*Material and method.* Lettuce seeds (*Laetuca sativa* var. Cabbage) were allowed to germinate in sterilized petridishes lined with filter paper and thoroughly moistened with each of the following solutions: 10 ml of niacin (500 ppm) combined with 10, 25, 50, 100 and 250 ppm of gibberellic acid ($GA_3$). For controls, separate petri dishes were used with filter papers moistened with niacin or $GA_3$ solutions in concentrations mentioned above or distilled water. Experiments were conducted in light (fluorescent tube light) at a temperature of 25 °C ± 2 °C.

*Results and discussion.* The effect of niacin (500 ppm) alone on growth of lettuce seedling is shown in the Table. It will be seen that the chemical, at the concentration used, strongly inhibited root growth[4]. $GA_3$ at concentrations between 25 to 100 ppm greatly increased the hypocotyl length, while the root growth was observed to be normal and similar to that of the seedlings grown in distilled water.

The combined effects of niacin (500 ppm) and $GA_3$ on root and hypocotyl growth have been recored in the Table. It is clear that $GA_3$, at the concentrations used (100 ppm and 250 ppm), greatly reversed the niacin-induced root inhibition. As a result the seedlings treated with $GA_3$-niacin combination indicated stimulation of root growth to the extent of 50% compared with those treated with niacin alone. It is, however, important to note that even an increase of $GA_3$ concentration up to 250 ppm in the presence of niacin (500 ppm) did not appear to affect hypocotyl growth to any significant degree. This would perhaps be of interest, since $GA_3$ is known to cause marked elongation in hypocotyl of lettuce seedlings[5,6].

The way in which niacin influences root growth is not clear. It is known, however, that niacin might disrupt the endogenous auxin levels by competing for tryptophan in the substrate induced biosynthesis[7,8]. On the other hand $GA_3$ is known to stimulate endogenous levels of auxins in many plant systems[9]. It would, therefore, appear possible that $GA_3$ reversed the inhibitory effect of niacin by affecting the endogenous auxin levels. This, however, remains to be tested in further experiments.

*Zusammenfassung.* Gibberellin und Niacin sind Antagonisten, Niacin hemmt das Wurzelwachstum von *Lactuca sativa*, während Gibberellin diese Wirkung teilweise aufhob.

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Combined effect of 500 ppm niacin and $GA_3$ on the growth[a] of radical and hypocotyl as compared with that of niacin and distilled water

| Treatment | Length in cm | |
|---|---|---|
| | Root | Hypocotyl |
| Distilled water control | 4.3 ± 0.3 | 2.6 ± 0.2 |
| Niacin (500 ppm) | 1.0 ± 0.2 | 2.4 ± 0.1 |
| Niacin + $GA_3$ | | |
| 500 + 10 ppm | 1.1 ± 0.1 | 3.1 ± 0.1 |
| 500 + 25 ppm | 1.0 ± 0.2 | 2.9 ± 0.2 |
| 500 + 50 ppm | 1.3 ± 0.1 | 3.4 ± 0.1 |
| 500 + 100 ppm | 2.5 ± 0.2 | 3.5 ± 0.2 |
| 500 + 250 ppm | 2.4 ± 0.2 | 3.4 ± 0.1 |

[a] Results indicate growth of four days old seedlings.

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## Pantothenic Acid Distribution and Protein Synthesis in the Particulate Fractions of Rat Liver

It was reported in earlier communications[1,2] published from this laboratory that pantothenic acid exerted a marked effect on the growth of rats and that it stimulated the synthesis of blood proteins.

The present paper gives an account of the distribution of pantothenic acid in vaious particulate fractions of liver of rats receiving pantothenic acid supplements in the diet.

*Materials and methods.* It was observed from the experiments conducted earlier[1] in respect of ad libitum feeding, that the food consumption of the animals fed on a pantothenic acid deficient diet was much less than than those maintained on the adequate diet, and their growth-rate was also lower. In order to eliminate the effect of inantion from that of panthothenic acid deficiency, pair-feeding experiments were carried out. 4–5 week-old male albino rats, weighing 35–40 g, were distributed according to the body weight and litter mates into 6 pairs. The control rat in each pair was fed on the pantothenic acid deficient diet as described in the previous paper[2] and its pair-fed experimental mate was fed on the diet supplemented with calcium pantothenate (20 mg/kg diet). The actual amount of deficient diet consumed by the control rat was determined daily and an equal amount of experimental diet was fed to its pair-fed mate on the following day. Both the diets were equicaloric. The pair-fed mates consumed the entire amount of food given to them.

