

*Studies on the Relationship between the Theiler Group of Viruses  
and the Intestinal Tract of the Mouse\**

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Following Theiler's<sup>1</sup> isolation of the virus which causes spontaneous encephalomyelitis in mice, it was shown by Olitsky<sup>2</sup> that this virus is present in the intestinal tract of almost all young adult mice. Olitsky<sup>2</sup> also reported that the virus, which he named the TO strain, is consistently absent from the intestines of mice younger than twelve days of age. Subsequently, Theiler and Gard<sup>3</sup> isolated the GD VII and FA strains of mouse encephalomyelitis virus. These latter viruses are not known to be present in the intestinal tract and are maintained by intracerebral passage in mice. Relative to the TO virus, these 2 strains are very virulent.

The purpose of this preliminary report is to present the results of two studies: (a) the isolation from adult mouse intestine of an inhibitor against the GD VII virus; (b) a comparison of the susceptibilities of mouse intestine and mouse central nervous system (CNS) to infection with the TO virus.

*Isolation of the GD VII Inhibitor:*—In the course of experiments with the GD VII virus, it had been observed that if virus is added to a mince of washed adult mouse intestine, there was a marked reduction in the infectivity titre of the virus. This observation was studied in greater detail with the following results: 1) If a water or saline extract of the tissue was used, a similar effect was obtained. 2) The inhibitory substance was found to be heat stable, non-dialyzable, insoluble in organic solvents such as acetone, ether, and chloroform.

The purification of the inhibitor was undertaken. Preliminary experiments indicated that the inhibitory activity was associated with the polysaccharide fraction of the crude tissue extracts. The commonly

used methods for polysaccharide purification were therefore followed to isolate the inhibitor. In essence, the method involves the removal of lipids by acetone and ether, removal of proteins by precipitation with trichloroacetic acid followed by denaturation of the residual protein by shaking with chloroform, removal of nucleic acids by precipitation with  $\text{CuSO}_4$ , and precipitation of the polysaccharide with ethyl alcohol.

In order to determine the effectiveness of the method, aliquots were collected after each step and the inhibitory activity determined. In the early stages of this problem, inhibitor activity was measured by *in vivo* titrations. For this purpose, Gard's<sup>4</sup> method for measuring virus titre, expressed

as the  $\frac{1}{T}$  value, was used. The activity was

estimated by the reduction in the  $\frac{1}{T}$  value produced by the inhibitor.

Lahelle and Horsfall<sup>5</sup> described a method for measuring the GD VII virus titre based on the agglutination of human erythrocytes by the virus. It was found that the intestinal inhibitor was effective in inhibiting hemagglutination as well as infectivity, and that this property can be used for the quantitative determination of the inhibitor. This technique was found to be not only more rapid, but also more quantitative than the *in vivo* method.

Wherever practicable, specific activity was determined in terms of hemagglutination-inhibition units per gram of dry weight. As an illustration of the data obtained, a protocol of one of the preparations is shown.

1. Wet weight of tissue	583 gms.
2. Acetone-ether dried powder	98
3. Alcohol precipitated powder	0.698
4. Final product	0.170
5. Final yield	29%
6. Purification	170-fold

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TABLE I—PROPERTIES OF THE THEILER GD VII VIRUS INHIBITOR

Distribution		Specificity	Carbohydrate Constituents**
Mouse Tissues	Intestine of Other Species		
Present: Adult Intestine	Present: Guinea Pig	Inhibits: GD VII	Present: Methyl Pentose Galactose Mannose Glucuronic Acid Hexosamine
Variable: Brain	Little or none: Monkey Rabbit	Does not inhibit: FA TO TO (Br)*	Absent: Glucose Ketohehexose
Little or none: Infant Intestine Adult Heart Adult Lung Adult Kidney Adult Liver Adult Spleen Adult Muscle	Hog	Lansing	
Stability		Activity	
Stable to: Autoclaving at neutral pH Prolonger storage at -25° C. Trypsin Cathepsin Ribonuclease Lysozyme		In vivo: 0.03 µg reduced 1/T from 0.30 to 0.14  In vitro: 0.025 µg inhibited hemagglutination of 8 units	
Unstable to: 100° C.—10 minutes in 1N HCl or NaOH			

\* TO virus after seven passages through mouse brain.

\*\* The analysis was kindly performed by Dr. Z. Dische of the College of Physicians and Surgeons of Columbia University.

An analysis of the final product showed that the major constituent was polysaccharide with about 10 per cent and 0.5 per cent nucleic acid also present.

Because of the presence of several molecular species, the question arose as to which one possessed the inhibitory activity. An attempt was made to answer this by chromatographic analysis. Using a paper strip impregnated with alumina and a phosphate buffer solvent, it was found that the carbohydrate and the inhibitory substance migrated at about the same rates. This may be considered as presumptive evidence that it is the polysaccharide which possesses the inhibitory activity.

The results of studies on the distribution and properties of the inhibitor are assembled in Table I.

It is interesting to note that on a dry weight basis infant mouse intestine shows considerably less inhibitory activity than adult intestine. It is also of interest that at approximately the same age level when TO virus first becomes detectable in the intestine, there is also a pronounced rise in the amount of inhibitory activity. These observations suggest a possible relationship between the receptor substance of the TO virus and the GD VII inhibitor. This hypothesis will be investigated.

A preliminary study of the mechanism of the inhibition reaction showed that virus can be inhibited only when it is inoculated simultaneously with, or shortly after, the inhibitor. This suggests that either, (a) virus and inhibitor combine to form a non-infectious and irreversible complex, or (b)

the inhibitor prevents the virus from reaching the receptor sites or some essential system of the host cells.

*Relative Susceptibilities of the Central Nervous System and the Intestine of the Mouse to the TO Virus.*—On the basis of Olitsky's observation<sup>2</sup> that very young mice are free of the TO virus, an attempt was made in 1947 to initiate a colony of Theiler-free mice. A number of 10-12 day old mice were decontaminated and transferred to an environment where contact with the TO virus was precluded. When the mice reached maturity they were tested and found to be free of TO virus. This colony of mice has been maintained in the Theiler-free state, under special precautions to prevent contamination, since the inception of this project.

The investigation of the relative susceptibilities of intestine and CNS was carried out as follows: Approximately four weeks old mice were divided into two groups. In one group the virus was titrated by the intracerebral inoculation of 0.03 ml. of appropriately diluted TO virus. The mice were observed for six weeks for symptoms of infection. In the second group the titration was carried out by feeding 0.3 ml. of the diluted virus preparation. One week after feeding, fecal samples were collected and tested for the presence of TO virus. The  $ID_{50}$  values were calculated on the basis of an inoculum of 0.03 ml. The values obtained for intestine and CNS were  $10^{-4.4}$  and  $10^{-3.8}$  respectively. This indicates that the intestine is more susceptible than the CNS. There is a possibility, however, that in the intestine there is also present a neuro-

tropic mutant of the intestinal virus. If this should prove to be the case, then a re-interpretation of the above results would be required.

#### SUMMARY

A substance has been isolated from adult mouse intestinal tissue which specifically inhibits the GD VII strain of Theiler's virus.

Presumptive evidence has been obtained that the inhibitor is a polysaccharide.

The amount of inhibitor that can be demonstrated in infant intestine is considerably less than that in the adult tissue.

Mouse intestine appears to be more susceptible than CNS to the TO virus when tested by parallel titrations in Theiler-free mice.

#### REFERENCES

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