IN THE LABORATORY

The Assay of New Rich Natural Sources of Ascorbic Acid

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Plant materials which are reported as containing very high concentrations of ascorbic acid include the hips of wild Alberta roses, *Rosa* sp. (1,300–3,500 mg./100 grams of flesh) (*5*); hips of a cultivated rose, *Rosa laxa* (3,000–5,000 mg./100 grams of flesh) (*5, 6*); green English walnuts, *Juglans regia* (2,000–3,000 mg./100 grams) (*8*); a gooseberry found in India and West China, *Phyllanthus Emblica* (920 mg./100 ml. juice) (*2*); and recently there has been reported the finding of 1,000–3,300 mg. of ascorbic acid/100 grams of edible matter in the West Indian cherry, *Malpighia punicifolia* (*1*).

It is well recognized that there exist in some fresh plant materials, notably green walnuts, certain non-vitamin C substances that reduce the dye, 2:6-dichlorophenolindophenol, on titration with which most published values for ascorbic acid in food materials depend. Means for differentiating true vitamin C from such non-vitamin C reductants are also well established (*3, 4, 9*).

We have shown, in the case of rose hips that non-vitamin C reductants, if present at all, are negligible in amount and that the dye titration provides an accurate index of their true ascorbic acid content (*6*). For English walnuts, which, through the kindness of J. R. van Haarlem, of the Horticultural Experiment Station, Vineland, Ontario, we had the opportunity of examining, the case is quite different, as our results in Table 1 show. The values shown for non-vitamin C reductants are based upon the method of Levy (*3*).

TABLE 1

VITAMIN C AND NON-VITAMIN C DYE REDUCTANTS IN GREEN WALNUTS (*Juglans regia*)

Tissue assayed	Weight in grams	Total dye reduction (mg. vitamin C/100 grams tissue)	True ascorbic acid (mg./100 grams tissue)	Non-vitamin C reductants (mg./100 grams tissue)	Non-vitamin C reductants (% of total dye reductants)
Whole nut.....	8.5	1,622	1,232	390	24
Whole nut.....	4.2	1,027	638	389	38
Mesocarp.....	6.1	2,114	1,830	284	13
Epicarp.....	6.7	1,312	576	736	56
Mesocarp.....	4.3	1,055	942	113	11
Mesocarp*.....	1.37	2,065	1,931	134	7
Epicarp*.....	2.13	956	578	378	39

* Tissues from the same nut.

Our assays show that the mesocarp contains more ascorbic acid than the epicarp and has a smaller proportion of non-vitamin C reductants. These findings are in accordance with the results of Wokes, *et al.* (*8*). It may also be observed that the percentage of non-vitamin C reductants varies from approximately 5 to 60 per cent of the total titration in the two tissues, and up to nearly 40 per cent in the whole green nut. The highest concentration of vitamin C in the mesocarp is nearly 2,000 mg./100 grams.

The work of Wokes, *et al.* (*7*) would suggest that the proportion of non-vitamin C reductants present in green walnuts decreases with increased maturity.

In view of such circumstances it would seem that values depending solely on the dye titration, reported for ascorbic acid in new food materials, should be accepted with much reservation.

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Autolyzed Brain Tissue as a Means of Facilitating Transmission of Experimental Poliomyelitis[1]

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Most attempts to infect various animals with poliomyelitis monkey-passage strains or infected human tissues failed until Armstrong (*1*) was able to adapt the Lansing strain from monkeys to cotton rats and from the latter to white mice. Since then many unsuccessful attempts to establish other monkey-passage strains in various rodents have been made, although the spreading factor of Duran-Reynals (*2*) and a variety of technics, such as rapid passage, brain trauma, hyperpyrexia, chilling, and use of immature animals, have been employed (*5*). Successful adaptation of a few other monkey-passage strains in cotton rats has been reported by Toomey and Takacs (*8*) and Jungeblut and Sanders (*4*).

In harvesting brains and cords from CFW (Carworth) Swiss mice paralyzed following intracerebral injection with the Lansing strain of poliomyelitis virus for the preparation of stock virus suspensions, we observed that autolyzed brain tissue removed from mice left in their cages for 7 or 8 hours after death appeared to accelerate the incubation period of

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the disease in passage mice. The autolyzed brain tissue suspensions were bacteriologically sterile. Preliminary experiments showed that the same effect could be produced by mixing sterile, autolyzed brain tissue from normal mice with stock virus suspensions. Mice and cotton rats inoculated with autolyzed, normal brain tissue alone remained normal.

