ABSORPTION SPECTRUM OF FURTHER PURIFIED CYTOCHROME C

As shown previously,[1, 2, 3] cytochrome c may be prepared from beef heart with an iron content of 0.34 per cent. This product, arrived at by different methods, was till now considered as pure. However, a thorough investigation of the cytochrome c by means of *Tiselius* electrophoretic apparatus revealed the possibility of a further purification. If electrophoresis experiments are carried out at pH = 10.5 (*i.e.*, higher than the isoelectric point of the cytochrome) two moving boundaries on each side of the U-tube appear, a colorless component migrating faster than the cytochrome. After complete removal of the colorless component, the cytochrome c had an iron content of 0.41 per cent. A further purification by means of electrophoresis at pH 7.3 raised the iron content to 0.43 per cent., corresponding to a molecular weight of 13,000. This value is definitely lower than one fourth of the molecular weight of the hemoglobin. The purified cytochrome c, contrary to the unpurified one, migrated in the electrical field as a homogeneous substance at all pH values investigated.

The purification had no influence upon the absorption spectrum in the visual region. The oxidized cytochrome shows different absorption bands, depending on the pH of the solution.[2] A reinvestigation of the visual part of the spectrum now revealed some new absorption bands and the existence of *four different forms* of the ferri-cytochrome c:

TABLE 1

Type	pH	Bands, mμ	Color of the solution	Dissociation constant, pK'
I ..	1	627; (540–490)	brown	I– II: 2.5
II ..	4–8	695; 560; 540–515	brownish red	II–III: 9.5
III ..	11	507–545	red	III– IV :12.7
IV ..	14	570; 530	brownish red	

The different types change reversibly one into another by changing the pH. A quantitative investigation, carried out by means of photoelectrical light absorption and pH measurements, showed that the different types go over one into another along common dissociation curves.

Solutions of an intermediate pH, types I and II both being present, were analyzed by means of the difference in light absorption at 530 mμ, $\beta_{I,\ 530} = 1.82 \times 10^7$; $\beta_{II,\ 530} = 2.68 \times 10^7$. The other curves were determined in an analogous manner at 650 mμ. The relative

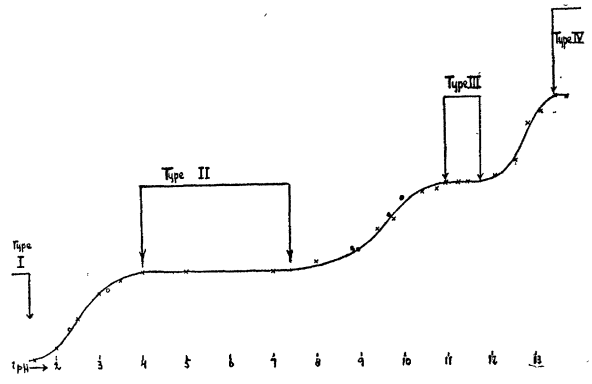

FIG. 1

amounts of the different types were plotted against the pH (Fig. 1).

The great stability of the cytochrome c, even at extreme pH values, depends very probably on the strong linkage of the protein component to the 2- and 4-standing side chains of the hemin.[4, 5] The authors hope that determinations of the magnetic properties of the iron atom in the four different types of the ferri-cytochrome c will give further evidence on the constitution of the cytochrome c.

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SODIUM CHLORIDE AND DIABETES INSIPIDUS

IT has been noted that the abnormally large fluid exchange of experimental diabetes insipidus (d.i.) varies directly with the amount of food the animal ingests.[1] In fasting and in sickness (and therefore probably anorexia), d.i. largely subsides.[2, 3, 4] By varying the NaCl intake of rats, in which d.i. had been experimentally produced by removal of the posterior hypophysis, we have found that the severity of the condition varies directly not with the intake of food in general but rather with the intake of the NaCl in the food. An NaCl-low diet[5] was used alone or to it were added known quantities of NaCl. Reduction of the daily NaCl intake (but not the total intake of food) from 250 mgms to 1.3 mgms caused a reduction of the daily fluid intake from the d.i. level of 60 cc to 25 cc. Equivalent results are obtained by fasting d.i.

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