tant intermediate host present. Of 385 voles examined by the writers during 1950, nine, or 2%, were infected. During 1949 and 1950, this vole was very abundant, apparently at a cyclic high level of population density, so that, although the relative number of infected voles is small, the actual number may be quite high. The other microtine rodents (*Clethrionomys* and *Dicrostonyx*) were at a low point in their cycle, and specimens could not be collected for study. Eighty-five ground squirrels were examined without finding any larval stage of *E. granulosus*. The larvae in the voles were of typical appearance, and ranged in size from 2 up to 3 cm in diameter (Fig. 1). The liver and mesentery are commonly involved. The effect of this parasite on the intermediate host will be discussed in a future publication.

It is well known that various species of mouselike rodents form the bulk of the diet of the arctic fox. It is also known that, particularly under conditions of high rodent populations (e.g., cyclic "high" of lemmings), dogs feed upon these easily obtained animals. It can be assumed that St. Lawrence Island dogs also might become infected from this source.

Man's association with the arctic fox is ordinarily not intimate enough to allow infection by its parasites. In this case, however, it appears that there might be a relationship that could account, at least in part, for human infections. The St. Lawrence Island people are hunters, depending almost entirely upon the flesh of sea mammals for their subsistence. During the summer months, however, a considerable quantity of a native green plant, as yet unidentified by us, is consumed. This plant is eaten in the field, unwashed and raw, as it comes from the ground. Arctic foxes are so abundant that it can be assumed that there is considerable contamination of the vegetation. It is of interest that Riley (*3*) stated that human cases of hydatid infection may be contracted by eating wild fruits contaminated by eggs from the feces of infected canids.

Not all factors involved in the epidemiology of the human infections are known. Some infections may result from ingesting eggs from the feces of dogs. Although they are perhaps not commonly infected now, it is possible that dogs harbored *E. granulosus* more often in former years when reindeer entered much more into the economy of the people. Reindeer serve as suitable intermediate hosts for this parasite in Alaska (*4*), and, when they were formerly slaughtered for food, waste parts were undoubtedly available to dogs. Organs such as lungs, in which the larval forms of *E. granulosus* often localize, are usually discarded, as would be anything of unusual appearance such as a large hydatid cyst. It is also possible that the water supply could afford opportunity for human infections, since water is obtained from sources open to fecal contamination by dogs and foxes.

Further investigation may disclose that hydatid disease in Alaska constitutes a serious public health hazard. On St. Lawrence Island, at least, in addition to the positive skin reactions, there is considerable clinical evidence of echinococcosis. Although a thorough clinical study of the reactors has not yet been made, there has been some opportunity for incidental observations. During the past two years two patients from the small population of St. Lawrence Island have undergone surgery for the removal of liver abcesses, and one man still has a post-operative draining sinus. Routine examination of various individuals by Alaska Department of Health personnel has indicated presence of an unusual number of nodular livers.

From the work so far, it is concluded that interstate shipment of dogs from arctic America should be prohibited. These investigations are being continued, and it is hoped that further work will disclose the over-all status of echinococcosis in Alaska, and lead to suitable control measures.

References

1. MAGATH, T. B. *Penn. Med. J.*, 1 (April 1941).
2. MCT. COWAN, I. *J. Wildlife Man.*, **12**, 105 (1948).
3. RILEY, W. A. *Minnesota Med.* **16**, 744 (1933).
4. HADWEN, S., and PALMER, L. J. *USDA Bull. 1089*, 1 (1922).

The Effect of Maleic Hydrazide on Certain Dehydrogenases in Tissues of Onion Plants[1]

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Maleic hydrazide in aqueous sprays applied to certain plants inhibits or reduces growth to an extent depending upon the concentration of the spray and the plant species involved. Tomato plants are very sensitive to this chemical. According to Schoene and Hoffman (*1*), the cupric and zinc salts of the acid at 2,000 ppm concentration stopped tomato growth for a period of 2 months, after which growth was resumed. Preliminary tests using the diethanolamine of maleic hydrazide on tomatoes confirmed the observations of Schoene and Hoffman concerning the retardation of growth. Onion bulbs have a much greater tolerance than tomato plants. Several spray applications with concentrations ranging up to 3,000 ppm did not cause necrotic spots on the leaves, and growth differences between the treated plants and the control plants were not visually apparent until 30 days after treatment. Plants treated with the chemical were not kept under observation long enough to ascertain if normal bulb formation would occur.

Since maleic and fumaric acids are isomeric, it was postulated that maleic acid might be interfering with the function of one or more enzymes in the respiration cycle. In order to test this hypothesis the activity of

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several dehydrogenases was determined in tissues of plants treated with various concentrations of maleic hydrazide, using a triphenyltetrazolium chloride technique.

