tive agent of the disease, the neurotropic filterable virus,[2] closely resembles in all its properties that of St. Louis and of the Japanese encephalitis except for its antigenic structure and certain other pecularities.

So far it has generally been accepted that the tick-borne encephalitis of man occurs only in thick forest (taiga) of the Far East. This view is partly refuted by our findings that similar diseases may occur beyond the woodland zone of the European part of the Soviet Union. The question is thus raised as to a more wide occurrence of these diseases, not only in the Soviet Union but in other countries as well, where the corresponding natural conditions and the *Ixodes* ticks are present.

In examining, May, 1939, two sera of blood of humans recovered from the so-called atypical poliomyelitis of adults (Perm district) the senior of the writers found a high content of antibodies to the Far-East strain of the virus of the tick-borne encephalitis of man.

This finding led us to a more extensive virusological (Chumakov) and clinico-epidemiological examination of the affections of the central nervous system diagnosticated as "atypical poliomyelitis," "serous meningitis," "post-grip encephalitis," the "Kojevnikov epilepsy," etc.

Through the collaboration of local neuropathologists we have learned that in several districts of the Soviet Union (Belarussia, Perm, Kirov, Sverdlovsk, Cheliabinsk district, Western Siberia, etc.) there occur every year, sporadic cases and small localized outbreaks of diseases that are quite similar in the clinical picture and epidemiology to the Far East tick-borne verno-aestival encephalitis. Numerous tests of the sera of typical reconvalescents invariably gave positive results as regards neutralization of the virus of the tick-borne encephalitis.

The disease is characterized by acute beginning high temperature, vomiting, meningeal and general cerebral phenomena, obscured consciousness and frequently by the development of flaccid pareses, paralyses and muscle atrophy, mostly of the upper limbs, bracial girdle and neck.

The season of the incidence is May to September. The disease affects wood cutters, hunters and similar persons attacked in the forests by the ticks one to two weeks prior to the beginning of the disease.

Numerous ticks, *Ixodes persulcatus,* were detected in the local forests (including those situated in direct vicinity to large inhabited localities). These ticks showed great activity in attacking man.

We have succeeded in isolating from the brain of three persons deceased from encephalitis and from the blood of one patient at the height of the disease, four strains of the neurotropic filterable virus (Ural and its neighborhood).

A similar virus was also detected in the local ticks, *Ixodes persulcatus,* which proved naturally infected at all stages of development and readily infected mice on which they fed. Most of the strains (above twenty) were obtained through a bite. It was also found that these ticks collected at the stage of fasting imago and fed on laboratory animals give rise to an offspring (larvae nymphs) which contains a highly active virus. Hence, in the ticks the encephalitis virus is transmitted through the ovum and during metamorphosis; it apparently propagates in the ticks without doing them any particular harm.

In another series of studies carried out in collaboration with N. N. Vorobieva and N. E. Sofronova we have succeeded in detecting a similar virus (four strains) in the brain of wild rodents killed in the endemic focus, *viz.,* hares and squirrels which are attacked by the ticks—the virus vectors.

A large group of strains was studied in detail by means of the following additional methods: (1) cross neutralization of the virus with specific immune sera (of man and animals) and (2) cross vaccination of mice against infection. All the isolated strains were found to be identical to one another as well as to the Far East strains of the tick-borne verno-aestival encephalitis.

In isolating the virus from humans and animals advantage was taken of the widely applied method of preliminary enrichment of the test material by cultivating it on embryonic tissues in Carrel dishes (after the Maitlands) or by inoculating into developing chick embryos.

Thus it was shown for the first time that in the European part of USSR and in Siberia there occurs a peculiar virus disease, *viz.,* the tick-borne encephalitis of man. Further study is necessary of its geographic distribution and of its relation to other neuro-virus infections transmitted by insects.

M. P. CHUMAKOV
N. A. SEITLENOK
ALL-UNION INSTITUTE OF EXPERIMENTAL MEDICINE, MOSCOW

VITAMIN B_1 IN RELATION TO THE GROWTH OF GREEN PLANTS[1]

THE demonstration of the significance of vitamin B_1 in the growth of pea embryos[2] and excised pea[3] and tomato[4] roots has raised the question of the role of

[1] Since completion of these experiments the writer has been informed of extensive experimentation on this question now in progress at the United States Department of Agriculture laboratories at Beltsville, Maryland.

[2] F. Kögl and A. J. Haagen-Smit, *Zeit. Physiol. Chem.,* 243: 209, 1936.

[3] J. Bonner, SCIENCE, 85: 184, 1937.

[2] References 3, 5 and 7, footnote 1.

vitamin B_1 in the nutrition of higher plants. The point of particular interest is whether intact green plants capable of synthesizing this vitamin can profit from its additions to an inorganic growth medium. This is obviously different from a demonstration of the need of excised roots or other isolated tissue for vitamin B_1 as its synthesis in higher plants takes place in green leaves under the influence of light[5] and this vitamin may thus be regarded as another among the organic substances for which roots depend on shoots for their supply—a relation not dissimilar to the general dependence of animals on green plants.

