LETTERS TO THE EDITOR

THE MODE OF ACTION OF VITAMIN D

SEVERAL hypotheses have been put forward from time to time to explain the mode of action of vitamin D, but none accounts satisfactorily for all or most of the observed phenomena. The earlier view that vitamin D directly influences absorption of calcium from the intestine has been disproved by evidence published by Patwardhan and Chitre.[1] Sufficient evidence on the other hand is forthcoming to show that vitamin D influences the retention of calcium and phosphorus in the body and thereby maintains a proper balance between the needs of the bones and soft tissues. That the vitamin does not influence deposition of bone by local action has been demonstrated by Robison and Rosenheim[2] with bone slices in calcifying solutions. Under these circumstances the suggestion made by Harris[3] that rickets is essentially a disease of blood rather than the bone seems to be worthy of consideration.

Investigation of the concentration of various chemical constituents of blood serum, *viz.*, Ca, P (acid-soluble, total and inorganic), Mg, Cl, total protein, albumin and globulin, total base, etc., in rachitic and normal children showed that apart from calcium and phosphorus of the serum no other constituent underwent any change which could be attributed to the influence of vitamin D.

That calcium and inorganic phosphorus singly or together decrease in vitamin D deficiency is well known. The diminution of total [Ca] without a simultaneous fall in the quantity or a change in the nature of serum protein would result in a decrease of $[Ca^{++}]$ of the serum [McLean and Hastings[4]]. This is followed by the fall of the ionic product of $[Ca^{++}] \times [HPO_4^{=}]$ and $[Ca^{++}]^3 \times [PO_4^{=}]^2$ leading to a state of undersaturation of the plasma with regard to either of these salts. In 1941, Freeman and McLean[5] showed that in induced rickets in puppies there existed a relation between the ionic product of $[Ca^{++}] \times [HPO_4]$ and calcification at the epiphysis. No such relationship could be found, according to them, with regard to the solubility product of $Ca_3(PO_4)_2$.

Our own observations on clinical rickets amply confirmed by a further study of experimental rickets in puppies show that, in fact, the calcification at the epiphyses and the solubility products of both the above salts are correlated. The critical values for the negative logarithms of the ionic products of $[Ca^{++}] \times [HPO_4^{=}]$ and $[Ca^{++}]^3 \times [PO_4^{=}]^2$ have been observed to be 5·7 and 23·0 respectively. The corresponding values for rachitic children and vitamin D deficient animals lie above and those for normal children and control animals lie below the critical values. This observation on the influence of vitamin D on the ionic products is significant and the authors feel that it should be capable of being applied to test the state of nutrition with regard to vitamin D, especially as no such test exists at the present moment.

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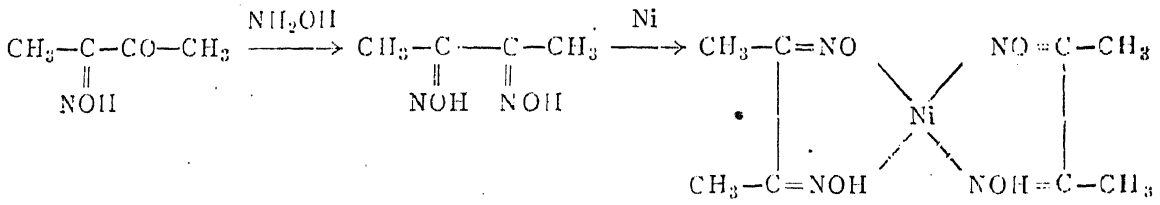
February 3, 1943.

1. Patwardhan and Chitre, *Ind. Jour. Med. Res.*, 1942, **30**, 81. 2. Robison and Rosenheim, *Biochem. Jour.*, 1934, **28**, 684. 3. Harris, *Lancet*, May 14, 1932. 4. McLean and Hastings, *Amer. Jour. Med. Sci*, 1935, **189**, 601. 5. Freeman and McLean, *Arch. Path.*, 1941, **82**, 387.

