without serum, none of these strains suffered any loss of their enhanced peripheral titer. Furthermore, protracted contact of such virus with sub-effective doses of an antiserum prepared by immunization of rabbits against one of the invasive strains failed to bring about a reversion to non-invasiveness. From what has been said it is obvious that the described variation is clear-cut and permanent when it takes place; however, the occurrence of failures on repetition under identical experimental conditions attests to the unpredictable nature of the biological process involved.

Serological tests were performed in order to determine the identity of one of the new viral forms produced by contact with normal rabbit serum. As may be seen from Table 1, the non-invasive standard

TABLE 1

CROSS NEUTRALIZATION OF NON-INVASIVE AND INVASIVE STRAINS OF THEILER'S VIRUS BY CORRESPONDING ANTIVIRAL IMMUNE RABBIT SERA IN INTRACEREBRAL AND INTRAPERITONEAL TESTS

Theiler's virus		Antiserum against non-invasive strain		Antiserum against invasive strain	
		Minimum lethal doses neutralized		Minimum lethal doses neutralized	
Phase	Potency	Intracerebrally	Intraperitoneally	Intracerebrally	Intraperitoneally
Non-invasive parent virus	i.c. 10^{-7} i.p. 10^{-1}	10^{1}	10^{4}*	10^{1}	10^{4}*
Invasive variant virus	i.c. 10^{-10} i.p. 10^{-9}	10^{4}	10^{10}†	10^{4}	10^{10}†

* Serum neutralized up to 1 : 1000 dilution.
† Serum neutralized up to 1 : 10 dilution.

GDVII strain was neutralized quantitatively as well by its homologus antiserum as by a hyperimmune serum produced against the invasive strain. Conversely, neutralization of the invasive strain by its homologous antiserum occurred at the same levels as that obtained with anti-GDVII serum. Neither strain of Theiler's virus, invasive or non-invasive, was neutralized when tested intraperitoneally with antisera produced against two strains of mouse-adapted human poliomyelitis virus (SK, MM).[4,5] It is clear from these data that the invasive variant of Theiler's virus was serologically indistinguishable from the non-invasive parent strain. It is also obvious that the invasive Theiler strain did not result from chance contamination with murine SK or MM virus.

In summary it may be said that rapid passage in mice of Theiler's virus of mouse encephalomyelitis induces, on certain occasions, a variation of the infectious agent. The most characteristic feature of this variation is an enhancement in the power of the virus to invade the central nervous system from peripheral channels of infection. The phenomenon apparently is aided by previous contact of virus with certain normal sera. The resulting variant seems to be stable since it retains its newly acquired properties over several mouse-passages. The available serological evidence indicates that the invasive strain is antigenically identical with the non-invasive present strain. The reported data support earlier observations on biological changes of Theiler's virus and throw new light on the inherent variability of the viruses belonging to the poliomyelitis group.

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FRUCTOSAN, A RESERVE CARBOHYDRATE IN GUAYULE, PARTHENIUM ARGENTATUM GRAY

IN the course of investigations on the carbohydrate metabolism of the rubber-producing plant guayule, indirect evidence was obtained for the presence of a polysaccharide having the properties of a fructosan. This constituent was isolated and identified as follows:

Two hundred grams of dry coarsely ground mixed guayule tissue was extracted with 80 per cent. ethanol until the percolate was colorless. The tissue was air dried, and extracted with 5 separate 300 ml portions of water at the temperature of the boiling water bath. The combined dark-colored water extract was then treated with excess neutral lead acetate, centrifuged, and deleaded with H_2S. After adjustment to pH 6, the solution was decolorized with charcoal at about 80° C and concentrated to 100 ml under reduced pressure.

Addition of 3 volumes of acetone to the concentrate caused the formation of a white precipitate, which was allowed to flocculate in an ice bath. This precipitate was centrifuged down, taken up in water at 80° C, treated with charcoal, and again precipitated with acetone in the cold. The resultant floc was taken up in water, treated with lead acetate, deleaded, and precipitated with acetone. After washing with acetone, the substance (I) was dried under vacuum.

The substance (I) was practically insoluble in water at room temperature, but dissolved readily in hot water to give a clear solution. It showed no color change with iodine, and was non-reducing. On mild acid hydrolysis the substance (I) gave rise to a

strongly reducing substance, II. Both I and II showed positive ketose tests with Seliwanoff's reagent, and were levorotatory. The osazone of II was identical with that of fructose.

On the basis of the above observations it is concluded that fructosan is one of the carbohydrate constituents of guayule. In so far as tests conducted to date indicate, this polysaccharide (I) appears to be inulin,[1] but its exact constitution remains to be determined.

Using a colorimetric method, values for the fructosan content in the stem and roots of guayule have ranged from 0.2 to 12 per cent. (dry weight basis), depending on the conditions under which the plants were grown. Evidence that this polysaccharide is the chief storage carbohydrate in this species will be presented in detail elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

NUCLEAR BEHAVIOR IN RELATION TO CULTURE METHODS FOR PENICILLIUM NOTATUM WESTLING

DIFFERENCES in the yield of penicillin from *Penicillium notatum* Westling occur among strains or in subcultures of the same strain, even when grown from single spore isolations. Clutterbuck, Lovell and Raistrick[1] reported difficulty in maintaining Fleming's strain of *P. notatum*. Currently Hansen and Snyder[2] attribute this variation to the dual phenomenon which they have found to be characteristic of many fungi.[3] To maintain an active culture these authors recommend selection of a suitable strain following single spore isolation and tests for yield. Subsequent transfers can then be made by mass spore transfer.

Foster *et al.*[4] recommend merely the selection of a high potency strain and its maintenance by mass spore transfers. An analysis of the 19 substrains they derived from 3 different parent colonies clearly suggests that association and dissociation of genetic factors can not be overlooked as an explanation of the varied results recorded by these and other investigators.

In none of these accounts has the nuclear behavior been taken into consideration. If heterokaryosis or the interaction of genetically different haploid nuclei in the same mycelium is responsible for the variation in penicillin production it is necessary to know whether *P. notatum* has a nuclear cycle capable of such behavior. The details of a cytological investigation of this fungus are now in press, to be published shortly.[5] It will suffice here to say that the conidia of this fungus are predominantly uninucleate although occasionally binucleate conidia occur. Using C and M to designate types, single conidia then could comprise either of these genetic factors or their possible combinations: C, M, CM, CC or MM, depending on the number of nuclei per conidium. If a spore is heterotypic then the genetic means of variation are present from the outset. If it is homotypic presumably the line can be developed monotypically provided no mutations occur. However, in mass spore transfers a few hours after germination there is marked anastomosis among the developing germ tubes, conidia and mycelia, giving abundant opportunity for nuclear interchange. Since analysis of cultural isolates indicates that the variations are due to a mixture of genetic factors following anastomosis and the establishment of heterokaryosis, it would appear that at present mass spore transfer methods would offer as certain a way as any of keeping active cultures. Unless a spore is binucleate and heterotypic, an infrequent condition in this fungus, the effect of heterokaryosis is eliminated by single spore transfer. Mass spore transfer increases the chances of nuclear mixing and consequently heterokaryotic vigor.

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[1] After this note was submitted for publication, an incidental statement (not supported by data) to the effect that inulin occurs in guayule was found in a report of the Experimental Chemical Laboratory of the Italian Ministry of War (Silvio Guglielminetti, *Azzurra Agricola-Floreale*, 16: 63, 1936).

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