concentration[2,3]. In this respect, cytochrome *c* resembles other trivalent hæmatin compounds such as methæmoglobin, parahæmatin and hæmatin. It is known, for example, that a parahæmatin compound has a characteristic absorption spectrum only within a certain limited range of hydrogen ion concentration[4]. On acidifying it or on making it alkaline the absorption spectrum of parahæmatin changes respectively to those of free acid and free alkaline hæmatins.

Although in acid solution cytochrome *c* shows an absorption spectrum similar to that of a free acid hæmatin with a characteristic band at 625 mμ, in a strongly alkaline solution, instead of showing the spectrum of a free alkaline hæmatin, it shows the spectrum of a parahæmatin.

On reducing cytochrome *c*, in either strongly acid or strongly alkaline solution, a compound is invariably obtained with the typical absorption spectrum of reduced cytochrome *c*. The absorption spectrum corresponding to that of a free hæm of cytochrome *c* has not so far been observed.

Interesting changes in the absorption spectrum of cytochrome *c* can be observed by treating it with sodium dodecyl sulphate (S.D.S.), a powerful surface active substance previously used in connexion with the study of other proteins[5]. On mixing 1 c.c. of a strong solution of *c* with 0·1 c.c. of 5 per cent neutralized solution of S.D.S., the absorption spectrum of parahæmatin is rapidly replaced by that of acid hæmatin. However, sodium hyposulphite reduces this compound, not to the ordinary reduced cytochrome *c*, but to a derivative showing two feeble and more diffuse bands at 563 mμ and 530 mμ, of which the first is stronger than the second. This derivative is autoxidizable, and forms with carbon monoxide a stable compound with absorption bands at 562 mμ and 532 mμ, the second band being now as strong as the first.

The effect of S.D.S. on cytochrome *c* is reversible, as after removing this reagent by dialysis cytochrome *c* reverts to its original state. On the contrary, S.D.S. added to a colloidal heart muscle preparation containing the complete cytochrome system clarifies this preparation and irreversibly destroys all the components of the system except *c*.

The absorption spectrum of the divalent derivative of *c* obtained in this way resembles that of a free hæm. On the other hand, the fact that it cannot be separated from its protein by either fractional precipitations, adsorptions or ultrafiltration shows that the hæm nucleus is still bound to the protein.

This derivative can therefore be considered as a hæm-protein compound, in which the protein has probably lost its connexion with the iron although it remains still linked with the porphyrin.

A similar compound can be obtained from methæmoglobin not treated with S.D.S. but acidified until the bands of acid hæmatin appear. On reduction with sodium hyposulphite this derivative, instead of forming hæmoglobin or hæmochromogen, gives a compound with the absorption spectrum of a hæm. On the other hand, when methæmoglobin is treated with S.D.S. it turns at once into a parahæmatin, which on reduction gives an ordinary globin hæmochromogen.

These observations strongly support the view that the protein and the prosthetic group in cytochrome *c* as well as in other natural hæmatin-protein compounds are bound by means of several links of very unequal stability and which are affected reversibly or irreversibly by different factors modifying simultaneously the general properties of these compounds.

The nature of these links in cytochrome *c* is, however, far from being understood, and this is not surprising considering how very little is known of the nature of links uniting hæm with globin in the hæmoglobin molecule.

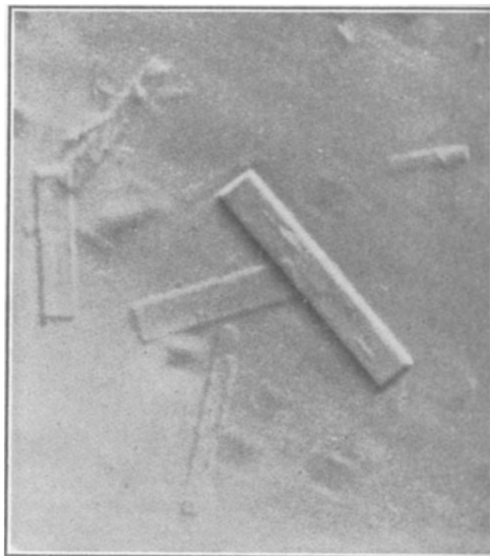
D. KEILIN.
E. F. HARTREE.

Molteno Institute,
University of Cambridge.
May 13.

[1] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.*, B, **122**, 298 (1937).
[2] Yakushiji, E., *Acta Phytochimica*, **10**, 127 (1937).
[3] Theorell, H., and Åkesson, Å., *Science*, **90**, 67 (1939).
[4] Keilin, D., *Proc. Roy. Soc.*, B, **100**, 129 (1926).
[5] Sreenivasaya, M., and Pirie, N. W., *Biochem. J.*, **32**, 1707 (1939).

A Crystalline Albumin Component of Skeletal Muscle

THE intracellular protein components of skeletal muscle are known generally as myosin, globulin *X*, myogen and myoalbumin. Myosin, the globulin which is probably directly related to the contractile process, is the major component, according to analyses carried out on rabbit and fish muscle. The remaining components have not been well characterized as individual proteins. For this reason it seemed desirable to attempt the crystallization of muscle albumin (myogen), adopting the technique employed by McMeekin[1] for the separation of the serum proteins.



A CRYSTALLINE ALBUMIN FROM RABBIT MUSCLE. (*c*. × 260.)

© 1940 Nature Publishing Group