30% H_2O_2, depending on the sample size. After cooling, 4.7 ml of triple-distilled water, 0.4 ml 10% $NH_2OH \cdot HCl$, 0.4 ml *o*-phenanthroline, and 6.0 ml 5 *N* NH_4OH 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 analyses by emission spectrography[3] 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.

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	
	7.9	.45	.057	
Virus	2.4	.41	.172	.015
	1.2	.22	.185	
	1.2	.17	.143	
	2.4	.29	.122	
	3.3	0.52	0.157	

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

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Syntheses of Methylamine-C^{14} and Diazomethane-C^{14} [1]

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Easily performed procedures for the preparation of the useful synthetic intermediates methylamine-C^{14} and diazomethane-C^{14} in yields, respectively, of 98% and 54% have been used with excellent results in these laboratories over the past two years.

Potassium cyanide-C^{14} 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-C^{14} hydrochloride was obtained by making the hydrogenation solution basic with potassium hydroxide and distilling the volatile base into a receiver containing hydrochloric acid.

(Methyl-C^{14})-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-C^{14})-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

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temperature. The fluffy solid produced by evaporation of the eluate was obtained in 77% yield from sodium cyanide-C^{14} (radiochemical yield, 76%).

Diazomethane was prepared by treating an ether suspension of the nitrosomethylurea with 50% potassium hydroxide for a short time and then codistilling the ether and product into dry-ice cooled traps containing reactants. The yield was 54% from sodium cyanide-C^{14} as determined by radiochemical analysis of methyl-C^{14} 3,5-dinitro-2-methyl benzoate (chemical yield, 60%). The radiochemical analyses were accomplished by oxidation of aliquots of the radioactive substance to carbon-C^{14} dioxide, the activity of which was determined by means of a vibrating Reed electrometer (*5*).

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Removal of Thiamin and Riboflavin from Milk for Dietary Use[1]

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In planning palatable experimental diets low in riboflavin and thiamin it is difficult to include adequate amounts of protein, calcium, and phosphorus from natural food sources. Recommended diets for children and adolescents, in particular, ordinarily contain large amounts of milk with its accompanying B vitamins.

Moody, Hauge, and Lundquist (*1*) have reported treatment of milk with florisil[2] in order to obtain a low-riboflavin milk for calf feeding. The same principle was used in this laboratory for removal of most of the riboflavin and thiamin from whole milk. The method uses florisil as an alternative to procedures involving irradiation of dry or whole milk (*1, 3, 4*). Main advantages of the florisil method are (1) treatment is rapid, (2) removal of thiamin and riboflavin is fairly complete, (3) there are no irradiation products, and (4) the taste of the milk is unaltered.

Two cotton gauze milk filter pads, such as are used by milk producers, are placed in a 6-in. Buchner funnel and sealed with water. Three hundred g 60/100 mesh florisil suspended in 2% acetic acid is poured into the funnel and allowed to drain (without suction). The florisil is washed three times by filling the funnel with water. This washing serves to remove both the excess acid and the florisil dust which cannot be retained by the filter pads.

Three gallons of whole, homogenized milk is then delivered from a separatory funnel into the Buchner at the rate of filtration. A small circle of glass should be laid on the florisil below the end of the separatory funnel to avoid formation of a hole in the florisil. Collection is begun as soon as all the water appears to have been expelled.

Filtration of 3 gal milk without suction requires 90 min; 87–91% of the riboflavin and 95–100% of the thiamin are removed. Removal of calcium, phosphorus, and protein was found to be negligible. Adsorption in absolute amounts of these substances may well be as great as or greater than that of riboflavin and thiamin, but, as the calcium, phosphorus, and protein concentrations in milk are of a much greater magnitude, the fraction of the whole which is removed is not enough markedly to impair the value of the treated milk as a source of these nutrients. Only the thiamin, riboflavin, calcium, phosphorus, and nitrogen contents of the treated milk were investigated. The possibility that certain of the other vitamins present are also removed to greater or less extent is recognized. The treated milk should not be assumed to be a source of any nutrient until this assumption is justified by analytical results.

Either aseptic procedures should be used throughout, or the treatment of the milk should be followed by pasteurization.

Because of the cost of florisil, it may be desirable to recover and reuse it. After the florisil has been washed free of milk, it is washed with 20% pyridine in 2% acetic acid, in order to elute the adsorbed vitamins. The pyridine solution is washed away with water, and the florisil is then heated overnight at 600° C (*5*). It is not necessary to remove the pyridine exhaustively by washing. In the course of the high-temperature treatment, the florisil will be freed of all pyridine and of any other residual materials that can be distilled or carbonized. Adsorptive power of the reclaimed florisil was found not to differ from that of the unused product. It has not been determined whether the florisil loses its adsorptive properties after being repeatedly reused and reactivated.

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[2] Florisil is a synthetic magnesium silicate obtainable from the Floridin Company, Warren, Pa. Particles which might conceivably remain in the treated milk would not make the milk unfit for human consumption. This compound and other fuller's earth–type materials are used for purification of pharmaceuticals and medicinally as antiacids and for treatment of peptic ulcers, etc. (*2*).