cloth-covered cage and sprayed with DDT as an additional precaution against possible contamination by unwanted insects.

Symptoms of phloem necrosis are seldom visible prior to mid-June. In late June 1947, therefore, all test trees were checked routinely for the possible appearance of disease. At this time one of the two seedlings placed under test on July 12, 1946, showed typical symptoms of phloem necrosis, and by August 1 the foliage was dead. The seedling was then removed and examined. Near the ground line, in certain portions of the phloem still alive, the symptoms were pronounced. Before this seedling was discarded, three patches of bark were removed and grafted into three healthy seedlings to determine whether the virus could be transmitted from the test seedling. In October one of these seedlings showed early symptoms of phloem necrosis. This seedling was then removed to a propagation room inside the laboratory, where it continued to grow until early in January 1947, when its foliage suddenly died. At this time the seedling showed typical late-stage symptoms of phloem necrosis.

On July 2, 1947, one of the seedlings placed under test on July 26, 1946, was found to have died so suddenly that its leaves had failed to abscise and still hung on—a phenomenon by no means uncommon among naturally infected trees growing in the open. Examination of the inner phloem of this seedling revealed typical phloem necrosis discoloration. These test trees developed symptoms, therefore, in less than a year after being exposed to infective insects. This period contrasts strikingly with a possible inoculation period of 5 years where species of *Erythroneura* were used.

The evidence favors transmission of the virus by this species of *Scaphoideus*, whereas transmission by species of *Erythroneura* is more doubtful. Among several hundred other test trees in the same and adjoining cages and among several thousand trees in a nearby nursery, there exists as yet no other evidence of insect transmission of the virus. As a further check on the significance of the results obtained with *Scaphoideus*, however, an extensive series of tests was established late in the 1947 season, but it is too early to report on these tests.

The individuals of *Scaphoideus* used in the 1946 tests were recovered and, after the development of disease symptoms in the test trees in 1947, were forwarded to the Division of Insect Identification. According to P. W. Oman, all were found to belong to the single species, *Scaphoideus luteolus* Van D. Oman's determination of these specimens was based on specimens compared with the type of *luteolus*. They therefore are not the same, according to him, as the species that DeLong (*2*) described as *luteolus*, but are the same as the species DeLong and Mohr (*3*) described as *baculus*. *Scaphoideus luteolus* is widespread throughout the region where phloem necrosis occurs, having been taken in surveys from Ohio on the east, to Kansas on the west, and to Jackson, Mississippi, on the south. That this species occurs in regions not yet known to harbor the virus is demonstrated by Oman's statement accompanying the determination, in which he reports having seen specimens from the following states outside the disease area: New Jersey, New York, Pennsylvania, Maryland, Virginia, Georgia, and Alabama.

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The Mechanism of Cysteine and Glutathione Protection Against Alloxan Diabetes

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The suggestion that alloxan may produce diabetes because of inactivation of essential sulfhydryl enzymes of the beta cells of the pancreas was made in an earlier paper by Lazarow (*5*). Inasmuch as large doses of alloxan also destroy other cells (liver, kidney, etc.), it also was postulated that the selectivity of this compound for the beta cells might be due to an especially low glutathione content which rendered the beta cells more susceptible to alloxan (*5*).

The injection of large doses of glutathione, cysteine, thioglycolic acid, and BAL protected rats against a diabetogenic dose of alloxan (*5*, *6*). By contrast, large doses of alanine, methionine, thiourea, and other compounds did not modify its diabetogenic effect. Protection against diabetes by these sulfhydryl compounds occurred only when they were given prior to the diabetogenic dose of alloxan. When glutathione or BAL was given 5 min after a diabetogenic dose of alloxan, no protection occurred. Since protection failed to occur when the sulfhydryl compound was given after the alloxan, it was suggested that, if the diabetogenic action of alloxan were due to a combination with essential sulfhydryl groups of enzymes, this reaction would not be as readily reversed as would be the case if the SH groups of the enzymes were simply oxidized to SS groups. However, a combination of alloxan with the SH groups would explain the failure to protect against diabetes when the sulfhydryl compound was given following the diabetogenic dose of alloxan.

Inasmuch as alloxan, dialuric acid (reduction product of alloxan), and derivatives of these compounds are unstable, the ultraviolet absorption spectra method was considered suitable for studying the possible reactions of alloxan with sulfhydryl molecules. This necessitated the study of the absorption spectra of alloxan and dia-

luric acid, which previously were reported as having no maxima (*4*).

