small, opaque, whitish pocks which are discrete and can be seen with the unaided eye. Microscopic section reveals the presence of cellular proliferation in the ectodermal layer of the chorioallantois, of marked cellular proliferation in the mesoderm with considerable cellular infiltration, and a slight proliferation of the endodermal layer. Section of rabbit's cornea also shows considerable epithelial cell proliferation. In the mouse brain an encephalitic process is noted. Detailed histological studies are under way, and experimental work is in progress with a view to establishing the identity of the virus.

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VARIABILITY OF THEILER'S VIRUS OF MOUSE ENCEPHALOMYELITIS[1]

ONE of the accepted characteristics of Theiler's virus[2] in albino mice is the high potency of the infectious agent—as demonstrated by intracerebral injection—and its limited infectivity—as determined by intraperitoneal inoculation. In other words, while the virus will abundantly multiply when sowed directly upon susceptible nerve tissue, it evidently has but little ability to reach the same tissue from peripheral channels of infection. The reasons for this discrepancy are not clear. For instance, the virus could be restrained in peripheral invasion by whatever individual protection the immune mouse may interpose; however, the response of Theiler-free cotton rats to intraperitoneal injection does not differ from that of Theiler-carrying albino mice. We are therefore dealing with an intrinsic property of the viral agent itself, probably brought about by prolonged interaction between virus and herd immunity within its natural host.

When Theiler's virus (GDVII strain) is serially passed through cotton rats, the virus will, on certain occasions, mutate and exhibit new biological properties.[3] For instance, cotton rat variants thus produced possess marked peripheral invasiveness for both cotton rats and albino mice; they may also prove capable of paralyzing other hosts, normally refractory to infection with Theiler's mouse virus, such as guinea pigs and rhesus monkeys. Data have recently been collected which show that similar changes can occur spontaneously during rapid mouse-passages of Theiler's virus (GDVII strain), especially following *in vitro* contact with certain normal sera. It is the object of this communication to briefly report these observations.

It has been our practice to maintain the virus in albino mice by serial intracerebral passage, transfers being usually separated by intervals of from two to four weeks. Occasional titrations over a period of almost two years indicated some fluctuation in potency, but, in general, the virus preserved its high intracerebral titer (10^{-6} to 10^{-8}) without gaining appreciably in virulence by intraperitoneal inoculation (10^{-1}). Irregularities were first observed when virus was harvested from mice—serving as controls in neutralization tests—which had become paralyzed following intraperitoneal injection with mixtures of virus and certain normal sera (rabbit, horse, guinea pig, man). The brains of such mice, on subsequent titration, often contained virus capable of paralyzing albino mice, intracerebrally as well as intraperitoneally, in extremely high dilutions. It was not immediately clear whether the observed phenomenon had occurred: (1) as the result of previous contact between virus and serum, (2) because virus was used which had been collected from intraperitoneally injected mice, or, (3) whether the rapid transfer of virus attending these operations, irrespective of derivation, had served to increase its virulence. Investigation of the three possibilities led to the following results: Ten experiments were carried out in which virus was exposed to contact with normal serum by means of intraperitoneal injection of virus-serum mixtures; in seven instances virus obtained from the brains of paralyzed mice reached intraperitoneal titers between 10^{-3} and 10^{-10}. Four experiments were run in which contact between virus and serum was established by intracerebral injection of virus-serum mixtures; in two instances titration of the resulting virus brains revealed intraperitoneal titers between 10^{-3} and 10^{-8}. Four experiments were finally conducted in which virus was passed rapidly, without serum contact, by either intracerebral or intraperitoneal injection. These last experiments were synchronous with an equal number of experiments in which virus was being transferred in combination with serum, the same batch of virus serving as source for both series. In one instance the virus showed unmistakable evidence of an increase in intraperitoneal titer (10^{-9}).

The use of the different methods mentioned yielded a total of ten viral substrains, all of which possessed high intraperitoneal potency for albino mice. When passed over three to five subsequent mouse-passages

[1] Aided by grants from the Dr. Philip Hanson Hiss, Jr., Memorial Fund, the Warner Institute for Therapeutic Research and anonymous donors.

[2] M. Theiler, SCIENCE, 80: 122, 1934; M. Theiler and S. Gard, *Jour. Exp. Med.*, 72: 49, 1940.

[3] C. W. Jungeblut, *Am. Jour. Publ. Health*, 33: 1227, 1943.

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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[4] C. W. Jungeblut, M. Sanders and R. Feiner, *Jour. Exp. Med.*, 75: 611, 1942.

[5] C. W. Jungeblut and G. Dalldorf, *Am. Jour. Publ. Health*, 33: 169, 1943; C. W. Jungeblut, *Am. Jour. Publ. Health*, 34: 259, 1944.

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