in any region may be taken from analysis of basal organic sediments in lakes on Mankato drift. Such dates supply only a minimum age, of course, because many lakes represent buried ice blocks that may have melted out long after disappearance of surface ice. Buried ice blocks of Cary age in this area thus may have survived the Two Creeks interstadial and the Valders cold interval as well. Two lakes (at Cedar Creek Bog and at Blomford) were sampled in an attempt to find a lake that originated immediately after withdrawal of surface ice; the Cedar Creek Bog lake sample is indicative.

Cedar Creek Bog is an ice-block feature in the Anoka sand plain in south-central Isanti County. An earlier sample had been taken by M. Buell from a depth of 28 feet from the pine-pollen zone of Lindeman (*8*) and was dated as 7988 ± 420 years old (sample C-332) (*9*). A new sample, however, collected by F. M. Swain and H. E. Wright from about the same spot at a depth of 30 feet just above the sand at the very base of the organic sediment, yielded at date of 11,830 ± 200 (sample W-466). The result suggests that the lake was already in existence during the Two Creeks interval. This date, in conjunction with the date from the Anoka sand itself at North Branch, only 13 miles to the east, seems to bracket the time of formation of the Anoka sand plain and further supports the late Cary correlation of the drift at Mankato.

The basal organic sediment of a second lake was also analyzed. This lake (near Blomford in central Isanti County) is a ground-moraine lake of the Grantsburg sublobe and should have started its existence very soon after the ice retreat. The date, however, is too late for consideration—4890 ± 200 years (sample W-465).

Glacial Lake Agassiz was initiated when the Des Moines lobe retreated into the Red River valley, which has a normal northward slope (*1*, p. 119). The early outlet was south by way of the Minnesota River valley (Glacial River Warren), but as the ice withdrew to the north it uncovered lower outlets across western Ontario to Lake Superior. Johnston (*10*) and Elson (*11*) have postulated a readvance of the ice to close the eastern outlets and raise the lake level to form Lake Agassiz II.

Whereas Leverett (*1*, p. 119) had considered Lake Agassiz to mark the retreat of the Mankato ice, radiocarbon dates suggest that it was older. Wood studied by Rosendahl (*12*) from a sewer excavation at Moorhead, Minnesota, beneath 25 feet of varved lake deposits (1800 varves) at a depth of about 45 feet below the lake plain was dated as 11,283 ± 700 BP (sample C-497) (*9*). A duplicate sample has recently been reanalyzed in Washington, D.C., as 9930 ± 280 BP (W-388).

Peat collected by Elson from Lake Agassiz II sediments near Rossendale in southern Manitoba was dated as 13,230 ± 600 BP by the solid-carbon method and 12,400 ± 420 BP by the more accurate acetylene method (sample Y-165), and shells from correlative sediment at a nearby locality as 11,230 ± 480 BP (Y-166) (*13*).

The Agassiz dates (except for the rerun of the Moorhead sample) suggest that the lake started its formation before the Valders advance, and even the Moorhead rerun is compatible with this hypothesis. The lake deposits were never overridden by ice in Minnesota, North Dakota, and adjacent southern Manitoba. The Des Moines lobe thus would also predate the Valders. Elson postulates that the Valders ice margin fell along certain moraines that blocked eastern lake outlets in central Manitoba and western Ontario and caused the growth of Lake Agassiz II. There are still difficulties with a Valders border this far north, inasmuch as drift of Valders age in the Superior sublobe and St. Louis sublobe have been identified in Minnesota (*4*), but the Lake Agassiz radiocarbon dates certainly point to a pre-Valders age for the Des Moines lobe proper and thus also for the drift at Mankato.

The radiocarbon analyses discussed in the preceding paragraphs all serve to date the drift at Mankato indirectly. Only one dated specimen of wood has been recovered from the Mankato drift itself. This was collected by R. Schneider from the surface drift near Redwood Falls, 60 miles up the Minnesota River from Mankato, and was dated as > 31,000 years old (sample W-99) (*14*). The drift is believed to be continuous with the surface drift at Mankato, but the date is so much greater than that anticipated for either Cary or Valders time that it must be considered anomalous; the wood may possibly have been derived from older drift. Wood collected by J. H. Zumberge from an oxidized drift that underlies the surface drift at Mankato itself was dated as > 37,000 BP (sample W-300, W-301). This drift must be pre-Wisconsin.