Bonner and Greene[5,6] have investigated the response of intact plants to additions of vitamin B_1 by treating a number of species grown from seed in soil and sand cultures with aqueous solutions of vitamin B_1 and found that certain plants (cosmos, cocklebur, mustard) responded with marked increases in growth, but that others (tomato, pea) were unaffected. In general, plants responding to additions of vitamin B_1 were characterized by a low concentration of this vitamin in the leaves in contrast with the high concentration found in the leaves of plants showing no response. The conclusion was reached by these investigators that the response of a given species to additions of vitamin B_1 is governed, in part at least, by the amounts synthesized in the leaves.

These findings seemed of special interest in the light of the experience of this laboratory, where many species of green plants have been grown with excellent results in metal or glass containers with inorganic nutrient solutions. The luxuriance of growth generally obtained under favorable environmental conditions without the addition of any organic nutrilites has lent support to the concept that under favorable climatic conditions and with an adequate supply of mineral nutrients and good aeration of the root system autotrophic plants are not limited in their growth by the inability to synthesize rapidly enough any of the essential organic growth substances. It was deemed desirable, therefore, to investigate under our conditions the effect of vitamin B_1 on several species of plants by growing them in carefully controlled nutrient solutions.

Among the plants selected tomato was already reported to give a negative response but cocklebur, cosmos and mustard a positive response[5,6] to additions of vitamin B_1 when grown in sand or soil.[7] In the present experiments all plants were grown by the water culture method in nutrient solutions of the following compositions: KNO_3 0.005 M, $Ca(NO_3)_2$ 0.002 M, $Mg(NO_3)_2$ 0.001 M, $(NH_4)_2HPO_4$ 0.001 M, K_2HPO_4 0.001 M, H_2SO_4 0.0005 M. (The sulfuric acid acidified the phosphates.) A supplementary solution[8] furnished 0.5 ppm each of boron and manganese, 0.05 ppm of zinc, 0.02 ppm of copper and 0.01 ppm of molybdenum. The solution was analyzed from time to time to insure adequate concentration of nutrients and changed as needed. All the cultures save those of cosmos and cocklebur were aerated by means of sintered glass aerators (preliminary experiments indicated that these two species did not respond to supplementary aeration under the experimental conditions employed.)

In the light of the known effects of a number of elements in minute quantity on plant growth it was undertaken to safeguard against the introduction of such micronutrients either as small impurities in the vitamin B_1 solution or as chance contaminations in the process of adding the vitamin solution to some cultures. To this end the special technique for growing plants for investigations on micronutrients,[9] which involves the use of Pyrex containers, purified chemicals and redistilled water was employed. Furthermore, each addition of vitamin B_1 was accompanied by a parallel treatment with an autoclaved solution of equal concentration of vitamin B_1 rendered alkaline (to phenolphthalein) prior to autoclaving. This destroyed the activity[10] of the vitamin but afforded a means of testing the possible effect of inorganic contaminants.

With the exception of cocklebur, which was grown only during the spring, all the other plants were grown at two or more seasons, in a series of successive experiments extending for more than a year. Vitamin B_1 was added in concentrations of 0.01 mg to a liter of culture solution. In some cases 0.05 mg to a liter was added, but the results were essentially similar. Table 1 gives some representative results, as averages of dry weight in grams for each treatment.

TABLE 1

	Tomato		Lettuce		Cosmos		Mustard		Cocklebur	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Control	3.6	0.72	3.4	0.44	0.54	0.07	2.5	0.41	3.3	1.06
Vitamin B_1 added	3.7	0.72	3.6	0.47	0.58	0.07	2.8	0.32	3.6	1.12
Autoclaved Vitamin B_1 added	3.7	0.78	3.1	0.45	0.62	0.07	2.7	0.35	4.23	1.09

[4] W. J. Robbins and M. A. Bartley, SCIENCE, 85: 246, 1937.

[5] J. Bonner and J. Greene, *Bot. Gaz.*, 100: 226, 1938.

[6] J. Bonner and J. Greene, *Bot. Gaz.*, 101: 491, 1939.

[7] The writer is indebted to Drs. E. J. Kraus and K. C. Hamner for furnishing the cocklebur and to Dr. James Bonner for the cosmos and mustard seed.

[8] The composition of this supplementary solution designated A5 is that of the previously described A4 solution (D. I. Arnon, *Am. Jour. Bot.*, 25: 322, 1938) plus 17.7 mgs of 85 per cent. MoO_3 per liter.

[9] P. R. Stout and D. I. Arnon, *Am. Jour. Bot.*, 26: 144, 1939.

