

cent and, later, when 500 mgm. calcium were added 23 per cent of ingested phytin-phosphorus appeared in the faeces. The increases suggest that the added calcium decreased the solubility of phytin and thus reduced the opportunity for hydrolysis. The percentages of dietary phosphorus voided in the faeces were 34, 36 and 40 during successive experiments with increasing levels of calcium intake. When these phosphorus excretions were corrected for unhydrolysed phytin it was found that phosphorus absorption was within normal ranges. It follows, therefore, that the phosphorus of hydrolysed oatmeal phytin is as available as the non-phytin-phosphorus of a mixed diet.

So far as calcium absorption is concerned our results are inconclusive. To some extent this was to be expected since calcium itself was the variable and, consequently, detection of any influence of phytin on calcium metabolism would be difficult unless such an effect were most marked. The alternative approach of varying the phytin intake by changes in the oatmeal consumption would have introduced a multiplicity of variables. Two of the subjects on the basal diet showed normal absorptions while the remaining two subjects showed subnormal absorptions. In the case of the latter subjects improvement in absorption was associated with increased calcium intake. In the former subjects the addition of calcium lowered absorption somewhat. While these results do not decide the problem of whether oatmeal phytin interferes with calcium absorption, we would like to direct attention to the long-term study of Steggerda and Mitchell⁴ who, in experiments designed to determine the calcium requirement of the adult, used a diet of low calcium content in which, incidentally, more oatmeal (284 gm. of oatmeal and oatcakes) was used than in our experiments. Notwithstanding the high oatmeal intake, Steggerda and Mitchell found the daily calcium requirement for equilibrium to be 9.2 mgm. per kgm. body weight, a value agreeing well with those of Leitch⁵ of 10 mgm., Mitchell⁶ of 9.75 mgm. and Outhouse *et al.*⁷ of 10.7 mgm. Further, recalculation of the data of Steggerda and Mitchell shows that in their experiments calcium absorption was not interfered with by the phytin of oatmeal.

The absorption of iron was higher than that found by Widdowson and McCance⁸ for subjects consuming brown bread diets.

The results reported in this note strongly suggest that the digestibility of phytin, and therefore probably its effect upon the absorption of minerals, varies from cereal to cereal.

E. W. H. CRUICKSHANK,
J. DUCKWORTH,
H. W. KOSTERLITZ,
G. M. WARNOCK.

Department of Physiology,
University of Aberdeen,
and Rowett Institute,
Bucksburn, Aberdeen. Sept. 1.

¹ McCance, R. A., and Widdowson, E. M., *Biochem. J.*, **29**, 2694 (1935); *J. Physiol.*, **101**, 44 (1942).

² Cathcart, E. P., Murray, A. M. T., and Beveridge, J. B., *Med. Res. Council Spec. Rep. Ser. No. 242* (1940).

³ Hutchison, R., *Trans. Highland and Agric. Soc., Scot.*, **2**, 1 (1868).

⁴ Steggerda, F. R., and Mitchell, H. H., *J. Nutr.*, **17**, 253 (1939).

⁵ Leitch, I., *Nutr. Abst. and Rev.*, **6**, 553 (1936-37).

⁶ Mitchell, H. H., "Act. Sci. et Ind.", No. 771 (Hermann and Co., Paris, 1939).

⁷ Outhouse, J., Breiter, H., Rutherford, E., Dwight, J., Mills, R., and Armstrong, W., *J. Nutr.*, **21**, 565 (1941).

⁸ Widdowson, E. M., and McCance, R. A., *Lancet*, **242**, 588 (1942).

Supplies of Crystalline Vitamin D₃

IN 1936, Windaus¹ and his co-workers isolated from the irradiation products of 7-dehydrocholesterol a dinitrobenzoate identical with the ester prepared almost simultaneously by Brockmann² from a concentrate of tunny fish-liver oil. The hydrolysates of these esters, though yielding no crystalline alcohol, clearly consisted of vitamin D₃ approaching purity. The crystalline vitamin itself was first prepared by Schenk³ in 1937 from the irradiation products of 7-dehydrocholesterol. This isolation was described more fully by Windaus, Deppe and Wunderlich⁴ in the same year. The same crystalline vitamin was prepared⁵ in the following year from fish-liver oil.

A few grams of crystalline vitamin D₃, of German origin, became available in Great Britain before the War, for experimental work on animals⁶ and man^{7,8,9}. It was then clearly shown that clinically and in rat assays there was no significant difference between the antirachitic or hypercalcaemic activities of vitamins D₂ and D₃. The very limited quantity remaining after the completion of these experiments was set aside as a British Standard for vitamin D₃ (chick tests); since 1939, no further quantity of crystalline D₃ has been available in Great Britain.

During the last decade in these Laboratories we have had fairly extensive experience of the isolation and purification of calciferol, both on the laboratory scale and on the relatively large scale of fine chemical manufacture. During the last four or five years frequent, if intermittent, attempts have been made by us to apply these methods to the isolation of pure vitamin D₃. Even the addition of seeds of the German sample mentioned above failed to yield us any crystalline material.

It is only during the past few months that the matter has been attacked again more systematically, and we have now been able, after rigorous purification of the dinitrobenzoate, to produce several small batches of crystalline material that undoubtedly consists of vitamin D₃. One of these batches has been submitted to biological assay, and was found to have an activity slightly greater than, but not significantly different from, 40 × 10⁶ i.u. per gram when assayed on rats by the usual line test procedure. All the batches had physico-chemical constants substantially the same as those claimed by the German investigators for the pure crystalline material.

We hope to prepare a series of larger batches, and to make a study of their physico-chemical constants, as well as of their biological activity, with the view of laying down a specification for the pure crystalline vitamin. Meanwhile, the small quantities of material at present in hand are available for research purposes and we should like this to be known to all workers in the vitamin field.

E. L. SMITH.
H. E. GLYNN.
P. A. WILKINSON.
R. W. PEEVERS.

Fine Chemical Department,
Glaxo Laboratories, Ltd.,
Greenford, Middx. Aug. 28.

¹ Windaus, A., *et al.*, *Z. physiol. Chem.*, **241**, 100 (1939).

² Brockmann, H., *Z. Physiol. Chem.*, **241**, 104 (1936).

³ Schenk, F., *Naturwiss.*, **25**, 159 (1937).

⁴ Windaus, A., Deppe, M., and Wunderlich, W., *Ann.*, **583**, 118 (1937).

⁵ Brockmann, H., and Büsser, A., *Z. physiol. Chem.*, **256**, 252 (1938).

⁶ Coward, K. H., *Bull. Health Org. League of Nations*, **9**, 425 (1940-41).

⁷ Morris, N., and Stevenson, M. M., *Lancet*, **237**, 876 (1939).

⁸ Himsworth, H. P., and Maizels, M., *Lancet*, **238**, 959 (1940).

⁹ Wilson, D. C., *Lancet*, **238**, 961 (1940).