The radiocarbon dates discussed here suggest that the surface drift at Mankato should be correlated with the Cary (pre-Two Creeks) rather than with the Valders (post-Two Creeks). This possibility was discussed by Horberg (*15*) and Flint (*16*). We therefore favor the use of the term *Valders* over the term *Mankato* for the last major substage of the Wisconsin. Adoption of this term would establish the several important intervals of the middle and late Wisconsin—Tazewell, Cary, Two Creeks, and Valders—as units of reference based on the activity of a single ice lobe (Lake Michigan lobe). Most of the stratigraphic and geomorphic relationships can be reconciled to a late Cary correlation for the Grantsburg sublobe, Anoka sand plain, the Des Moines lobe proper, and Lake Agassiz I. The St. Louis sublobe is considered to be Valders in age, but its relationship to Lake Agassiz is not certain at the present time.

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4 September 1956

Structure of Small "Spherical" Viruses

The recent x-ray diffraction study by Caspar (*1*) on a crystal of tomato bushy stunt virus (TBSV) and the electron microscope studies by Williams and Steere (*2*) and by Rice, Kaesberg, and Stahmann (*3*) on frozen-dried preparations of several so-called "spherical" viruses suggest that these particles have a remarkably symmetric structure. The x-ray diffraction study shows that the TBSV particle has twofold and threefold symmetry axes and quite possibly also fivefold axes. The electron microscope studies show that tobacco ringspot virus (TRSV) and squash mosaic virus (SMV) often appear hexagonal in contour and TBSV sometimes appears hexagonal. All of these viruses cast shadows having several straight sides. On the other hand, a polyhedral contour has not been demonstrable previously in frozen-dried preparations of polio virus (*4*) or in turnip yellow mosaic virus (*5*). No detailed electron microscope structure has been reported for any air-dried preparations of small (around 300 A in diameter) viruses, probably because of the distorting effects of surface tension.

Lightly shadowed viruses can be imaged clearly in properly adjusted microscopes, but their shadows are not sufficiently well defined to be very useful. However, *heavy* shadowing with a mate-

rial such as uranium provides sufficient contrast in the shadows, so that they are clearly delineated, although, in this case, the viruses themselves are distorted in appearance. It can be shown from geometric considerations that the metal deposited on the viruses themselves, if it remains fixed where it lands, does not affect the shape of the shadows cast by the viruses.

In this laboratory, frozen-dried preparations of purified turnip yellow mosaic virus, squash mosaic virus, wild cucumber virus, and brome grass mosaic virus have been examined recently (*6*). Lightly shadowed preparations almost invariably suggest a polygonal contour. Hexagonal contours are often seen in the latter three viruses and occasionally in turnip yellow mosaic virus. Figure 1*a* shows three particles of brome grass mosaic virus, each lying in a different orientation with respect to the shadowing direction. The two central particles are clearly hexagonal. The hexagonal contour of the topmost particle is not very evident in the print, particularly because its south-pointing sides are not clearly demarcated. The lower double particle is discussed in a subsequent paragraph. Measurements of the distances between the numerous small bumps on the carbon substrate film show that the microscope can readily resolve 30 A under these experimental conditions.

The shadows of all four preparations usually show four or five straight sides. Figure 2*a* shows particles of turnip yellow mosaic virus which have been shadowed heavily with uranium and printed to a very high contrast. The contrast owing to the uranium is sufficiently high so that only those parts of the viruses on which uranium is deposited are visible. Although the particle images are much distorted and thus do not reveal the true particle contours, it is evident that the shadows are clearly delineated. Such pictures, together with the x-ray and electron microscope work cited here, suggest that these viruses and perhaps all small viruses have approximately the shape of symmetric polyhedrons.

There are only five kinds of polyhedrons that are made up of identically shaped plane faces and vertices in equivalent positions: a tetrahedron (made up of four triangular faces), a cube (six squares), an octahedron (eight triangles), a dodecahedron (12 pentagons), and an icosahedron (20 triangles). The tetrahedron and the cube may be eliminated from consideration, because their contours as viewed from above are clearly at variance with the observed images. Both the octahedron and the icosahedron have hexagonal contours, while the dodecahedron has a ten-sided contour, which might, however, not be resolved clearly in the microscope.

A comparison of the shapes of shadows

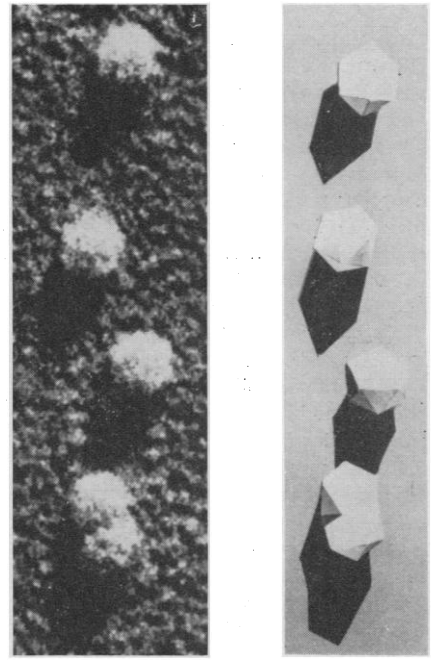

Fig. 1*a* (left). Electron micrograph of lightly shadowed brome grass mosaic virus. Because of the light shadowing, the shadows will not be clearly delineated, but the virus particles themselves should be reproduced faithfully. (× about 240,000.) Fig. 1*b* (right). Icosahedral models of the particles of Fig. 1*a*.

