by diagnostic, clinical laboratories on sera of patients suffering from infectious diseases seldom, if ever, include determinations for incomplete antibody. If present, such antibody might provide a more useful measure of the progress of the infection. Potuzník and Gavlík(7) have published data comparing incomplete and complete Vi antibody in serum of typhoid patients and carriers, and found that incomplete Vi antibody appeared in higher titers in almost every case, particularly among the carriers.

It is generally believed that during the many years of its use, typhoid vaccine has afforded man a significant degree of protection against natural infection. The fact that Vi antibody, as determined by the usual procedures, appears in a relatively small percentage of immunized individuals has led a number of investigators to question the importance of Vi antibody in protection of man against this disease. In the light of the present work, however, it might be profitable to reexamine sera from typhoid-vaccinated individuals for incomplete Vi antibody. Such a study is currently in progress in this laboratory.

Summary. Immunization with purified Vi antigen elicited incomplete Vi antibody in the Cinnamon strain, and complete Vi antibody in BALB/c mice. Incomplete Vi antibody alone accounted for protection afforded by serum from Cinnamon mice against challenge with fully virulent typhoid bacilli. Absorption tests verified the specificity of incomplete Vi antibody and protection it afforded.

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Growth and Passage of Variola Virus in Mouse Brain.* (25922)

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Variola virus has been distinguished from other pox viruses by its poor growth on primary inoculation into laboratory animals, and its failure to become adapted to laboratory animals on subsequent passage(1,2,3). Furthermore, it has been reported that mice inoculated intracerebrally (IC) with variola virus succumb in erratic death patterns(4), and that lethality disappears after the first passage(3). Under proper conditions to be described here, it will be shown that these distinguishing characteristics may not serve as absolute markers for differentiating variola from the other pox viruses. Data are presented which show that variola virus does grow well when inoculated IC in mice, and that it can be serially passaged.

Materials and methods. The Yamada strain of variola virus was used. To determine whether multiplication of virus occurred, a large number of Swiss mice were inoculated IC. At selected sampling intervals, the brains of 3 sacrificed mice were pooled, homogenized as 20% suspensions in heart infusion broth, and assayed for virus titer on the chorio-allantoic membrane (CAM) of 11-day-old embryonated eggs(5). Ten eggs were used for each serial 10-fold dilution. Titers were expressed

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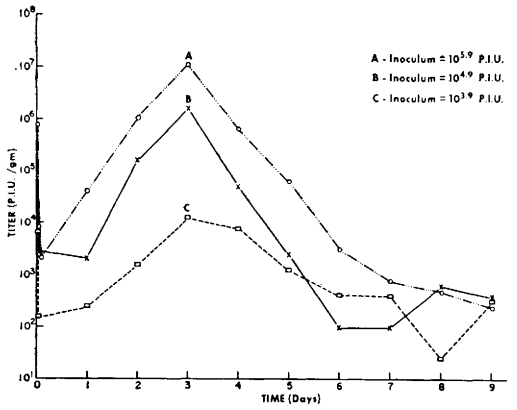

FIG. 1. Growth of variola virus in mouse brain.

as number of pock infectious units (p.i.u.)/g of tissue.

Results. The results of a typical experiment on growth of virus in the brain of IC inoculated mice weighing 12-14 g† are shown in Fig. 1. Titer of the virus decreased 91-99% in the first 5 minutes, rose to a peak between 48 and 72 hours, then decreased. In experiments using younger mice (8-10 g), this final rate of decrease was more gradual. The peak titer was generally proportional to inoculating dose and occurred on the same day irrespective of size of inoculum. These 2 features are not characteristic of most viruses. Generally, peak titer is independent of inoculating dose and is usually delayed with low doses. The growth curve obtained with the highest dose confirms Helbert's results(6) who was one of the first to give quantitative growth data in mice in a comparative study of variola and alastrim.

