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

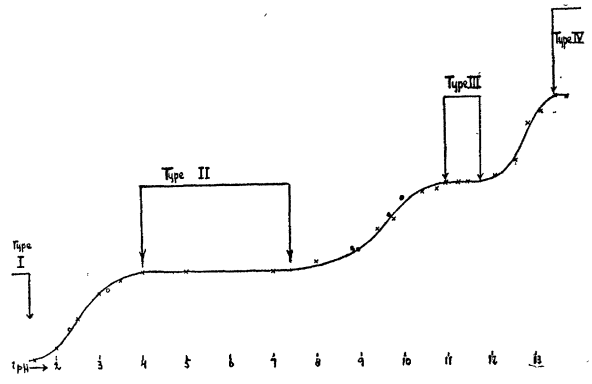

FIG. 1

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.

1 Theorell, *Biochem. Zeits.*, 279: 463, 1935.
2 *Ibid.*, 285: 207, 1936.
3 Keilin and Hartree, *Proc. Roy. Soc., London*, B, 122: 298, 1937.
4 Theorell, *Biochem. Zeits.*, 298: 242, 1938.
5 *Ibid.*, in press.

1 C. P. Richter, "The Pituitary Gland," Baltimore, 1938.
2 P. Bailey and F. Bremer, *Arch. Int. Med.*, 28: 773, 1921.
3 G. M. Curtis, *Arch. Int. Med.*, 34: 801, 1924.
4 C. Fisher, W. R. Ingram and S. W. Ranson, *Arch. Neurol. and Psychiat.*, 34: 124, 1935.
5 E. Orent-Keiles, A. Robinson and E. V. McCollum, *Am. Jour. Physiol.*, 119: 651, 1937.

rats. Conversely, in both types of experiment it is possible to cause a full return of the d.i. by giving 250 mgms of salt per day without any food. Similarly, excess NaCl administration is well known profoundly to aggravate an existing d.i.[6] All the above experiments were done with rats in the permanent phase of d.i. We have found, however, that the transient phase is not dependent on the rate of NaCl ingestion; it occurs even though the NaCl content of the diet has been previously reduced or, as is well known, in fasting.

Although the rate of ingestion of NaCl greatly influences the permanent phase of d.i., we do not think it exclusively responsible for the condition. If the NaCl intake is reduced to zero by fasting, a degree of d.i. still recurs at the time of the onset of the permanent phase. At this time, the fluid exchange of the d.i. animals rises to about three times normal, as contrasted with seven times normal when such animals are fed. In d.i. rats, therefore, a large portion of the abnormal fluid exchange which commences with the permanent phase can be ascribed to the return of the animal's appetites with consequent ingestion of NaCl in the animals' food.

Using Richter's[7] technique for determining appetites in the rat during deficiencies of various kinds, we have also found that there is in d.i. in the rat a considerable appetite for salt. Diabetic rats ingest approximately three times the quantity of salt that the normal rat ingests when given a choice between water and physiological saline to drink.

Summary: The severity of permanent diabetes insipidus in the rat is directly related to the amount of NaCl ingested; if little or no NaCl is taken in, the condition exists in only a mild form. The effect of fasting on diabetes insipidus can be attributed to the withdrawal of NaCl rather than to the withdrawal of food in general. Also, the diabetic rat shows a considerable appetite for NaCl. These findings, coupled with the fact that the diabetic animal is abnormally sensitive to NaCl administration, suggest that in diabetes insipidus the primary defect is some abnormality in NaCl metabolism.

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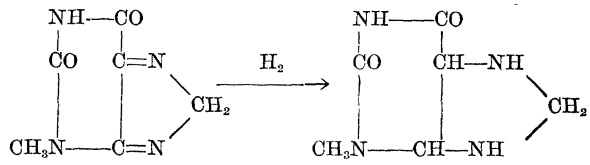
THE ATTEMPTED HYDROGENATION OF 3-METHYLXANTHINE[1]

THE observation by vanVeen and coworkers that *toxoflavin* $C_6H_6O_2N_4$ (I), the highly toxic substance produced by the bacterial action of *Bacterium cocovenenans* on certain food products originating in the commercial production of cocoanut oil in Java,[2] is reduced catalytically in the presence of platinum oxide to a purine containing a saturated glyoxaline ring (II), stimulated the authors to investigate the behavior of 3-methylxanthine (IV) on catalytic reduction.



I II

The graphical formula (I) proposed by vanVeen to express the structure of *toxoflavin* is isomeric with that of 3-methylxanthine (IV), and is the first representative of the desmotropic formulation for purine (III) to be described in the chemical literature.

III IV

It is a well-known fact that xanthine (V), 3-methylxanthine (IV) and several other alkylated xanthines undergo reduction electrolytically[3] with absorption of hydrogen at the 6-position of the purine cycle. This treatment leads to the hydrogenation of the carbonyl group (CO) to a methylene radical (CH_2) and formation of a desoxypurine derivative (VI). There was no attack by hydrogen on the imidazole cycle of the purine nucleus of the various xanthine compounds subjected to this electrolytic technique of reduction. Uric acid is also reduced in a similar manner.[4]

V VI

The authors now desire to report that it has been their experience that both xanthine (V) and 3-methylxanthine (IV) are extremely resistant to structural changes when subjected to hydrogenation in the pres-

[6] H. G. Swann, *Endocr.*, 24: 253, 1939.

[7] C. P. Richter, *Endocr.*, 21: 50, 1937; *ibid.*, 22: 214, 1938.

[1] Researches of pyrimidines, CLXII.

[2] vanVeen and Mertens, *Rec. trav. chim.*, 53: 402, 1934; vanVeen and Baars, *Kononklijke Akad. Wetenschappen Amsterdam*, 40: 6, 1937; vanVeen and Baars, *Rec. trav. chim.*, 57: 248, 1938.

[3] Tafel and Weinschenk, *Ber.*, 33: 3370, 1900; Tafel and Ach, *Ber.*, 34: 1166, 1901. See also Johnson, "The Chemistry of Pyrimidines, Purines and Nucleic Acids," Gilman's "Organic Chemistry," Volume II. John Wiley and Sons, Inc., New York City, New York, 1938.

[4] Biltz, *Jour. pr. Chem.*, 145: 83, 1936.