Mattson, Jensen, and Dutcher (*2*) suggested that certain pyridine nucleotide dehydrogenases reduced triphenyltetrazolium chloride to the red water-insoluble formazan. Kun and Abood (*3*) reported that succinic dehydrogenase activity could be determined in rat tissue homogenate by a colorimetric measurement of the amount of formazan formed from the tetrazolium salt at pH 7.4. Mattson and Jensen (*4*) found that reducing sugars could be determined at higher alkalinity by means of this salt. The method used in this study was that of Kun and Abood (*3*), with certain modifications making it suitable for the determination of several dehydrogenases in plant tissue.

Determinations attempted on tomato tissue were not successful, since the chlorophyll present prevented accurate reading in the colorimeter, and a suitable solvent was not found to remove the chlorophyll without inactivating the dehydrogenases. For that reason onion tissues were used, since the bulb and the underground portions were free of chlorophyll. The bulbs (sets 1/2 in. in diameter) were planted in pots, and one lot grown in full sunlight in the greenhouse and another in complete darkness. When the shoots had attained a height of approximately 3 in. they were sprayed with maleic hydrazide solutions in concentrations equivalent to 0 (control), 1,000, 2,000, and 3,000 ppm of the free hydrazide for 3 successive days. The etiolated plants were tested at the end of 7 days, and the green-leaved plants at the end of 20 and 50 days after the first spray application. The entire plant of the etiolated onions was tested, but only the underground chlorophyll-free parts of the green plant were used. The samples were washed in distilled water to free them of soil, cut into small fragments, and ground into a homogeneous mass. Ten average onion bulbs yielded 12 ml of homogenate, to which 4 drops of distilled water was added to reduce slightly the plasticity of the mass.

The reagents used were: the diethanolamine of maleic hydrazide (30% maleic hydrazide) diluted to 1,000, 2,000, 3,000 ppm of the hydrazide; 0.2*M* monopotassium phosphate buffer (pH 7.4); 0.2*M* solutions of sodium succinate, sodium fumarate, sodium malate, and sodium pyruvate, all adjusted to pH 7.4; and 0.2*M* glucose and alcohol solutions. The dye was a freshly prepared 0.1% solution of triphenyltetrazolium chloride. Purified acetone was used as a solvent for the formazan produced.

Each reaction tube contained 0.5 ml of buffer, 0.5 ml of substrate, 1 ml of the tissue homogenate, and 1 ml of triphenyltetrazolium chloride reagent. By trial it was determined that volumetric aliquots of fresh tissue homogenate of both treated and control plants were of similar dry weight. The tubes were placed in a 38° C oven for a period of 20–24 hr, after which 7 ml of acetone was added to dissolve the formazan produced. The solution was filtered to obtain a clear tissue-free solution and read in a Coleman spectrophotometer at 490 mμ. A blank of normal onion tissue homogenate which had been heated to 82° C for 5 min produced no formazan. Likewise, 0.5-ml samples of 1,000, 2,000, 3,000 ppm of the maleic hydrazide solutions did not reduce the tetrazolium salt at pH 7.4. A standard curve was prepared using solutions of 100, 200, 300, and 400 μg of triphenylformazan in acetone.

TABLE 1

MICROGRAMS OF TRIPHENYLFORMAZAN PRODUCED BY DEHYDROGENASES IN ONE ML OF ONION HOMOGENATE IN 20 HR AT 38° C FROM PLANTS TREATED WITH 0, 1,000, 2,000, 3,000 PPM OF MALEIC HYDRAZIDE. TESTED 20 DAYS AND 50 DAYS AFTER FIRST APPLICATION

Substrate	Control	Maleic Hydrazide Concentration		
		1,000 ppm	2,000 ppm	3,000 ppm
	Triphenylformazan in μg			
20 days after first application				
Succinate	190	145	135	130
Fumarate	195	155	125	135
Malate	165	135	135	125
Pyruvate	130	90	90	100
50 days after first application				
Succinate	125	45	20	63
Fumarate	143	33	20	30
Malate	155	33	30	43
Pyruvate	73	30	30	35
Glucose	55	25	40	30
Ethyl alcohol	63	30	20	35

The results obtained from onions treated with maleic hydrazide and grown in full daylight are presented in Table 1, where the quantity of triphenylformazan produced by the several dehydrogenases is indicated. In all samples of onions treated with maleic hydrazide, the amounts of triphenylformazan produced by the dehydrogenases were apparently less than in the untreated samples.

At the time of the 50-day test the leaves of the treated onions were approximately 5 in. shorter than the leaves of the control plants; the roots of the former were about half as long as the roots of the latter, and appeared thicker and less fibrous. The bulbs of the treated plants appeared swollen and

TABLE 2

MICROGRAMS OF TRIPHENYLFORMAZAN PRODUCED BY DEHYDROGENASES IN ONE ML OF ETIOLATED ONION TISSUE HOMOGENATE IN 20 HR AT 38° C FROM PLANTS TREATED WITH 0, 1,000, 2,000, AND 3,000 PPM OF MALEIC HYDRAZIDE. TESTED 7 DAYS AFTER FIRST APPLICATION

Substrate	Control	Maleic Hydrazide Concentration		
		1,000 ppm	2,000 ppm	3,000 ppm
	Triphenylformazan in μg			
Succinate	180	185	10	60
Fumarate	135	165	35	115
Malate	157	5	10	15
Pyruvate	105	10	15	5
Glucose	15	0	0	20
Ethyl alcohol	0	20	15	0

flabby, with air spaces between fleshy leaf sheaths. The proportional reduction in the amount of triphenylformazan produced in the treated samples was more pronounced after 50 days than it was on the twentieth day after treatment.