Preliminary experiments suggested that the marked initial decline in titer was neither due to dilution effect(7) nor to very rapid and efficient adsorption to susceptible cells. The first explanation was ruled out when almost complete recovery of unadapted Newcastle disease virus was obtained 5 minutes after IC inoculation. The second explanation was considered unlikely because of the well documented reports on slow and inefficient adsorption of pox viruses(8,9), and the reproducibly lower decrease in titer obtained with brain-adapted vaccinia virus in the same interval of 5 minutes (average 80%, vaccinia; average 95%, variola). The possibility for existence of a virus inhibitor in mouse brain is being investigated.

The virus was uniformly lethal for mice when $10^{5.5}$ p.i.u. or more were inoculated intracerebrally. With smaller doses, mortality rate and death pattern became erratic (See also(2) and (6). For example, although doses as low as 10 p.i.u. infected 50% of the mice inoculated, as determined by resistance to challenge with neurotropic vaccinia virus after 3 weeks, the percentage dying varied from one experiment to another. Furthermore, in any given experiment, percentage of dead mice from this very small dose was sometimes the same or greater than percentage dying from a larger dose.

Rough estimates indicated that weanlings (6-8 g) were approximately 1000 times more susceptible to the lethal effect of the virus than older (12-14 g) mice. Quantitative pock counts showed that the younger mice consistently produced 90% more virus in brain tissue. Marshall and Gerone (manuscript in preparation) have found that suckling mice 6-22 hours old give consistently uniform death patterns such that confident assays may be carried out in this host.

Serial passage of variola virus by the IC route was next attempted. The brains of three 8-10 g mice, each inoculated with $10^{5.5}$ p.i.u., were excised at time of peak titer (3rd day) and homogenized as a 20% suspension in heart infusion broth. Inoculation of these homogenates proved to be toxic for a certain proportion (10-30%) of the mice which succumbed within the first 15 minutes. The toxic factor was associated with uninfected mouse brain, but by repeated low speed centrifugation, the toxicity of the supernatants was substantially reduced. Passages were made, therefore, employing both supernatants and whole brain homogenates. Ten consecutive passages were made. The results of one experiment (Table I) indicated that variola virus may be continuously propagated by intracerebral passage provided the requirements of dosage and time of harvest are met. The erratic lethal pattern persisted when titrations

† 12-14 g = 28-30 days; 8-10 g = 21-23 days.

TABLE I. Passage of Variola Virus Intracerebrally in Mice. 10 successive passages.

Series 1 Homogenized brain suspension passaged	Series 2 Supernatant of brain suspension passaged
Titer p.i.u./g	
1.2×10^7	
7.0×10^6	1.1×10^5
1.7×10^7	1.5×10^6
1.0×10^6	2.2 "
4.0 "	2.0×10^4
8.0×10^5	4.0×10^2
2.3×10^6	2.5 "
4.9×10^7	1.5 "
3.5 "	6.0 "
1.6 "	8.0 "

of passage material were made. It is noteworthy in the passages made with supernatants, that once a decrease in titer occurred (5th passage), the titer remained low and could not be restored. This occurred twice in 6 passage series. In both cases, when the titer fell below a critical level of about 10^4 p.i.u./g, it decreased further on passage and remained at a very low level.

Attempts were made to confirm the identity of the tenth passage virus and to determine if a variant of variola virus might have emerged. The results of these experiments (Table II) identified the passaged virus as variola virus. Mice surviving infection with the passaged virus were immune 3 weeks later to IC inoculation of 3000 MIC LD_{50}s of a mouse neurotropic strain of vaccinia virus. Pocks that formed on the CAM by the tenth passaged material were the same in size, color and morphology as those formed by the parent variola strain. The passaged virus, like the parent, was incapable of forming plaques on monolayers of chick fibroblasts. Pirsch and Mika (10) have found that with the exception of variola and alastrim, all the classical pox viruses tested form distinct plaques on chick fibroblast monolayers. This appears to be an important new characteristic differentiating variola from other members of the pox group. These and the remaining data in Table II support the fact that a variant had not emerged during the 10 mouse brain passages. Passaged mouse brain material and an egg seed prepared from it gave identical results.