For the present studies the autolyzed brain tissues were obtained from CFW normal mice that were killed by trauma and kept at room temperature for about 16 hours. Ether should not be used for killing because it seems to interfere with autolysis. A 10 per cent suspension by weight of autolyzed brain in nutrient broth (Baltimore Biological Company, pH 6.8) was then prepared by grinding in a sterile mortar with alundum and filtered through sterile gauze. The pH of the autolyzed brain suspensions ranged from 6.9 to 7.1. The suspensions were used immediately after preparation or stored overnight in the refrigerator. All autolyzed brain tissues used were bacteriologically sterile. Adjustment of the autolyzed brain suspension to pH 4.0, using the buffer solution recommended by Hammon (*3*), did not alter its effect. The virus suspensions were prepared from the pooled brains and cords of mice paralyzed 2–5 days after inoculation in the same manner as described in a previous publication (*6*).

Combined results of 8 to 10 different experiments with Lansing poliomyelitis virus comparing the incubation period and the LD_{50} titer for CFW Swiss mice, using autolyzed brain suspension, buffered saline pH 4.0, and normal mouse serum diluents mixed with equal parts of 10 per cent virus suspension, are shown in Table 1. Groups of 8 or more mice were em-

TABLE 1

Material inoculated	No. of mice	LD_{50} titer of virus	Range of incubation period (days)*	Incubation time (days) to produce 50% mortality
Lansing virus + autolyzed brain	154	1×10^{-5}	1–9	3
Lansing virus + buffered saline pH 4.0	76	1×10^{-4}	2–18	7
Lansing virus + normal mouse serum	48	0.5×10^{-3}	3–20	8.5
Autolyzed brain control	48	0	0	0

* Number of days between inoculation and onset of paralysis.

ployed in each experiment. Control animals inoculated with autolyzed brain alone remained normal, and sections of their brains and cords were negative.

Five hamsters were inoculated intracerebrally (0.05 cc.) with 10 per cent Lansing strain in autolyzed brain diluent. Two became paralyzed after 24 hours, a third on the 2nd day, while the remaining animals were paralyzed on the 3rd and 15th days, respectively. In each instance the virus was successfully transferred to 8 mice and was neutralized by human poliomyelitis convalescent serum known to contain protective antibodies against the Lansing strain. We failed to produce infection in hamsters inoculated repeatedly with mouse-passage Lansing strain suspended in buffered saline pH 4.0 or normal mouse serum, although successful transmission to this species has been reported previously (*7*).

Preliminary results in rhesus monkeys inoculated intracerebrally with a 10 per cent suspension of the BK, Leon, and McK[2] strains of monkey-passage virus and the Lansing mouse-passage strain suspended in autolyzed brain diluent have shown a fulminating infection with rapidly progressing flaccid paralysis and a very short incubation period. Histopathologic studies of the sections of brains and cords of these monkeys showed lesions typical of severe poliomyelitis.

Transfer of monkey-passage Leon strain to CFW Swiss mice. Ten per cent infected cord suspension from the 12th monkey-passage Leon strain[3] of poliomyelitis virus mixed with equal parts of 10 per cent autolyzed brain suspension was inoculated intracerebrally into each of 8 mice. On the 15th day one mouse developed paralysis of both front and hind legs, and the rest remained well. This animal was sacrificed, and its brain and cord suspension mixed with autolyzed brain was passaged to 8 additional mice. Two mice developed complete paralysis on the 13th and 21st day after inoculation, respectively, while the rest remained well. All of 8 mice inoculated with the third-passage virus mixed with autolyzed brain developed paralysis of one or more limbs in from 8 to 32 days. A cotton rat inoculated intracerebrally with the third-passage virus developed flaccid hind leg paralysis on the 17th day. The fourth passage was also made with autolyzed brain diluent, the 8 inoculated mice developing paralysis in from 4 to 17 days. Sections made of the brains and cords of fourth-passage mice showed lesions typical of severe poliomyelitis. The fifth passage was made with virus suspended in buffered saline pH 4.0 and inoculated intracerebrally into 8 mice, a cotton rat, and a rhesus monkey. The mice all developed paralysis in 2 to 9 days. The cotton rat showed paralysis of both fore limbs on the 8th day. The rhesus monkey had a temperature of 104.5°F. on the 5th day, paraplegia of the arms, and a right facial paralysis. On the following day this animal developed a complete quadriplegia and was sacrificed. Lesions typical of severe poliomyelitis were seen in sections of the brain and cord. Virus obtained from this monkey was passaged to a second monkey and 8 mice with positive results. The sixth-passage virus was completely neutralized by 1/100 dilution of human immune serum globulin, while control mice developed paralysis.

In cross-immunity tests mice immunized with three weekly intraperitoneal injections (0.25 cc. each) of 10 per cent active Lansing and Leon mouse-adapted strains and challenged intracerebrally on the 21st day with 10^{-2} dilution of virus were immune to the homologous strain. Lansing-immunized mice were immune to challenge with the Leon strain, but Leon-immunized mice showed no immunity to the Lansing strain.

