Immunization of Mice with Inactivated Herpes Simplex Virus.* (26673)

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While several reports indicate that inactivated herpes simplex virus is effective for immunization of experimental animals(1-4), recent articles cast doubt as to its efficacy (5-7). The immunologically related herpes B virus, after inactivation with formalin, is of low potency as an immunizing agent for its natural host, the monkey, but produces effective immunity in rabbits(8). As herpes simplex virus infection is a prototype of mammalian virus latency, it is especially desirable to understand its immunogenic characteristics. The evidence presented here indicates that immunization of mice with noninfective virus may be accomplished with the production of neutralizing antibody in this host.

Materials and methods. The strain of herpes simplex virus used was isolated on chick embryo fibroblast culture in this laboratory in Jan., 1957, from a herpes simplex labial lesion and has undergone 12 serial passages on chick embryo fibroblast or rabbit kidney cell monolayer cultures. It is neutralized by rabbit herpes simplex antiserum.

Male CFW albino mice of 18-20 g weight obtained from Carworth Farms, New City, Rockland Co., N. Y. were used throughout these experiments.

Neutralizing antibody titers are expressed as that dilution of serum neutralizing 100 TCD_{50} or 10 TCD_{50} of herpes simplex virus on human liver cell cultures. Titers were determined for individual animals and no dilution below 1:8 was tested because of the small amount of serum available.

Procedures and materials varied in some respects in each of 3 mouse immunization experiments (Table I). The virus was grown on rabbit kidney cell monolayers with a maintenance medium of 2% calf serum in 199† and containing 100 units of penicillin and 100 μg of streptomycin per ml. This tissue culture fluid was found to have a titer of $10^{5.5}$ TCD_{50}/ml on human liver cell cultures in Exp. I. The titers were $10^{7.0}$ and $10^{7.5}$ TCD_{50} respectively on rabbit kidney cell cultures in Exp. II and III. Titration end points of herpes simplex virus are 1.5 and 2.0 logs higher with rabbit kidney than in liver cell culture. Hence, these 2 virus suspensions were of approximately equal initial infectivity titer.

Virus was inactivated in 10 ml lots in a 9.5 cm Petri dish, using a Westinghouse Sterilamp G8T5 (u.v. germicidal lamp), at 4 inches distance for 13 minutes. Subsequent inoculation of this preparation undiluted or in 10^{-1} dilutions on rabbit kidney monolayers gave no cytopathic effect. Cultures in thioglycollate broth and in blood agar showed no bacterial growth. Uninoculated maintenance medium was removed from rabbit kidney cell monolayers and irradiated for use as a control in Exp. III.

Results. (Table I) in Exp. I, 2 groups of 5 mice each received 0.2 ml of inactivated virus into the tail vein followed in 11 days by 0.5 ml of inactivated virus intraperitoneally. Intracerebral challenge with 333 LD_{50} or 33 LD_{50} or herpes simplex virus caused no deaths in either immunized group. None of the control mice which received 333 LD^{50} and only one control mouse injected with 33 LD_{50} survived.

In Exp. II, 2 groups of 15 mice immunized as above, were injected intracerebrally with herpes simplex virus in a calculated dosage of 1700 LD_{50} or 170 LD_{50}. Only one death occurred in each group of immunized mice while 14 deaths occurred in 15 control mice which received 1700 LD_{50} and 8 of 15 unimmunized mice died which received 170 LD_{50}. It was surprising that only 8 of the 16 control mice died from the calculated 170 LD_{50} dose. However, this LD_{50} had been deter-

* This investigation was supported in part by grants from Nat. Inst. Health, Division of Allergy and Infect. Dis., and from Am. Cancer Soc.

† Microbiological Associates, Inc., Bethesda, Md.

TABLE I. Immunization of Mice with Inactivated Herpes Simplex Virus.

Exp.	Antigen	Intracerebral challenge (LD_{50})	S/T*	%
I	Inact. HSV†	3.3×10^2	5/5	100
	None	3.3×10^2	0/5	0
	Inact. HSV	3.3×10^1	5/5	100
	None	3.3×10^1	1/5	20
II	Inact. HSV	1.7×10^3‡	14/15	93
	None	1.7×10^3‡	1/15	7
	Inact. HSV	1.7×10^2‡	15/16	93
	None	1.7×10^2‡	8/16	50
III	Inact. HSV	10^3	10/14	71
	Cell culture fluid	10^3	0/12	0

* S/T = Survivors/Total experimental animals.
† Herpes simplex virus.
‡ LD_{50} previously determined in younger mice (see text).

mined 3 weeks earlier on mice of the same shipment, and it has been shown that susceptibility of mice to herpes simplex virus infection decreases significantly with age(9).

