Reports and Letters

Determination of Magnesium in an Insect Virus

A semiquantitative emission spectrographic analysis (*1*) of *Bombyx mori* L. (silkworm) polyhedral bodies and of virus revealed iron and magnesium as the only metals present in appreciable quantities. A note on the iron content has been published (*2*). The quantitative determination of magnesium presented great difficulties, owing to the low magnesium concentration and the small quantities of virus available. The low concentration excluded the emission spectrographic method, because, with the available electrodes, it was not possible to burn several milligrams of protein and obtain reproducible results. Practically all the numerous colorimetric methods described in the literature, which are very well summarized by Mitchell (*3*), as well as several combinations of them, were tried. Combustion of the organic matter presented the greatest problem. Dry ashing in quartz tubes at temperatures ranging from 400° to 600°C did not yield reproducible results because of the formation of insoluble oxides and/or volatile products. Effective wet ashing requires high concentrations of acids, which results in high salt concentrations that interfere seriously with most colorimetric determinations. Nitric or hydrochloric acids, which can be evaporated after ashing, are unsuitable because of heavy losses during evaporation.

Table 1. Magnesium content of polyhedral bodies and polyhedral virus of *B. mori*. The titan yellow and thiazole yellow methods were used.

Method	Wt. (mg)	Mg (μg)	Mg (μg/mg)	Mean Mg (%)
Polyhedral bodies				
Titan	2.70	2.1	0.78	
Titan	4.40	3.6	0.82	
Titan	7.50	6.5	0.87	
Titan	11.95	10.2	0.85	
Thiazole	2.41	2.10	0.87	
Thiazole	2.64	2.00	0.76	
Thiazole	4.19	3.65	0.87	
Thiazole	5.54	4.41	0.80	
Thiazole	2.50	2.00	0.80	
Thiazole	3.39	2.95	0.87	
Thiazole	3.08	2.65	0.86	0.083
Polyhedral virus				
Thiazole	2.60	0.90	0.35	
Thiazole	4.41	1.35	0.31	0.033

However, the titan yellow method as described by Orange and Rhein (*4*) combined with that of Young and Gill (*5*) using ghatti gum as color stabilizer is useful for the determination of small quantities of magnesium in proteins. Yet this method is unsuitable for virus samples because of interference by nucleic acid and other materials present. Intensive investigations trying numerous combinations (*6*) resulted finally in a greatly simplified yet sensitive procedure that is particularly useful for determination of magnesium in comparatively pure nucleoproteins and viruses. It is based on the well-known thiazole yellow method, follows partly the procedure of Young and Gill (*5*), and uses glycerol as color stabilizer as recommended by Hanssen *et al.* (*7*). The use of "compensating solutions" against interfering ions was found to be unnecessary since the samples were comparatively pure. Various other "color stabilizers" increased the blank readings and were found to be unreliable and unnecessary for quantities up to about 10 μg of magnesium when glycerol was used.

Samples up to 10 mg, dry or in solution, were placed in quartz digestion tubes, and 0.25 ml of concentrated H_2SO_4 was added to each. Blanks and standards were prepared similarly and all samples were dried for about 8 hr at 150°C in a drying oven. They were then ashed at 210°C (silicone bath) for 8 hr; 1 drop of H_2O_2 (30-percent) was added every hour except the last. After cooling, 3.25 ml of distilled water and 1.5 ml of glycerol-water (1:1) were added, and the samples were well mixed. Next 0.15 ml of 0.02-percent thiazole yellow (*8*), prepared freshly daily was added; immediately after, 1.0 ml of 10*N* NaOH (carbonate-free) was added; and the two were well mixed. The samples were allowed to stand for 25 minutes and were then read in a spectrophotometer at a wavelength of 510 mμ and a light path of 5 cm. With the Beckman instrument, the blanks had an optical density of 0.450, and the standards had an optical density of approximately 0.050 per microgram of magnesium. A standard solution containing 5 μg of magnesium per milliliter was prepared by dissolving 51.25 mg of $MgSO_4 \cdot 7H_2O$ in 1 lit. It is most important that all glass and quartz ware be carefully cleaned with chromic acid and well rinsed. Although reproducibility of the method is about ± 5 percent, it is advisable to run standards with the samples.