THE COLORIMETRIC ESTIMATION OF HYDROXYLAMINE

THE several reactions which can be used for the qualitative detection of hydroxylamine have been reviewed by Blom (1928).[1] There exists, however, no satisfactory method for the quantitative estimation of this substance in biological material (*cf.* Lemoigne, *et. al.*, 1935).[2] We describe here a procedure which has been worked out for the colorimetric

estimation of hydroxylamine in the study of nitrogen metabolism in plants. The method is based upon the conversion of hydroxylamine by condensation with diacetylmonoxime to dimethylglyoxime which is then determined by its highly specific reaction with nickel.



By the use of nickel solution oxidised by bromine as employed by Feigl (1924)[3] for the estimation of nickel, the nickel dimethylglyoxime is obtained as a pink solution which can be estimated colorimetrically.

Reagents

A. Hydroxylamine standard, containing 0·01 mg. NH_2OH-N in 1 ml. (4·953 mg. of $NH_2OH.HCl$ in 100 ml.). The solution is neutralised to pH 7·2 with ammonia. The standard is stable for over two weeks. A standard of half this strength can also be used, but with slightly less accuracy.

B. Diacetylmonoxime in N/10 ammonia—3·6 g. dissolved in 10 ml. of N ammonia and diluted with water to 100 ml.

C. Oxidised nickel solution—0·4479 g. of $NiSO_4$, $6H_2O$ in 1 litre. For each estimation 10 ml. of the solution are treated with saturated bromine water added drop by drop till a distinct excess is present. After standing for 15 minutes, N-ammonia is added drop by drop till the excess bromine is decolorized.

Procedure

The test solution containing between 0·025 and 0·075 mg. of hydroxylamine-N is neutralised to pH 7·2 by the addition of ammonia and then treated with 1 ml. of the diacetylmonoxime solution (B). After allowing to stand for half-an-hour at room temperature, 10 ml. of the oxidised nickel solution (C) are added and mixed and after five minutes made up to 25 ml. The rose-red colour which develops is compared with the standard hydroxylamine solution (A), 5 ml. of which are subjected to the same treatment as the test solution.

Table I shows that there is a reasonably good proportionality between colour produced and concentration of hydroxylamine for ranges of concentration from 50 to 150 per cent. of the standard with an error ranging from 0·5 to 3·25 per cent. The smallest concentration of hydroxylamine that can be satisfactorily determined is 0·0005 mg.—N per ml. using a standard of half the strength described above. The errors in this case are slightly higher being of the order of 1 to 4·5 per cent.

The chief conditions for satisfactory results are (1) that the condensation between hydroxylamine and diacetylmonoxime should be carried out at room temperature and a pH of 8·8 to 9, and (2) that a considerable excess (about 150 times the theoretical amount) of diacetylmonoxime should be present. These conditions are satisfied with the quantities and procedure given here. The colour produced is stable for 20 minutes.

In experiments on the hydroxylamine content of root-nodules the method gave 98 per cent. recovery of added hydroxylamine.

Full details with the results obtained on plant materials will be described elsewhere.

Table I

Proportionality between Colour and Hydroxylamine Concentration

Standard and test solution made up to 25 ml. before colour comparison.

Standard: 0·05 mg. NH_2OH—N. Reading of Standard—20.

Test solution (mg. NH_2OH-N)	0·020	0·025	0·030	0·035	0·040	0·045	0·055	0·060	0·065	0·070	0·075
Readings found	..	41·3	34·3	29·3	25·3	22·4	18·3	16·9	15·7	14·6	13·7
Readings calculated	50·0	40·0	33·3	28·6	25·0	22·2	18·2	16·7	15·4	14·2	13·3
% Error (−)	..	3·25	3·00	2·40	1·20	0·90	0·55	1·20	1·94	2·80	3·00

University Biochemical Laboratory, Madras, *February* 18, 1943.

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1. Blom, *Biochem. Zeitschr.*, 1928, **194**, 385. 2. Lemoigne M. Mongaillon, P., and Desveaux, R., *Compt. Rend.*, 1935, **201**, 1067. 3. Feigl, *Ber.*, 1924, **57**, 758.

GROWTH IN SOME STOMATOPODS

In a previous note[1] we recorded the metamorphosis of the larvæ of five species of *Squilla* and assigned them to their respective species. Since then work on similar lines has been continued and five more species of pelagic larvæ have been successfully reared through their metamorphosis, post-larval and pre-adolescent stages, and have been identified as