[10] R. R. Williams and T. D. Spies, "Vitamin B_1 and Its Use in Medicine," Macmillan, 1939.

The results obtained do not support the view that, for the species investigated, intact plants grown from seed can benefit from additions of vitamin B_1 to an otherwise favorable nutrient medium. Such fluctuations in dry weight as were obtained are probably within limits of experimental error. It appears that under the conditions of these experiments the rate of vitamin B_1 synthesis was not limiting growth for any of the species investigated or, stated in other terms, such differences as do exist in the rate of vitamin B_1 synthesis among these plants are apparently compatible with their different needs.

Although these findings are regarded as favoring the contention that green plants when propagated under favorable conditions from seed are capable of synthesizing adequate amounts of vitamin B_1, they do not bear directly on the question of the effect of vitamin B_1 on the rooting of cuttings.[11] An important distinction should be made in this connection between sexual and vegetative propagation. The seed is a storage organ for vitamin B_1 (and is valuable for that among other reasons in animal nutrition) and other growth and food substances essential for the initial growth of the seedling, which soon embarks on photosynthesis, inorganic nutrient absorption and the other metabolic activities of the growing plant. A cutting, on the other hand, is a vegetative organ, not characterized by the storage of nutritive and growth substances to the extent observed in the seed. Cuttings of different species, or for that matter cuttings of the same species, taken under varying conditions may differ in vitamin B_1 content which in some cases may conceivably become a limiting factor in root development. Thus the beneficial effect of vitamin B_1 on the rooting of certain cuttings is not inconsistent with the finding that, when propagated from seed, green plants are capable of synthesizing their own vitamin B_1 requirements. In the light of the observation that in higher plants vitamin B_1 is synthesized in the green leaves under the influence of light[5] it is interesting to note that the presence of leaves on cuttings usually promotes root formation.[12]

This discussion is based on work done with several species of plants grown under favorable nutrient and climatic conditions. Whether under conditions adverse to the synthesis of vitamin B_1, these and other plants, even when grown from seed, may respond favorably to additions of vitamin B_1 from without, is a question of potential agricultural interest which can not be answered definitely at this time. Evidence was obtained, however, to show that several widely differing species, when grown from seed under favorable conditions, are not limited in their growth by an inherently low rate of vitamin B_1 synthesis.

D. I. ARNON

UNIVERSITY OF CALIFORNIA

[11] F. W. Went, J. Bonner and G. C. Warner, SCIENCE, 87: 170, 1938.

[12] Review by H. L. Pearse, Imp. Bur. Hort. and Plantation Crops, Tech. Com. 12, 1939.

SOME DIFFICULTIES ENCOUNTERED IN THE EXTRACTION OF GROWTH HORMONES FROM PLANT TISSUES[1]

ALTHOUGH there are nearly as many methods of extracting plant growth hormones (auxin) as there are investigators, there is no satisfactory quantitative method. For over two years the author has been engaged in studying growth hormones in a number of plants, and during that time he has tried out many of the existing methods. Van Overbeek's[2] method has been used as the standard against which the others have been compared. It has proven to be as satisfactory as any, but it does not extract the auxin quantitatively from the material under investigation. The trouble has been that only after weeks or months of extraction was a point reached where no more auxin was obtained from the material.

The auxin assay has been carried out in the usual way with *Avena* coleoptiles under standard conditions of humidity and temperature and calculations made according to the equation formulated by Van Overbeek. In his publication of the method, Van Overbeek stated that he obtained complete auxin extraction in a period of 24 hours, and that succeeding periods gave no further active material. The writer has not found this to be true with his material, even when the extraction was done on an agitator, as shown in Table I.

TABLE I

AUXIN CONTENT IN OVARIES OF THE SUNFLOWER. THE AUXIN CONCENTRATION IS DENOTED IN TERMS OF INDOLE ACETIC ACID EQUIVALENTS, AND THE FIGURES ARE GAMMAS PER KG OF FRESH MATERIAL

Extraction	Time of extraction in hours	Amount of auxin obtained H_1	Amount of auxin obtained H_6
1	37	0.6	2.22
2	25	1.74	1.46
3	26	0.81	0.97
4	27	0.70	0.77
5	37	1.04	1.81
6	45	1.21	1.63
7	27	1.17	0.75
8	24	1.35	0.44
9	20	0.74	0.99
10	22	0.85	0.98
11	23	0.41	1.22

Note: During the next two weeks several more extractions were made and each showed the presence of auxin.

Table II gives the data from an experiment with young tomato plants.

These two experiments are typical of the many that have been performed during the past two years. It is obvious that not all the auxin, which is finally extracted

[1] This investigation has been financially aided by the Horace H. Rackham Trust Fund of the University of Michigan.

[2] J. Van Overbeek, *Proc. Nat. Acad. Sci.*, 24: 42–46. 1938.