

capsules were all empty, but those only partly expanded had the full complement of 6–8 seeds attached to the two parietal placentae. Some of these plants were brought into the laboratory and placed in shallow water. After 24 hr, water drops falling from 3 ft caused seeds to be thrown horizontally up to 16 in. Some seeds had not completely abscised, the plants were less turgid than in the field, and some difficulty was experienced in keeping the capsules as strictly upright as they are when growing naturally; otherwise it is probable that seeds would have been ejected for greater distances. *Chrysosplenium* should be easily grown in the greenhouse and would serve well to demonstrate the mechanism to students.

Chrysosplenium is well suited to this dispersal mechanism since it grows frequently in the splash from waterfalls, below moist cliffs, or in swampy woods where even a drizzling rain will fall from the trees in large drops.

On June 18 it was noticed that all capsules of *Mitella diphylla* L. that were approaching maturity had become vertically oriented even on a plant whose stem had been bent to the horizontal. Changed orientation of capsules on plants brought into the laboratory indicated that the vertical disposition is due to a phototropic response. Although the mature capsule of *M. diphylla* is somewhat flattened, rather than circular, in plan view, it has the form of a deep cup with a widely flaring lip and appears well adapted for splash dispersal of the seeds, which are held in clusters at the two ends of the cup. The seeds were all firmly attached at this time and no test could be made. It was not possible to visit colonies of this plant again until July 4. Heavy rain had fallen most of the preceding night, and the majority of capsules were empty. Plants with some immature capsules were collected and kept in water for 48 hr. The capsules were then subjected to water drops falling from distances of 3 ft 4 in. to 3 ft 8 in., depending upon the position of the capsules on the stalk. Although some capsules shriveled without the seeds abscising, seeds were ejected from several of them, to distances of 4–29.5 in., the average distance being 11.6 in. With a free fall from the canopy of the mature deciduous woods in which *M. diphylla* commonly grows the seeds are presumably thrown appreciably further.

In the writer's experience the capsules of *Mitella nuda* always become erect at maturity, and this fact is borne out by examination of herbarium specimens ranging from Alberta to Nova Scotia. Less abundant material of *M. breweri*, *M. ovalis*, and *M. pentandra* suggests that the same is true of these species. The mature capsules of all these species have much the same form as those of *M. diphylla*, and it is probable that they function similarly.

Examination of available herbarium material indicates that in some species of *Heuchera* (e.g., *H. americana*) the mature capsules are more or less erect, whereas in others (e.g., *H. glabra*) they are not. In all species the capsules open apically but with a con-

stricted throat, resulting in a deep vessel that probably functions as a very inefficient splash cup even when it is suitably oriented. It is probable that in the Saxifragaceae the splash mechanism originated in *Heuchera*. In the related *Tolmiea menziesii* the capsules are more or less erect but are more poorly shaped than those of most *Heuchera* spp. In *Saxifraga* the mature capsules are generally erect and form a somewhat better cup with less constriction at the throat, but too broad at the base for high efficiency. From *Heuchera* or *Tolmiea* there appear to have been two evolutionary lines: one leading to *Saxifraga* and related genera with axile placentation, but poorly developed splash cups; and one, perhaps through *Tellima*, to *Mitella* with parietal placentation, but efficient splash cups. *Chrysosplenium*, also with parietal placentation, has perhaps been derived from the latter line, but it is so reduced and specialized morphologically that its affinities are obscure.

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Manuscript received August 4, 1952.

Iron Content of an Insect Virus¹

C. F. Holoway and G. H. Bergold

Department of Chemistry, Sault Branch,
Michigan College of Mining and Technology,
Sault Ste. Marie, Michigan, and
Laboratory of Insect Pathology,
Sault Ste. Marie, Ontario, Canada

Glaser and Chapman (1) reported that polyhedral bodies of *Bombyx mori* L. (silkworm) contained .496% iron, at a time when the nature of the polyhedral bodies and their relation to the virus itself were unknown. Bergold (2) first isolated and demonstrated the virus particles and showed that the polyhedral bodies of *B. mori* consist of about 95% non-infectious, homogeneous polyhedral protein with a molecular weight of about 378,000, and about 5% infectious virus material. In view of these findings it seemed of interest to investigate the distribution of iron in the polyhedral bodies.

The quantitative determination of iron was difficult since only small quantities (1–3) mg of virus were available. Comparison of the numerous methods described in the literature was undertaken, and finally the o-phenanthroline method of Saywell and Cunningham (3) and a wet-ashing procedure² were adopted. The following procedure was developed: To samples (up to 20 mg) containing 0.1–1.5 µg Fe, 2 ml of 9 N H₂SO₄ is added and dried for 5 hr at about 150° C. They are incinerated at 210° C for 3 hr, or until colorless (up to 7 hr), adding a total of 4–10 drops of

¹ Contribution No. 75, Division of Forest Biology, Science Service, Department of Agriculture, Ottawa, Canada.

