the reversible reduction with hyposulphite. The absorption spectrum is practically identical with that of riboflavin[2] (peaks of the three absorption bands at 270, 360 and 445 mμ respectively). The fact that the prosthetic group is insoluble in benzyl alcohol indicates that it is not riboflavin but more likely the corresponding phosphorylated compound[3].

Milk flavoprotein was found to catalyse the oxidation of reduced coenzyme I (prepared by reduction either with dehydrogenase systems or with hyposulphite) by carriers such as methylene blue, riboflavinphosphate and cytochrome *c*. Calculation showed that in presence of a large excess of reduced coenzyme I, each molecule of milk flavoprotein catalysed the oxidation by methylene blue of about a thousand molecules of reduced coenzyme per minute at 38°. A positive catalytic effect was observed with as little as 0·02 γ flavin equivalent per ml.

In the course of its catalysis, milk flavoprotein did not appear to undergo a cycle of reduction and oxidation. This consideration leads us to believe that milk flavoprotein acts catalytically not by serving as an intermediary between reduced coenzyme and the carrier, but rather by forming a complex with the reductant and the oxidant and thereby facilitating the transfer of hydrogen.

Since our best preparations contain only 0·06 per cent flavin contrasted to 0·6 per cent in the purest preparations of yeast flavoprotein, the possibility still exists that the catalytic effects observed may be due to some persistent colourless impurity. However, the parallelism between catalytic activity and concentration of flavoprotein makes the impurity hypothesis unlikely.

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[2] Kuhn *et al.*, *Ber.*, **66**, 1035 (1933).
[3] Emmerie, NATURE, **141**, 416 (1938).

A New Enzyme of the Glycosidase Type

I SHOULD like to report the occurrence of what appears to be a new enzyme of the glycosidase type.

This ferment, which is readily prepared from celery seed using Tauber's technique,[1] hydrolyses naringin, the bitter glycosidic principle of grape-fruit (*Citrus decumana*) *in vitro* at 37° and *p*H 7. The products are the insoluble aglycone naringenin (or naringetol) in crystalline aggregates belonging to the hexagonal system (I mention this because I have seen no reference to its crystalline form, and have myself only succeeded in producing the amorphous form together with glucose and rhamnose by acid hydrolysis of naringin), and in addition, a seemingly new disaccharide. That this latter is not rutinose (β . 1 . rhamnopyranosido - 6 . *d* . glucopyranose) follows from the work of Rabaté,[2] whose results I have confirmed, namely, that rhamnodiastase, the enzyme which removes rutinose from its glycosides, will not decompose naringin. Further, since repeated attempts to hydrolyse naringin with emulsin have failed, it is probable that the disaccharide is akin to rutinose but has an α- instead of a β-linkage between its constituent sugars, or the only alternative, that it is glucosidorhamnose, the aglycone linkage being through rhamnose instead of glucose.

It is hoped to establish this definitely later on, and meanwhile it is interesting to record yet another instance of the occurrence together in the same species, but not necessarily in the same organ, of substrate and enzyme, which latter can be prepared from the leaves of the tree *Citrus decumana*, again using Tauber's method. The enzyme is absent from the rag, peel and seeds of the fruit, but occurs in small amount immediately between the flavedo and albedo, where very possibly it plays some part in ripening, since it is known that the bitterness then decreases in the fruit.

It would seem, therefore, that this represents the first occasion on which naringin has been hydrolysed by an enzyme *in vitro*, and possibly this new enzyme can be employed to study further the metabolism of rhamnosides in plants.

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[2] Rabaté, *Bull. Soc. Chim. Biol.*, **17**, 314 (1935).

Quantitative Measurement of Vitamin B_1 by the Thiochrome Reaction

IT has been known to us for more than a year that there is a large discrepancy between the results of biological vitamin B_1 assay and of determination of aneurin by the original method of Jansen[1], when both methods are applied to animal tissues. We did not like to report on these observations before having succeeded in determining vitamin B_1 quantitatively by the thiochrome method. Immediately after the publication of a paper by M. A. Pyke[2], however, it was clear to us that he had estimated only a very small part of the total amount of vitamin B_1 actually present in the animal tissues investigated. Dr. Pyke's letter in NATURE of June 25, p. 1141, prompts us to make the following remarks.

Westenbrink and Jansen[3] described the determination of the two forms of vitamin B_1 known with certainty up to this moment, aneurin and cocarboxylase or aneurin-pyrophosphoric acid, separately by means of the thiochrome method at the meeting of the Dutch Society of Physiology and Pharmacology of April 2, 1938. This method is based on the fact that thiochrome formed from aneurin is extracted quantitatively from the aqueous reaction mixture by isobutanol, whilst the thiochrome-pyrophosphoric acid formed from cocarboxylase remains quantitatively in the aqueous layer[4].

Since that time we have applied this method to various animal tissues. When the tissues are finely minced and suspended in water and vitamin B_1 is absorbed on franconite at *p*H 3, the aqueous and isobutanol layers both show a blue fluorescence even without oxidation by ferricyanide. In most cases this 'blank' fluorescence is even stronger than the fluorescence observed after oxidation of the vitamin by ferricyanide. However, the 'blank' fluorescence may be reduced to a very small remainder by digesting the tissue proteins by pepsin, previous to the adsorption of the vitamin on franconite.

After this observation we were able to carry out aneurin and cocarboxylase determinations in animal tissues. Certainly the method is still capable of improvement, but it was sufficiently accurate already to provide the following results :