By methods which will be published in detail at a future date, it was found that alloxan showed an inflection at 270 mμ (ε about 90) below pH 5.0 and an inflection which became a maximum at 245 mμ as the pH was raised above pH 6.0–7.4 (ε = 4,800 at pH 7.4). The decomposition of alloxan at pH 7.4 was found to be a first-order reaction, the half-life of alloxan being just under 1 min at 37° C and about 2 min at room temperature. Below pH 5.0 alloxan was relatively stable.

Dialuric acid showed a maximum at 270 mμ (ε = 3,200) in 1% HCl and at 275 mμ (ε = 16,500) at pH 7.4. Dialuric acid is oxidized rapidly by air in dilute solution but can be stabilized by the addition of cysteine.

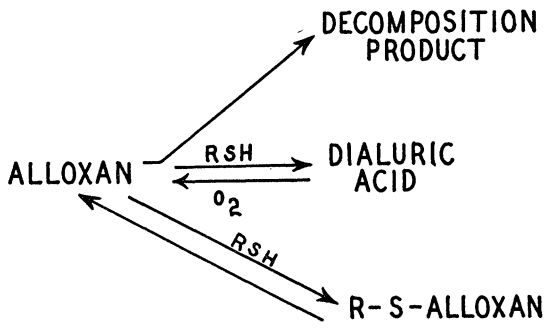

FIG. 1

Alloxan was found to react with cysteine to give a spectrum similar to that found when dialuric acid was stabilized with cysteine. With time, at pH 7.4 both dialuric acid and alloxan decomposed in the presence of cysteine, and a broad band appeared between 340 and 360 mμ (ε = 50–650, depending on conditions).

Alloxan reacted with glutathione to give maxima at 275 mμ and 305 mμ. The maximum at 275 mμ was observed with a high glutathione to alloxan ratio (9:1, alloxan = M/5,000). On standing, the maximum at 275 mμ disappeared, whereas that at 305 mμ became more intense (ε = 17,400). When equimolecular proportions of alloxan and glutathione were mixed (M/5,000), a maximum at 305 mμ was observed. In 3 min the extinction at 305 mμ reached 75% of the highest extinction obtained for this ratio.

Dialuric acid and glutathione gave essentially the same final result as did alloxan and glutathione. Oxidized glutathione did not react with alloxan. Alloxan (or dialuric acid) reacted much more slowly with glutathione in the presence of cysteine, to give a maximum at 305 mμ, than was the case when cysteine was absent.

Alloxan added to crystalline egg albumin, denatured with "Duponal P.C." to free the sulfhydryl groups, gave a spectrum with a maximum at 305 mμ. The spectrum was determined by comparing with a blank containing the protein and Duponal.

The data presented suggest the following conclusions, which are illustrated in Fig. 1.

Alloxan is rapidly decomposed at pH 7.4. It is also reduced to dialuric acid by sulfhydryl compounds. With alloxan and glutathione (as compared with cysteine), the formation of a maximum at 305 mμ is striking, and is thought to be due to formation of an addition product which would appear to be similar to that described by Schubert (*8*) in his study of the reaction of carbonyl groups with sulfhydryl. Since the addition of cysteine will convert alloxan to dialuric acid, the fact that added cysteine slows down the formation of a product with an absorption spectrum maximum at 305 mμ indicates that the mechanism involves a reaction of glutathione with alloxan rather than with dialuric acid. Since oxidized glutathione fails to react, the addition product is believed to take place through the sulfhydryl group of the glutathione.

The mechanism by which cysteine protects against alloxan diabetes is probably due to the reduction of alloxan to an nondiabetogenic compound, dialuric acid. Although dialuric acid can be reconverted to alloxan, this reconversion is also slowed by cysteine, thus preventing the formation of an effective concentration of alloxan. In addition, glutathione removes alloxan from the field of action by forming a new compound with an absorption spectrum maximum at 305 mμ. These results might explain why the administration of a large dose of sulfhydryl, given 5 min after a diabetogenic dose of alloxan, fails to protect rats from diabetes. For, if the alloxan has already reacted with proteins as indicated in the data (presumably with the sulfhydryl groups of essential enzymes), the addition of sulfhydryl might not be expected to reverse this reaction. The data also support the contention that dialuric acid itself is not diabetogenic, but that the diabetes observed after the injection of dialuric acid (*1*) is due to the conversion of dialuric acid to alloxan in the body.

Since it has been reported that an enzyme contained in dog liver can convert uric acid into dialuric acid (*7*), and since the injection of dialuric acid produces diabetes, presumably by conversion to alloxan, there is a reasonable chance that alloxan may also occur in man (*2*).

In a preliminary note Griffiths claimed the production of diabetes by the injection of massive doses of uric acid into a glutathione-depleted rabbit (*3*).

Should alloxan prove to be a factor in human diabetes, the reaction between glutathione and alloxan herein reported would have an important bearing on its etiology and prevention.

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