² A procedure for dry-ashing will be described by the senior author at a later date.

30% H₂O₂, depending on the sample size. After cooling, 4.7 ml of triple-distilled water, 0.4 ml 10% NH₂OH·HCl, 0.4 ml *o*-phenanthroline, and 6.0 ml 5 *N* NH₄OH are added, resulting in a total volume of 12.0 ml, pH about 10. After 30 min the sample is cleared by high-speed centrifugation, and the absorption is measured in a photoelectric spectrophotometer equipped with a blue filter (4) and using cells with 5 cm light path. Amounts of 0.1–1.5 µg Fe can be determined with an accuracy of about ± 0.02 µg. Special care is necessary to avoid contamination.

The isolation and purification of the virus and polyhedral protein were carried out as described previously (2, 5). Samples of polyhedral protein and virus yielded the same results when washed repeatedly or dialyzed. Results are summarized in Table 1. Parallel

TABLE 1
IRON CONTENT OF POLYHEDRAL BODIES, POLYHEDRAL
PROTEIN AND VIRUS OF *B. mori*

	Sample size (mg)	µgm Fe	µg Fe/mg	Mean % Fe
Polyhedral bodies	17.5	0.89	0.051	0.005
	19.4	1.11	.057	
	8.2	0.41	.050	
	19.9	0.86	.043	
	18.4	1.01	.055	
	13.9	0.70	.050	
	17.4	.82	.047	
Polyhedral protein	8.0	.45	.056	.005
	13.5	.89	.066	
	16.1	.96	.060	
	8.6	.42	.049	
	4.8	.28	.056	
	9.7	.53	.053	
	10.9	.59	.059	
Virus	7.9	.45	.057	.015
	2.4	.41	.172	
	1.2	.22	.185	
	1.2	.17	.143	
	2.4	.29	.122	
	3.3	0.52	0.157	

analyses by emission spectrography³ were in good agreement. From Table 1 it can be seen that highly purified polyhedral bodies of *B. mori* contain only .005% iron instead of .496%, as reported by Glaser. It is of interest to note that the iron content of the virus is about three times as great as that of the surrounding polyhedral protein. The interpretation of the function of such a small amount of iron is probably not advisable. However, .015% still represents about 750 atoms of Fe per virus particle (particle weight 45.6 × 10⁻¹⁷ g), which would be sufficient for a cytochrome system. Since insect viruses are considered to be organisms (6), the iron content might be of some biological significance.

So far as is known, iron has never previously been reported as a constituent of any purified virus.

³ The authors wish to express their appreciation to Dr. MacNamara and E. Herbert, of the Algoma Steel Corporation, Ltd., Sault Ste. Marie, Ontario, for the spectrographic results.

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Manuscript received December 23, 1952.

Syntheses of Methylamine-C¹⁴ and Diazomethane-C¹⁴¹

A. Russell Jones and Walter J. Skraba

*Chemistry Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee*

Easily performed procedures for the preparation of the useful synthetic intermediates methylamine-C¹⁴ and diazomethane-C¹⁴ in yields, respectively, of 98% and 54% have been used with excellent results in these laboratories over the past two years.

Potassium cyanide-C¹⁴ was prepared by a modification of the Loftfield (1) procedure which utilizes the reaction of carbon dioxide and ammonia with a potassium mirror at 630° C. The sealed tubes were made of quartz, which eliminated the strains to which Pyrex is subject. No tube failure has occurred since this change in over a hundred experiments. The formation of the potassium mirror was accomplished simply by warming the lower end of the evacuated tube containing the potassium with a small burner while blowing a stream of cooled air on the upper walls of the tube. The nozzle of the air tube was directed by hand so as to cause condensation of the potassium progressively downward from the top.

The cyanide was readily reduced at atmospheric pressure by hydrogen and platinum oxide in dilute sulfuric acid solution. A 98% yield of methylamine-C¹⁴ hydrochloride was obtained by making the hydrogenation solution basic with potassium hydroxide and distilling the volatile base into a receiver containing hydrochloric acid.

(Methyl-C¹⁴)-urea was quantitatively obtained by heating the distillate (vol reduced to 5 ml) for 30 min with a slight excess of potassium cyanate in a boiling water bath. The procedure of Arndt and Amende (2) was more easily adapted to the small-scale preparation required than the methods for the preparation of diazomethane precursors involving the reactions of methylamine hydrochloride with urea (3) and of methylamine with mesityl oxide (4). The purified product was eluted from the dried reaction residue with boiling anhydrous methanol.

N-nitroso-N-(methyl-C¹⁴)-urea was obtained by the usual procedure, although with inverse addition of the reagents. It was filtered and freed of inorganic material by elution with anhydrous methanol at room

¹ This document is based upon work performed under Contract Number W-7405-eng-26 for the Atomic Energy Commission at the Oak Ridge National Laboratory.