After the 10th week, when the typical symptoms of pantothenic acid deficiency, such as bloody whiskers and reddening of paws, were developed in the control group, the animals in both groups were sacrificed by decapitation and allowed to bleed profusely. The livers were quickly excized and placed in an ice-cold normal saline. They were blotted between the filter papers and weighed. The homogenates (10%) were prepared with isotonic sucrose (0.25 *M*) + $CaCl_2$ (0.0018 *M*) solution in Potter and Elveh-

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Table I. Effect of pantothenic acid on protein content of particulate fractions of liver in rats (10 weeks)

| Diet | Mean with S.E. (6 animals/group) Weight of rat (g) | Weight of liver (g) | Weight of liver (g/100 g body wt.) | Protein (mg/g fresh liver) H | N | M | S |
|---|---|---|---|---|---|---|---|
| Basal (deficient) | 105 ± 5.81 | 4.14 ± 0.08 | 3.95 ± 0.07 | 206 ± 2.55 | 47 ± 1.39 | 56 ± 1.20 | 96 ± 2.10 |
| Basal + Ca-pantothenate | 148 ± 5.83 | 5.01 ± 0.19 | 3.39 ± 0.08 | 233 ± 1.40 | 47 ± 0.90 | 60 ± 0.02 | 114 ± 2.07 |
| Statistical analysis | | | | | | | |
| Values of $t$ | 5.22 | — | — | 5.84 | 0.00 | 1.70 | 6.10 |

Values of $t$ equal to and greater than 3.17 are significant at 1% level.

H, homogenate; N, nuclear fraction; M, mitochondrial fraction; S, supernatant fraction.

Table II. Pantothenic acid content of particulate fraction of liver in rats (10 weeks)

| Diet | Mean with S.E. (6 animals/group) Total pantothenic acid (μg/g fresh liver) H | N | M | S |
|---|---|---|---|---|
| Basal (deficient) | 27.0 ± 0.30 | 7.1 ± 0.39 | 7.2 ± 0.14 | 12.7 ± 0.27 |
| Basal + Ca-pantothenate (experimental) | 87.1 ± 1.19 | 7.2 ± 0.55 | 18.2 ± 0.92 | 61.8 ± 0.54 |

H, homogenate; N, nuclear fraction; M, mitochondrial fraction; S, supernatant fraction.

jem type homogenizer consisting of a smooth walled glass tube fitted with an electrically driven teflon pestle. The various particulate fractions of liver homogenate were isolated by differential centrifugation by the method of SCHNEIDER and HOGEBOOM[3,4]. 3 fractions consisting of 1. nuclear, 2. mitochondria and 3. supernatant fraction (microsomes and soluble portion) were isolated.

Protein content of the homogenate and the particulate fractions was determined by the standard Biuret reaction[5]. An aliquot of the liver homogenate and particulate fractions were taken for the assay of total pantothenic acid. A double enzyme treatment described by NOVELLI and SCHMETZ[6] was given to liberate bound pantothenic acid. The vitamin was assayed microbiologically using *L. arabinosus* 17/5 as test organism[7].

*Results and discussion.* It will be seen from the results presented in Table I that the addition of calcium pantothenate to the deficient diet resulted in the elevation of liver protein content. The difference between the values of liver protein in the control and experimental groups is significant at 1% level. The increase in the liver protein content in experimental group is reflected in the elevation of protein in the supernatant fraction. The stimulatory action of pantothenic acid on liver protein formation may have been mediated indirectly through its bound form (Coenzyme-A) which activates amino acids.

The percent distribution of pantothenic acid (Table II) is also more in the supernatant fraction as compared with that in nuclear and mitochondrial fractions in both the deficient and experimental groups. The supplementation of Ca-pantothenate resulted in the increase of the pantothenic acid concentration in the supernatant fraction of liver from 47% (deficient rat) to 71% (experimental mate) of the total pantothenic acid content of the liver.

It appears, therefore, that the function of this vitamin is confined more in the supernatant fraction of the liver than that of nuclear and mitochondrial fractions.

*Zusammenfassung.* Ratten wurden während 10 Wochen mit durch Pantothensäure angereicherte Nahrung oder mit Pantothensäure-Mangeldiät gefüttert. Im Anschluss daran wurden die durch Differentialzentrifugation gewonnenen Fraktionen von Leberhomogenat auf ihren Protein- und Panthothensäuregehalt untersucht. Die Resultate deuten darauf hin, dass Panthothensäure die Synthese von Leberproteinen, insbesondere in der Überstands-Fraktion, stimuliert.

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