STUDIES ON THE INTER-RELATIONSHIP AMONG VITAMINS AND AMINO-ACIDS

Influence of Desoxy-pyridoxine on the Bio-synthesis of Nicotinic and Ascorbic Acids in Germinating Pulses

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TRYPTOPHANE-NICOTINIC acid interrelationship first demonstrated with rats,[1,2] has been extended to other mammals[3,4,5] plants and micro-organisms, and such studies have been widened to include other vitamins and amino-acids. It has been shown for example that tryptophane serves as a niacin precursor in the case of the fungus neurospora by way of kynurenine and 3-hydroxy anthranalic acid.[6,7] Seeds are known to give rise to increased amounts of thiamine,[8] nicotinic acid and riboflavin,[9] pyridoxine,[8] and ascorbic acid,[10] during germination. But it was found that a tryptophane-niacin relationship did not exist in the case of bean seedlings grown without their cotyledons on synthetic media.[11] Nason[12] has, however, shown that a tryptophane-niacin relationship similar to that found in certain mammals and *Neurospora,* does exist in higher plants like the corn and has studied the effect of *l*-tryptophane and vitamin B_6, individually as well as in combination. He concluded that niacin synthesis is a direct function of the supply of *l*-tryptophane and is independent of the vitamin B_6 used, since addition of vitamin B_6 to the medium did not significantly increase niacin synthesis. Banerjee and Banerjee[13] have shown that in the case of *Phaseolus mungo* addition of vitamin B_6 to the medium does not enhance the bio-synthesis of niacin. In the case of ascorbic acid, however, the effect of the addition of several nutrients during germination on the bio-synthesis of this vitamin has been studied.[10,14,15,16] But there appears to be no general agreement on the specific constituents which promote such bio-syntheses. Thus, the beneficial influence of salts, of manganese and mannose have been shown by some, while others have not been able to confirm these observations. In the case of rat, some data of a controversial nature, as regards ascorbic acid content in various tissues have been obtained in 1939 by Sure and his associates.[17] Studies have, therefore, been undertaken first with germinated seedlings with a view to elucidate these problems. Cereals and pulses contain appreciable amounts of vitamin B_6[18,19] which increases during germination.[8] Any study of the influence of vitamin B_6 on the inter-relationship between tryptophane and nicotinic acid and on the bio-synthesis of ascorbic acid should therefore take into account the initial presence of B_6 in the seed before germination. In order to counteract the influence of vitamin B_6 already present, a competitive inhibitor like desoxy-pyridoxine[20,21] was added to the medium during germination. It was then found that the bio-synthesis of nicotinic and ascorbic acids could be greatly influenced by vitamin B_6 during the course of germination of several pulses.

EXPERIMENTAL

(*a*) *Germination.*—Green-gram (*Phaseolus mungo*), cow-pea (*Vigna sinensis*) and red-gram (*Cajanus cajan*) have been used in the course of these experiments. Seeds (5 gm. for nicotinic acid and 2 gm. for ascorbic acid) were sterilised with 0·1 per cent. mercuric chloride solution, washed and transferred into sterile petri-dishes containing a layer of acid-washed (B.D.H.) sterile sand. Sterile glass-distilled water was added to each dish in requisite amounts. Necessary amounts of the sterilised solutions of the chemicals were added to particular dishes. The petri-dishes were kept away from direct sunlight in a sterile chamber. The mercuric chloride washed seeds on crushing and plating out in a nutrient medium were found to be free from any bacterial contamination. The germination was carried out for the number of hours specified in the data presented (Tables I and II).

(*b*) *Estimation of nicotinic acid plus nicotinamide in dry and germinated seeds.*—Dry powdered seeds were directly weighed out (5 gm. lots) into conical flasks. After requisite period of germination the seeds from each petri-dish were separately ground with 30 ml. water and transferred quantitatively into conical flasks. 10 ml. of 12 N. sulphuric acid were added, boiled over a water-bath, centrifuged after cooling, made up to 50 ml., washing the residue twice or thrice. The nicotinic acid was determined colorimetrically using lead acetate and zinc sulphate for the deproteinisation; the colour produced by cyanogen bromide and aniline was measured in a photo-electric colorimeter according to the method described by Hawk, Oser and Summerson.[22]

(*c*) *Estimation of ascorbic acid.*—Samples, before and after germination, were ground in a mortar to a fine paste with 6 per cent. freshly

TABLE I

NICOTINIC ACID

(Figures are expressed in γ per gram of dry material)

Dish No.	Substances used in the medium	Hours of Germination								
		Phaseolus mungo-nicotinic acid in dry seed-20·8γ/g.			*Vigna sinensis*-nicotinic acid in dry seed-15·4γ/g.			*Cajanus cajan*-nicotinic acid in dry seed-19·6γ/g.		
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
1	30 ml. Water (control)	24·7	30·5	39·8	18·4	23·9	27·2	22·3	27·9	30·6
2	20 ml. Water *plus* 400γ desoxy-pyridoxine in 10 ml. Water	19·6	22·3	28·2	15·2	17·4	19·8	19·9	23·7	23·8
3	10 ml. Water *plus* 400γ desoxy-pyridoxine in 10 ml. Water *plus* 7 mg. pyridoxine in 10 ml. Water	24·0	29·9	38·3	17·9	24·1	26·3	21·8	25·9	29·5
4	20 ml. Water *plus* 20 mg. *dl*-Tryptophane in 10 ml. Water	25·8	31·6	40·2	19·1	25·2	27·4	23·2	28·2	30·1
5	10 ml. Water *plus* 20 mg. *dl*-Tryptophane in 10 ml. Water *plus* 400γ desoxypy-ridoxine in 10 ml. Water	20·1	23·2	29·3	15·8	17·1	20·2	19·4	24·1	23·6
6	20 mg. *dl*-Tryptophane in 10 ml. Water *plus* 400 γ-desoxy-pyridoxine in 10 ml. Water *plus* 7 mg. pyridoxine in 10 ml. Water	24·2	30·5	38·9	18·4	24·3	25·6	22·1	26·3	29·1