Table 2 gives the results found with onions grown in the dark and tested on the seventh day after maleic hydrazide treatment. In general the dehydrogenases were less active in the etiolated plants than in the plants that received full sunlight. These plants grew rapidly, and probably the waxy coating on the epidermis was not so thick as that on the plants grown in full sunlight. This condition may have favored the entrance of the chemical into the plant tissue. The 3,000 ppm sprays of maleic hydrazide frequently had less effect on the dehydrogenases than the sprays of 2,000 ppm. This might be attributed to local toxic effects severe enough to retard hydrazide translocation. Morphological differences were not apparent between the treated and control plants.

The results indicate that maleic hydrazide sprayed upon the foliage of plants affects respiration through the partial inactivation or inhibition of one or more of the dehydrogenases. The rapidity of the effects of the chemical apparently are governed by the rate of its absorption into the plant.

References

1. SCHOENE, D. L., and HOFFMAN, O. L. *Science,* **109**, 588 (1949).
2. MATTSON, A. M., JENSEN, C. O., and DUTCHER, R. A. *Science,* **106**, 294 (1947).
3. KUN, E., and ABOOD, L. G. *Science,* **109**, 144 (1949).
4. MATTSON, A. M., and JENSEN, C. O. *Anal. Chem.,* **22**, 182 (1950).

The Effect of Desiccated Thyroid, Iodinated Casein, and Casein on a Rachitogenic Diet

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At Michigan State College a modified Steenbock rachitogenic ration is used for the assay of vitamin D milks. This ration is somewhat goitrogenic, as well as deficient in one of the essential amino acids—namely, lysine. Rats fed the Steenbock ration are found to have thyroid enlargements with hyperplasia. This is to be expected, since the diet on the average has a content of 15 γ iodine/g as determined by Levine, Remington, and von Kolnitz (*1*). The daily intake of iodine necessary to yield a concentration of 1% iodine in the thyroid (dry basis), which is essential to normal thyroid functioning, was found by the same workers (*2*) to be approximately 1γ/day. This same group found that the low-iodine goiter is produced when the iodine content in the gland is 0.03% or less (dry basis), and this corresponds probably to an iodine intake from known sources of 0.3 or 0.4γ/day. Therefore, inasmuch as lack of uniformity in the growth of animals might be due to variations in thyroid activity, it was considered likely that the use of desiccated thyroid or iodinated casein would influence the growth of rats fed the rachitogenic diet and result in more uniform growth. Because desiccated thyroid is expensive and its potency variable, iodinated casein, as prepared by Reineke (*3*), was also used, since it provided a comparatively inexpensive product of nonvariable high-thyroid activity.

With the knowledge of the amino acid requirements of the rat, Francis (*4*) tabulated the amount of the individual essential amino acids present in the Steenbock ration and found it to be deficient in lysine. The addition of 0.5% lysine effectively increased the growth rate in addition to reducing the incidence of respiratory infections. Therefore, a study was also made of the value of adding casein to increase the supply of lysine, one of the limiting amino acids.

Throughout the pre-rickets-producing period the rats were maintained on a diet that provided for normal development in all respects except that the supply of vitamin D was limited so that the rats would develop severe rickets in 21 days. For the rickets-producing period a rachitogenic ration consisting of table corn meal 72%, wheat gluten 20%, yeast 1%, and NaCl 1% was used, as well as the modifications indicated in Table 1.

TABLE 1

Ration No.	Modifications
I.....	Basal (the above ration)
II.....	1 kg basal + 5 gr desiccated thyroid*
III.....	1 kg basal + 10 " " "
IV.....	1 kg basal + 25 " " "
V.....	Basal + 1% iodinated casein
VI.....	Basal + 0.05% " "
VII.....	Basal + .025% " "
VIII.....	Basal + .0125% " "
IX.....	Basal + 0.025% iodinated casein – 5% casein
X.....	Basal + 5% casein

* ½ grain commercial desiccated thyroid tablets were used.

Young albino rats of the Michigan State College strain, weighing from 44 to 60 g and 21–25 days old, were assembled into groups of 2 or 3 and fed the rachitogenic diet or one of the above modifications for 21 days and water, *ad libitum*. They were then placed in separate cages 1 day prior to the beginning of the 10-day assay period, so as to adequately adjust themselves to their new surroundings. The animals received a supplement of homogenized vitamin D milk supplying 4 U.S.P. units of the vitamin. In each series some animals were included as negative controls. At the end of the assay period the animals were killed, and the distal ends of the radii and ulnae removed and cleansed of adhering tissue. The bones were then permitted to remain at least overnight in 95% ethanol; "line tests" were then run.

The criteria employed were growth during the experimental period, general appearance of the lines,