The polyhedral bodies, polyhedral proteins, and polyhedral virus were purified and prepared as described previously (*9*). The polyhedral protein was dialyzed against a total of 40 lit of distilled water with four changes for several days in the cold room, and the virus was washed four times by high-speed centrifugation. The results of the magnesium determination summarized in Table 1 show that *B. mori* polyhedral bodies contain about 0.083 percent magnesium and that the virus contains about 0.033 percent magnesium. Ten samples of purified polyhedral protein weighing between 3 and 9 mg each yielded no magnesium. This indicates that practically all the magnesium found in the polyhedral bodies is either adsorbed or loosely bound by a salt linkage that is split during the dissolution of the polyhedral bodies by weak alkali (0.006*M* Na_2CO_3). The latter possibility is more likely, since a major part of the phosphorus present in the polyhedral bodies is also loosely bound and dialyzable. It is possible that the magnesium and phosphorus are essential for crystallization of the polyhedral protein.

It is impossible to dialyze the virus particles effectively without destroying their structure and infectivity. However, four washings with 20 ml of distilled water would be expected to remove most of the magnesium adsorbed on the virus surface. It is felt, therefore, that the magnesium found is a normal constituent of the virus. From the amount of magnesium and a virus particle weight of 45.6×10^{-17} g, one can calculate that each virus particle contains about 3500 atoms of magnesium, which could be of biochemical significance.

C. F. Holoway*
Department of Chemistry, Michigan College of Mining and Technology, Sault Ste. Marie

G. H. Bergold
Laboratory of Insect Pathology, Sault Ste. Marie, Ontario

References and Notes

1. Contribution No. 235 of the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Ont. We wish to express our appreciation to J. MacNamara and E. Herbert of the Algoma Steel Corporation, Ltd., Sault Ste. Marie, Ont., for the spectrographic results. We also wish to thank Lorna Callahan for her technical assistance.
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* Present address: George Williams College, Drexel at 53 St., Chicago, Ill.

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Induction of Flowering in Pineapple by Beta-Hydroxyethylhydrazine

Some years ago, Rodriguez demonstrated that ethylene could induce pineapple plants to flower (*1*). Subsequently, it was found that acetylene could also "force" differentiation of flower buds in the pineapple plant (*2*). With these exceptions, the chemical materials reported to induce flowering in the pineapple have had the chemical structure characteristic of plant growth regulators and have given positive results in the various tests for such properties (stimulation of cell elongation, initiation of roots, and so forth). Such flower-inducing materials as 2,4-dichlorophenoxyacetic acid, 1-naphthaleneacetic acid, and 2-naphthoxyacetic acid have a ring system nucleus, a double bond in the ring, a side chain containing a carboxyl group (or structure readily converted to it), and, presumably, the necessary spatial relationship between the ring and the carboxyl group (*3*). They are also active in the Went split-pea-stem test (*4*).

Beta-hydroxyethylhydrazine, H_2NN-HCH_2CH_2OH, gave no activity in the split-pea-stem test or in the *Avena* test and has none of the structures associated with plant-growth-regulator activity. However, it has induced early flowering in two tests at different seasons in the pineapple, *Ananas comosus,* variety smooth Cayenne (*5*). The chemical was applied in water by sprinkling can to 20 plants at each treatment rate in each test. Plants of test 1 were planted in May 1953; they were treated 18 May 1954, and the buds were counted 23 July 1954. Plants of test 2 were planted in October 1953; they were treated 15 Sptember 1954, and the buds were counted 15 November 1954 (Table 1).

Table 1. Induction of flowering in the pineapple with beta-hydroxyethylhydrazine.