There is always a hazard that a latent mouse neurotropic virus will be uncovered in attempting to adapt a monkey-passage strain to mice. The subsequent successful transfer to monkey of the fifth mouse passage of the Leon strain and also neutralization in high dilution with human immune serum globulin rules out the possibility of accidental contamination by a recognized spontaneous neurotropic virus in our stock mice. Furthermore, Leon-immunized mice were not immune to inoculation with Theiler's mouse encephalomyelitis virus (FA strain).

Attempts to transfer virus from other monkey-passage

[2] The BK, Leon, and McK strains were obtained through the courtesy of Dr. John F. Kessel.

[3] Isolated by Dr. John F. Kessel from human autopsy in 1937.

strains and human cases of poliomyelitis to white mice and cotton rats, using autolyzed brain diluent, are now in progress.

Summary. Autolyzed brain tissue diluent shortens the incubation period and facilitates the transfer of poliomyelitis virus to CFW Swiss mice, hamsters, and rhesus monkeys. The Leon monkey-passage strain of poliomyelitis virus was successfully adapted to CFW Swiss mice by means of this technic.

Since this manuscript was submitted we have isolated several strains of poliomyelitis virus from infected human feces and spinal cord in CFW Swiss mice by means of this technic.

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Semiquantitative Determination of Traces of Uranium: A Fluorophotometric Method for Field Use

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The method described below was devised in an attempt to meet a need for a lightweight portable apparatus for determining traces of uranium. Restrictions of portability were such as to eliminate most chemical and physical methods from consideration. The procedure adopted is an adaptation of that employed by Hernegger and Karlik (*1*) and by Hoffmann (*2*), who succeeded in determining quantities of uranium of the order of 1×10^{-4} μg.

The method used by the above-mentioned workers involves spectrophotometric measurement of the brightness of the fluorescence of a sodium fluoride bead containing traces of uranium, presumed to be in solid solution. A successful adaptation of this technique for use in a portable field kit was achieved by substituting a cast disc of more fusible material for the sodium fluoride bead and by employing a simple visual comparator. Certain features of two fluorophotometers constructed and a brief summary of the results obtained appear to be of sufficient interest to warrant publication.

The first fluorophotometer was constructed for laboratory use as follows: A General Electric AH-8 mercury lamp, powered by a constant-voltage transformer, was employed as a source of near-ultraviolet radiation. An aspheric condensing lens of optical glass was used to focus an enlarged image of the lamp on the fluorescent object. Next to the lens were placed glass filters to isolate the 365-mμ line. Corning Glass Works filters Nos. 585 and 986 were found to be satisfactory by spectrographic tests. Light of lower wave length (254 mμ) was found to excite fluorescence other than that due to the uranium-alkali-fluoride system and would also require use of optical materials other than glass. The fluorescent disc, a blank, and a reference disc of canary glass were held in a sliding carriage oriented at 45° to the axis of the illuminating system so as to permit fluorescent and reflected light to enter a Weston Photronic cell oriented at right angles to the illuminating beam. The carriage was constructed of blackened aluminum and served to mask the sometimes irregular edges of the discs. Between the carriage and the cell were placed two filters, which isolated the green fluorescence and prevented ultraviolet and blue light from entering the cell. Corning Glass Works filters Nos. 351 and 428 were employed. The output of the cell was taken through an Ayrton shunt to a sensitive galvanometer (0.001 μA/mm./M). The entire instrument, exclusive of the galvanometer and shunt, was contained in a hardwood box provided with light-tight ventilators. The integral lamp unit was lined with sheet aluminum.

TABLE 1

RELATIVE FLUORESCENCE OF NaF-NaCl DISCS* CONTAINING URANIUM

Added element	Relative fluorescence	Remarks
None	1.0	
Al	0.9	
As	0.9	Disc fragile
B	1.0	
Ba	1.0	
Be	0.9	
Bi	—	Disc fragile and discolored
Br	1.0	
Ca	1.0	
Cb	1.0 (+?)	
Cd	—	Disc adheres to platinum
Ce	0.5	Disc yellowish
Co	0.4	Disc gray
Cr	0.2	Disc pronounced yellow
Cu	0.9	Disc slightly gray
Fe	0.5	Disc yellowish
Hg	0.9	
I	1.0	
Mg	1.0	
Mn	—	Disc blue-green and adheres to Pt
Mo	1.0	
Ni	1.0	
Pb	0.3	Disc fragile and yellowish
Sb	0.3	
Si	1.0	
Sn	1.0	
Sr	1.0	
Ta	1.0	
Th	1.0	
Ti	1.0	
Tl	1.0 (+?)	
V	0.9	
W	0.9	
Zn	0.6	
Zr	1.0	

* The discs contained 35–100 μg. uranium/gram flux and, initially, a threefold excess by weight of added element. Discs were fused 10 minutes at bright red heat and weighed 1.9 grams.

This instrument was used for preliminary studies on the effect of temperature of fusion, composition, and thickness of the alkali fluoride discs, and for subsequent studies on interfering substances. It was found that a mixture of 5.75 parts

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