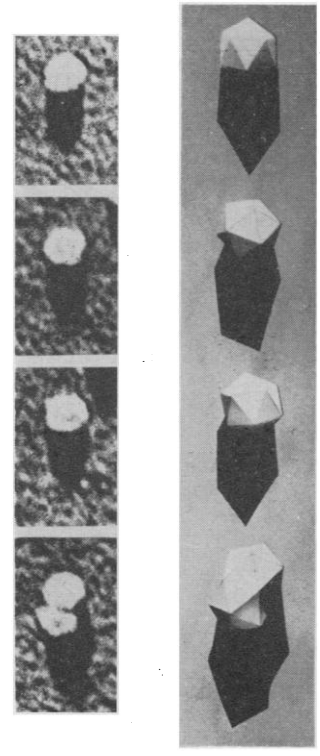

Fig. 2*a* (left). Electron micrographs of heavily shadowed turnip yellow mosaic virus. Because of the heavy shadowing and high contrast printing, the particle contours are much distorted; however, the particle shadows should be clearly delineated. (× about 150,000.) Fig. 2*b* (right). Icosahedral models of the particles of Fig. 2*a*.

of the four virus preparations with those that could be produced by the symmetric polyhedrons again unambiguously eliminates the tetrahedron and cube, and also the octahedron, from consideration as suitable models. Shadows cast by the dodecahedron and the icosahedron often can be approximately matched with virus shadows. However, the pointed shadow (the topmost particle in Fig 2*a*), which is sometimes seen, cannot be matched by a dodecahedron lying on any of its faces. Furthermore, a suitably positioned dodecahedron is capable of casting a shadow that is rarely (if ever) seen.

These facts, coupled with the frequency of appearance of hexagonal contours, suggest that these four viruses may have approximately the shape of symmetric icosahedrons. Judging from the particle contours, this approximation is fulfilled best by BGMV and least by TYMV. Figures 1*b* and 2*b* show models of icosohedrons that have been positioned to correspond to the virus particle orientations and show the extent to which agreement can be obtained. It should be emphasized that the heavy shadowing and high-contrast printing in Figure 2*a* preclude any very precise agreement between particle and model contours; the shadow contours should, however, be directly comparable.

Two particles in contact are seen often, particularly with brome grass mosaic virus and turnip yellow mosaic virus. The distance between the extreme ends of such dimers is usually about 20 percent less than twice the particle diameter. Furthermore, it is not possible to get agreement between the shapes of shadows cast by such dimers and the shadows cast by two polyhedrons in contact. Reasonably good agreement is obtained, as is shown in the figures, with a dimer model that consists of two icosahedrons interpenetrating sufficiently to eliminate one corner from each particle and to have five of the corners of one in a common position with five of the corners of the second. It is thus necessary to conclude that these dimers have been very much squashed together at some stage in their preparation or that perhaps they were produced in the plant cells as Siamese twins. Experiments are in progress which are designed to detect and isolate such twins in solution.

It would be desirable if the geometric models considered here could be replaced with more realistic models, perhaps consisting of subunits (*7*) (of protein) surrounding a core (of nucleic acid). In place of the icosahedron, one could, for example, assume a model consisting of identical spherical subunits placed at each of the 12 corners of the icosahedron. This would be a close-packed arrangement, each unit being in contact with five others. (The twins would consist of 17 units, a unit of each

twin being absent, and five units being held in common.) However, such a model casts shadows that are in poorer agreement with the virus pictures than does the icosahedron. It would seem necessary to conclude that the subunits are either much larger in number than 12 or are shaped properly to contribute to the icosahedral appearance. Caspar's x-ray data on bushy stunt virus suggest that the most likely number of subunits is 60. His results would be consistent with the electron micrographs if, for example, each of the icosahedral vertices consisted of a cluster of five subunits.