Material	Concn. (%)	Plants with flower buds (No.)
Test 1		
Beta-hydroxy-ethylhydrazine	0.001	0
	0.01	0
	0.06	18
	0.12	20
Untreated control		1
Test 2		
Beta-hydroxy-ethylhydrazine	0.01	0
	0.06	1
	0.12	13
	0.23	18
Untreated control		0

It would be of interest to determine the effect of this chemical on long- or short-day plants undergoing inductive periods to ascertain whether it has the properties of an auxin antagonist.

DONALD P. GOWING
ROBERT W. LEEPER
Pineapple Research Institute of Hawaii, Honolulu

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5. We wish to express our appreciation to the Olin Mathieson Chemical Corporation for providing the beta-hydroxyethylhydrazine and to Martha J. Kent for the split-pea-stem and *Avena* assays. This report is published with the approval of the director of the Pineapple Research Institute of Hawaii as technical paper No. 236.

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Effect of Stress on an Extinguished Fear Response

Gellhorn (*1*) has reported a series of studies that were made to determine whether convulsions induced by Metrazol, electroshock, or insulin hypoglycemia would effect the acquisition and/or extinction of a simple conditioned avoidance response. By means of a two-compartment box, a barrier, and shock, rats were taught a jumping response that enabled them to escape shock in one of the compartments. A bell was generally used as the conditioning stimulus, although in some cases a light was used. A number of Gellhorn's findings are pertinent to the present study. Gellhorn found that a conditioned avoidance response, extinguished by lack of reinforcement, could be reinstated following three to five convulsions that were induced by electroshock or Metrazol. Reinstatement lasted 5 to 10 days, after which a gradual decrease in the frequency of the response occurred. Gellhorn reported that with the use of insulin, only in the case of production of a coma state was the extinguished avoidance response reinstated. In this case, the recovered response lasted as long as several months without reinforcement. Evidence was presented that was interpreted as indicating that the coma state was the essential condition for reinstatement of the avoidance response.

The present study (*2*) reports the effect of stress induced by treadmill running on an extinguished conditioned fear response.

A modified Miller box (*3*) was used for the acquisition of a conditioned fear response. This apparatus consisted of a rectangular box, the over-all dimensions of which were 28 by 7½ by 11½ inches, that was divided into two equal chambers by a partition containing a manually operated vertical sliding door. One compartment, painted white, contained a charged grid floor and a lead weight that was suspended by a wire to the left of the intercompartment door. The second compartment was painted black; it contained no suspended weight and its grid floor was uncharged.

By means of standard conditioning procedure, ten male inbred albino rats were taught to escape shock in the white compartment by hitting the suspended weight with their forepaws. On performance of this act, the experimenter opened the sliding door and allowed the subject to escape to the black compartment. All animals were conditioned to hit the weight and escape to the black compartment immediately on placement in the white compartment, in the absence of shock.

After this habit had been acquired, the response was extinguished by lack of reinforcement. The subjects were then exposed to treadmill (*4*) running, with noninjurious shock as a motivator, for a 5-minute period daily for 1 week. Subsequently, the animals were replaced in the conditioning apparatus. Ten matched male albino rats used as controls were not subjected to the treadmill running.

We have previously reported (*5*) that some rats that were subjected to treadmill running became so highly agitated that they exhibited full pattern convulsions, including tonic, clonic, and comatose stages. In the present study, four of the ten experimental animals showed complete pattern fits on each exposure to the treadmill. The remaining six rats, although they were agitated, did not show seizures. It was found that after exposure to the treadmill running the extinguished fear response was reinstated in all experimental subjects. After a period of 1 week, the fear response of the six animals not showing the convulsions gradually disappeared; the fear response was not evidenced in any of these subjects 14 days following the treadmill experience. In the case of animals showing full pattern fits, the reinstated fear response continued to be manifested with regularity, and in the absence of reinforcement, for a period of 3 weeks following treadmill experience.