In Exp. III, a group of 15 mice immunized by the schedule previously described were challenged intracerebrally 28 days after the second injection with 1000 LD_{50} of herpes simplex virus. A group of 15 control mice were injected with irradiated cell culture fluid and similarly challenged. One mouse of the immunized group and 3 control mice died of undetermined cause prior to intracerebral challenge. Ten of 14 immunized mice survived while all 12 control mice died within 7 days.

A second group of 15 immunized mice and 15 control mice was bled for antibody determination 28 days after completion of immunization. Sufficient serum for antibody studies was obtained from 14 of the immunized mice and 9 of the control mice. No antibody could be demonstrated against 100 TCD_{50} of herpes simplex virus, although there was delayed viral cytopathic effect in the presence of serum from immunized mice as compared to serum from control mice. Neutralizing antibody was demonstrated against 10 TCD_{50} of herpes simplex virus in all but one of the 14 immunized mice tested. None of the 9 control sera had neutralizing activity against herpes simplex virus.

Discussion. The successful attempts at herpes immunization reported earlier were done with virus grown in intact animal hosts and inactivated with formalin or phenol. In our experiments the use of ultraviolet inactivated virus from relatively simple cell culture fluids obviated the introduction of animal tissue and formalin into the immunizing materials.

In only 2 instances was neutralizing antibody demonstrated by previous investigators following immunization with inactivated virus, both times in the guinea pig(2,10). We were able to demonstrate antibody in mouse serum as might be expected, but this has not been reported by others.

Comparison of Exp. I and II with Exp. III (Table I) suggests that the immunity conferred by inactive virus may fall rapidly. Ninety to one hundred per cent survival was seen in animals challenged one week after completion of immunization, while only 71% survived when challenged 28 days after completion of immunization. In earlier studies the challenge interval has been only 10 days to 2 weeks.

It is suggested that cell culture materials may confer a greater immunity than is seen with antigen from animal sources. Animals immunized with inactivated virus from animal brain or skin lesions withstood 10 to 100 MLD while 71% of those immunized with inactivated cell culture virus were able to withstand 1000 LD_{50} in the present study. This may be related to the higher pre-inactivation titers of the cell culture virus suspensions. While our studies were in progress a report of similar findings appeared in which mice immunized with inactivated herpes simplex virus from rabbit kidney cell culture fluid withstood a challenge of 10,000 LD_{50} (10). There are several possible explanations of the failure of inactivated herpes simplex virus produced in eggs to induce immunity. Jawetz, Allende and Coleman(6, 7) employed material of lower pre-inactivation virus titer than is obtainable in cell culture materials. Hence the amount of antigen injected may have been less than that

in cell culture materials. A second possibility is that the inactivation procedures used may have destroyed antigenicity as well as infectivity. It is also possible that along with the well recognized alteration in virulence of herpes simplex virus on egg passage, there is some accompanying change in antigenic potency. In fact, a recent report indicates that herpes virus grown in eggs may be less active antigenically than virus from cell culture or mouse brain even if pre-inactivation titers are comparable(10).

It is clear from our experiments and those of Chu and Warren(10) that injection of inactivated herpes simplex virus in mice can protect against a large intracerebral challenge. Production of demonstrable neutralizing antibody also results from these injections.

Summary. Ultraviolet inactivated herpes simplex virus prepared in rabbit kidney cell cultures was injected intravenously and intraperitoneally into mice. The majority of mice so immunized survived a 1000 LD_{50} intracerebral herpes simplex virus challenge and were shown to possess neutralizing antibody against the virus. These findings are contrary to the findings of studies in which inactivated allantoic fluid virus was used as antigen. However, they are in agreement with early experiments which employed inactivated herpes simplex virus prepared in mouse or rabbit brain and a recent report in which antigen was also prepared in rabbit kidney cell culture.

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Received March 23, 1961. P.S.E.B.M., 1961, v107.

Effect of Hyperthyroidism on Body Weight Gain and Feed Consumption in Male and Female Rats.* (26674)

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Normal thyroxine secretion rates (TSR) of many female rats of Sprague-Dawley-Rolfsmeyer (S-D-R) strain have been determined in this laboratory(1,2). TSR varied from 0.25 μg to 2.0 μg/100 g B W, with mean of about 1.0 μg. A significant increase in TSR was observed in lactating rats(3). Lactating rats showed a range up to 3.0 μg. Use of 3.0 μg/100 g B W has been shown to stimulate significant increases in mammary gland growth(4,5) and lactation(6). Unpublished data on TSR of normal male rats of this strain show a range of 0.5 μg to 1.5 μg/100 g B W with a mean of about 1.0 μg/100 g B W.

The object of the present experiment was to relate mean and maximum TSR of this strain of rats with optimal level of hyperthyroidism for weight gain. It was considered that injection of graded levels of thyroxine above mean and maximum TSR would be reflected in changing weight gain in such a manner as to indicate optimal hyperthyroidism and transition to a state of thyroxine induced stress.

Material and methods. Male and female

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2288. Approved by Director.

† This investigation was supported in part by a grant from U. S. Atomic Energy Commission.