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Histochemical Evidence of Protein-Bound SH Groups in Plant Tissues with 4-Iodoacetamido-1-naphthol

Dickens (*1*) and Rapkine (*2*) studied inhibition of glycolysis by iodoacetate and iodoacetamide and reported that inhibition was a result of an alkylation reaction with sulfhydryl (SH) groups of reduced glutathione. Young and Conn (*3*) obtained almost complete inhibition of wheat-germ glutathione reductase with iodoacetic acid ($10^{-2}M$) and iodoacetamide ($10^{-3}M$). Barrnett and Seligman (*4*) found complete inhibition of SH staining with 2,2′-dihydroxy-6,6′-dinaphthyl disulfide (DDD) in tissues pretreated with iodoacetate ($0.1M$). However, Barron (*5*) stated that iodoacetate is not SH specific and will react with amino groups of amino acids at a physiological *p*H. Barrnett, Tsou, and Seligman (*6*) reported the results of preliminary histochemical experiments with 4-iodoacetamido-1-naphthol (IAN).

Experiments were conducted with IAN to determine the SH specificity of this reagent in a diazo-coupled reaction in plant tissues. *Zea mays* L. embryos were excised in early stages of germination, when the coleorhiza had initially split the pericarp of the grain. The specimens were fixed for 24 hours in a 2-percent solution of trichloroacetic acid in 80-percent ethyl alcohol. The specimens were dehydrated, imbedded in paraffin, and sectioned at 15 μ. The sections, mounted on slides with albumin, were stained by a modification of the diazo-coupling method of Barrnett and Seligman (*4*) for DDD. In this method, the slides were incubated 2 hours at 60°C in a mixture consisting of 35 ml of Michaelis barbital buffer (*p*H 8.55) plus 15 ml of absolute ethyl alcohol containing 35 mg of 4-iodoacetamido-1-naphthol (*7*). In the coupling reaction the slides were stained in 3 to 5 minutes with tetrazotized diorthoanisidine. Inhibition was achieved by pretreatment of controls for 24 hours in an aqueous solution of N-ethyl maleimide ($0.1M$).

The highest concentration of protein-bound SH was observed in the promeristem of the radicle, and the staining diminished rapidly back from the apex. A high concentration was also observed in the promeristems of the paired adventitious roots located above the level of the scutellar node. Moderate-to-strong staining was observed in the procambial strands throughout the embryo. The embryonic vascular bundles of the epicotyl and coleoptile are similarly stained in cross section. Although the entire embryo is diffusely stained, those areas indicating the least SH include scutellum, coleorhiza, coleoptile, and scutellar node.

The results obtained with IAN are in complete agreement with results that I reported previously (*8*) for TCA-pretreated specimens stained with nitroprusside reagent and Bennett RSR reagent, respectively. Furthermore, I have obtained an identical staining pattern for these tissues with DDD (*9*). We can therefore conclude that the results obtained with this new SH reagent are valid, inasmuch as these findings correspond with results obtained by different types of histochemical reactions (mercaptan, disulfide, and alkylation reactions).

There is, however, the possibility that this reagent may couple with amino groups. Danielli (*10*) has suggested the use of a specific and readily removable NH_2-blocking agent to achieve SH specificity. The Danielli dinitrofluorobenzene method for tyrosine, SH, and NH_2 is based on a series of specific blocking agents. This line of approach has not, in general, been successful (*11*), and I have not used any NH_2-blocking agents with IAN.

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Odostomia impressa Parasitizing Southern Oysters

Recently Loosanoff (*1*) has reported observations on the pyramidellid snail *Odostomia* (*Menestho*) *bisuturalis* Say as an "obscure oyster enemy" in New England waters. According to Abbott (*2*) and Miner (*3*), *O. bisuturalis* has its southern limit at Delaware Bay. It should now be recorded that *Odostomia* (*Menestho*) *impressa* Say, which ranges from Massachusetts Bay to the Gulf of Mexico, has similar habits. A hundred or more of these snails may be found holding to the extreme margins of an oyster's shell, each inserting its proboscis between the valves whenever the oyster opens to feed. G. Robert Lunz, director of the Bears Bluff Laboratories, demonstrated this to P. Korringa of Holland and myself when we visited South Carolina in 1948. On the very day that Loosanoff's article appeared in *Science*, I arrived at Bears Bluff to begin a long-planned study of this interesting parasite.

Observations and experiments on *Odostomia impressa* to date indicate that its behavior differs somewhat from that of *O. bisuturalis* as described by Loosanoff. Rather than attacking young oysters, like the northern species, *O. impressa* works mostly on large oysters. When numerous snails are placed in the middle of aquaria containing adult oysters at one end and shells covered with spat (3 to 19 mm long) at the other end, the majority of the snails go to the large oysters, and this majority gradually increases as snails desert the spat and collect on the large oysters. Snails placed in an aquarium with single oysters of graded sizes, 26 to 76 mm long, assemble in the largest numbers on the largest oysters and in proportionately smaller numbers on the smaller oysters. Rough surfaces, such as the outside surfaces of oyster shells, attract or retain more snails than smooth surfaces, such as the inside surfaces of oyster shells. Shells of living oysters, bearing many attached snails, lose these snails when the oyster is carefully opened