TABLE II

ASCORBIC ACID

(Figures are expressed in mgm. per 100 gram of dry material)

Dish No.	Substances used in the medium	Hours of Germination								
		Phaseolus mungo (Ascorbic acid in dry seed = 6·37 mg./100 g.)								
		Water in the medium			5 ml. of 2½% sol. of Glucose in the medium		5 ml. of 5% sol. of Glucose in medium	5 ml. of 2½% sol. of Mannose in the medium		5 ml. of 5% sol. of Mannose in medium
		24 hrs.	48 hrs.	72 hrs.	48 hrs.	72 hrs.	48 hrs.	48 hrs.	72 hrs.	48 hrs.
1	15 ml. Water (control)	32·7	42·31	51·81	30·73	36·78	30·81	34·65	50·05	34·60
2	10 ml. Water *plus* 125γ desoxyp-yridoxine in 5 ml. Water	31·0	19·16	19·65	18·73	22·06	18·69	34·33	47·43	34·05
3	5 ml. Water *plus* 125 γ desoxyp-yridoxine in 5 ml. Water *plus* 1250 γ pyridoxine in 5 ml. Water	31·26	41·05	54·49	28·93	35·35	27·05	34·36	49·39	33·77

prepared *meta*-phosphoric acid, glass-distilled water being used throughout these experiments. The ground sample was transferred quantitatively to a 50 ml. centrifuge tube and the contents centrifuged, the supernatant decanted and the residue again extracted three times in a similar manner. The total extract was made up to a known volume and titrated against 0·2 ml. of the standard indophenol dye according to the method of Harris and Olliver.[23] These values were confirmed by the colorimetric method of Stotz and Robinson.[24]

RESULTS AND DISCUSSION

The results obtained for the bio-synthesis of nicotinic acid and ascorbic acid during germination of some of the pulses are presented in Tables I and II respectively. It will be seen from Table I that during germination of pulses in water medium alone there is a gradual increase of nicotinic acid. The addition of desoxy-pyridoxine during germination markedly inhibits this increase, but the decrease in value does not fall below the original nicotinic acid value of the seed. The addition of pyridoxine hydrochloride in the concentration specified abolishes this inhibitory effect of desoxy-pyridoxine. The presence of *dl*-tryptophane in the medium to the extent of 20 mg. exerts only a slight influence, if at all, on the increase in the nicotinic acid. The more sensitive microbiological method for the detection of these smaller differences in nicotinic acid is proposed to be used in future experiments to confirm these results. But, as in the case of water medium, addition of desoxy-pyridoxine to the tryptophane medium exerts a similar deleterious effect on the increase of nicotinic acid which, however, is counteracted when pyridoxine is simultaneously added into the medium along with desoxy-pyridoxine. These results, therefore, clearly suggest that B_6 influences the conversion of tryptophane to nicotinic acid. The fact that *dl*-tryptophane added to the medium does not increase the nicotinic acid shows that the seed has enough of this amino-acid liberated or made available during the course of its germination. However, some preliminary experiments indicate that when *l*-tryptophane is added to the medium in similar amounts there is greater increase in the nicotinic acid of these pulses during germination, even though there is enough of this amino-acid originally in the seed. Whether the conversion of *l*-tryptophane to nicotinic acid is more rapid than with *dl*-tryptophane and whether vitamin B_6 has any role to play as a "coracemase" as suggested by Snell[25] remains to be investigated.

The results for ascorbic acid content of pulses under different conditions of germination are presented in Table II. As in the case of nicotinic acid, there is a steady increase in the vitamin C content during germination, though the increase is not as large as reported by other workers. But, what perhaps has been observed for the first time is the fact that by the addition of desoxy-pyridoxine, the influence of vitamin B_6 has been considerably affected on such a synthesis and only a small increase in the vitamin C content is observed. The large addition of pyridoxine to the medium, however, counteracts the anti-vitamin effect of desoxy-pyridoxine and the normal increase of vitamin C content during germination is again noticeable.

Another interesting observation made in the course of these studies was the effect of added glucose and mannose into the medium during germination. It will be seen from the results that when 5 ml. of water is replaced by an equal volume of 2·5 per cent. solution of glucose, the deleterious effect of desoxy-pyridoxine was observed, while with mannose solution no such deleterious effect could be noticed. These results indicate that vitamin B_6 influences the first stage of conversion of glucose into mannose but not the conversion of mannose into ascorbic acid. Whether vitamin B_6 is acting here also like a "coracemase"[25] by influencing isomerisation *in vivo* of glucose to mannose remains to be elucidated. Further work is in progress.

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