examples are yet known in which such a strict comparison is possible; for in our opinion the value for this purpose of measurements on cultured tissue-cells, growing under conditions which are certainly unphysiological, is very doubtful.

The first example is the hepatoma, to which Berenblum *et al.* take exception on the grounds that aerobic glycolysis may sometimes be lacking in hepatomata, and that normal liver has variable and often considerable aerobic glycolysis. Both in our own experience, however, and in the recorded values, we have always found the aerobic glycolysis of liver to be small compared with its respiration, especially when it is remembered that the often large formation of acetone bodies by this tissue must be taken into account. The second example is the transformation of skin epithelium into Shope papilloma, studied by Berenblum, Chain and Heatley[3]. Some experimental data are not detailed in this paper, such as the medium used, the number of observations and the consistency of the results; also it is not stated if normal tissues give the same values of aerobic glycolysis and respiratory quotient in the minute pieces used as they do in the usual larger-scale experiments. The purity of the papilloma tissue used for comparison with 100 per cent pure epithelium and the stage of malignancy arrived at are also desirable data. However, in the one example given, there was no change of metabolism other than a slight general lowering of all the figures, including respiratory quotient, when a tumour had resulted.

In our opinion, these two cases counterbalance one another, and no general conclusion can as yet be drawn as to the occurrence of an alteration of metabolism in the development of cancer.

F. DICKENS.
H. WEIL-MALHERBE.

Cancer Research Laboratory,
North of England Council of the
British Empire Cancer Campaign,
Newcastle-upon-Tyne.
April 22.

[1] Boyland, E, NATURE, **145**, 513 (1940).
[2] Weil-Malherbe, H., *Biochem. J.*, **32**, 2257 (1938).
[3] Berenblum, I., Chain, E., and Heatley, N. G., *Amer. J. Cancer*, **38**, 367 (1940).

Chemical Estimation of Vitamin B_6 in Foods by Means of the Diazo Reaction and the Phenol Reagent

THE recent investigations of Kuhn and his co-workers[1] and Harris and Folkers[2] have shown that vitamin B_6 is 2-methyl-3 hydroxy-4 : 5 di(hydroxy-methyl)-pyridine. It contains a hydroxy group in the β-position of the pyridine ring, which gives the characteristic red coloration of true aromatic phenols with ferric chloride. The vitamin also gives colour reactions with diazotized aromatic amines and the phenol reagent of Folin and Ciocalteu[3].

A method has been developed for the estimation of the vitamin in biological materials using diazotized sulphanilic acid or the phenol reagent. Since these reagents are not specific for vitamin B_6, it is necessary to remove all interfering substances before colorimetric estimations can be carried out. By the procedure outlined below it has been found possible to obtain values for the vitamin B_6 content of various foods which appear to correspond approximately to those obtained by other workers using biological methods.

A suitable quantity (2–50 gm. containing 10–20 rat units of vitamin B_6[4]) of the finely minced or powdered test material was digested with pepsin for twenty-four hours. Protein and its derivatives were removed by tungstic acid. Purine, pyrimidine and imidazole bases were precipitated with silver nitrate and barium hydroxide and the excess silver removed. The solution was adjusted to *p*H 1–2 and the vitamin B_6 present was adsorbed on clarite (2 gm.). The vitamin was eluted from the clarite with hot barium hydroxide and the silver precipitation was repeated. The solution was then adjusted to *p*H 6, concentrated to 25 ml., and treated with sodium nitrite and acetic acid for 15 minutes to destroy any amino group that might be present. After bringing the *p*H to 7, the solution was filtered and made up to 50 ml. 10 ml. aliquots were treated with diazotized sulphanilic acid and the azo colour formed estimated colorimetrically by comparison against a standard of 20 μgm. of pure vitamin B_6 treated in the same way. The method is highly sensitive. 10 μgm. of vitamin B_6 is easily estimated and the colour obtained is proportional in intensity to the amount of vitamin present. The values for fifteen foodstuffs are given in the accompanying table.

VITAMIN B_6 CONTENT OF VARIOUS FOODSTUFFS

Foodstuff	*Vitamin B_6 μgm./gm.*
Yeast, dried (brewer's)	54·0
Yeast, dried (brewer's) (autoclaved at *p*H 9·4)	53·0
Rice polishings	13·4
Liver, sheep	13·4
Muscle, sheep	4·5
Milk, cow's	1·7
Maize, yellow, whole	7·1
Wheat, whole	7·6
Cholam (*Sorghum vulgare*)	8·0
Rice, husked	6·6
Rice, highly milled, raw	3·0
Soya bean	8·0
Beetroot	1·3
Cabbage	3·1
Plantain, ripe	1·3

Known amounts of vitamin B_6 were added to weighed quantities of foodstuffs and the recovery was good in all cases, ranging from 70 to 100 per cent.

The method described is applicable to all types of foodstuffs. It is probable that in some cases it may be abbreviated. For example, treatment with nitrous acid has been found to be unnecessary in the case of yeast. Possibly the second silver precipitation is not always necessary A variety of different phenolic reagents may be used and many modifications in detail are possible. For example, with yeast and potatoes, comparable values have been obtained using diazotized sulphanilic acid and *p*-nitroaniline and the phenol reagent, nitrous acid treatment being omitted.

A fuller account of these investigations will be published in the *Indian Journal of Medical Research*.

M. SWAMINATHAN.

Nutrition Research Laboratories,
Indian Research Fund Association,
Coonoor, S. India.
March 27.

[1] Kuhn, Westphal, Wendt, and Westphal, *Naturwiss.*, **27**, 469 (1939). Cited in *Nut. Abstr. Rev.*, **9**, 606 (1940).
[2] Harris and Folkers, *J. Amer. Chem. Soc.*, **61**, 1245 (1939).
[3] Folin and Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).
[4] Birch, György and Harris, *Biochem. J.*, **29**, 2830 (1935).