Summary. Variola virus was lethal for mice by intracerebral route when relatively high doses were inoculated. Lower doses produced erratic death patterns. Virus proliferated in mouse brain reaching peak titer between 48 and 72 hours. Amount of virus at peak titer was dependent on quantity of virus inoculated. Ten consecutive passages of the virus in mouse brain were successfully carried out. The most important factor which appeared to influence successful serial passage of the virus was level of titer attained in preceding passage. This titer was, in turn, dependent on size of inoculating dose, time of harvest and age of mouse. The passaged virus was identical with parent strain with respect to a

TABLE II. Properties of Parent and Mouse Brain-Passaged Variola Virus.

Property	Parent virus	Passaged virus
Surviving mice challenged with neurotropic vaccinia virus (3000 MIC LD_{50}s)	Induces immunity	Induces immunity
Pocks on CAM	White convex .9 mm 2 days 1.4 3	White convex .9 mm 2 days 1.4 3
Plaques on chick fibroblasts	None	None
Lethality for mice	Erratic death pattern (see text)	Erratic death pattern
Growth response in mouse brain	Same as Fig. 1	Same as Fig. 1
Ratio p.i.u./g / r.s.l.p.$_{50}$/g*	$10^{2.5}$	$10^{2.5}$
Hemagglutinin titer	1/160	1/160
50°C inactivation rate (first 30 min.)	1.7 log/15 min.	1.6 log/15 min.

* p.i.u./g = pock infectious units/g of CAM seed; r.s.l.p.$_{50}$/g = median rabbit skin lesion-producing dose/g of CAM seed.

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A New Insight into Pathogenesis of Carbon Tetrachloride Fat Infiltration.* (25923)

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Recent investigations in a number of laboratories have made possible the formulation of a simple hypothesis regarding the underlying functional derangement responsible for accumulation of fat in the liver following carbon tetrachloride poisoning. This hypothesis is as follows: The liver is constantly secreting large quantities of triglycerides into the plasma. Carbon tetrachloride poisons the secretory mechanism and as a result triglycerides accumulate in the liver. Key observations which led to formulation and testing of this hypothesis were the following: It was observed(1) that following intubation of carbon tetrachloride into the stomach of rats, the hydrocarbon reached its peak concentration in the liver within 1 to 2 hours. In another study(2) it was shown that liver triglycerides were elevated 34% within one hour and 195% within 3 hours following carbon tetrachloride poisoning. Furthermore, we have shown that mitochondrial oxidation of octanoate, and mitochondrial respiratory control, are completely unaffected 2 hours after carbon tetrachloride poisoning at which time liver triglycerides are elevated 70% (Recknagel, R. O., and B. Lombardi, unpublished). These observations in conjunction with our earlier studies(3,4) of mitochondrial function following carbon tetrachloride poisoning, as well as related work of others(5) appeared to us to demonstrate convincingly that failure of fat oxidation, or uncoupling of oxidative phosphorylation, could not account for the early rise in liver triglyceride content. It was further observed(6) that 4 hours after carbon tetrachloride poisoning, hepatic glucose-6-phosphatase, a microsomal enzyme, was depressed to 59% of control levels. Subsequently, (Recknagel, R. O. and B. Lombardi, unpublished) it was shown that this enzyme was depressed 30% 2 hours after carbon tetrachloride intoxication. Neubert and Maibauer (7) had previously noted that the hepatic microsomal enzyme system involved in aminopyrine detoxication was destroyed in carbon tetrachloride poisoned rats at a time when no derangement in mitochondrial oxidative phosphorylation was evident. These observa-

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