TABLE III. Response of Tissue Constituents in Adrenal Demedullated Rats 72 Hours after Intravenous Alloxan.

Tissue constituents	Controls (10)	Alloxan inj. (10)	"P" value*
Part A. Liver lipids fractions (g %)			
Total lipids	5.22 ± .11	6.42 ± .22	.0002
Phospho-lipids	4.20 ± .10	4.42 ± .08	ND
Total cholest.	.20 ± .004	.24 ± .01	.002
Free "	.19 ± .003	.21 ± .008	.055
Ester "	.01 ± .004	.03 ± .002	.015
Neutral fat	.75 ± .05	1.74 ± .14	<.0001
Part B. Liver and plasma electrolytes,† water, glucose			
Liver:			
% water	69.3 ± .3	69.6 ± .4	ND
K	86.2 ±1.0	77.7 ±1.4	.0002
Na	25.0 ± .8	30.7 ± .9	.0005
Glycogen (g %)	3.60 ± .22	1.56 ± .28	<.0001
Plasma:			
% water	92.8 ± .09	91.9 ± .23	.02
K	5.26 ± .05	5.87 ± .08	<.0001
Na	169.9 ±2.0	155.6 ±2.2	.0004
Glucose (mg %)	126 ±6	500‡	<.0001

All values are mean ± S.E. No. of animals in each group is figure in parentheses.

* Difference between means occurring by chance compared with controls.

† Liver and plasma electrolytes are expressed as mEq/kg wet tissue and mEq/kg plasma water, respectively.

‡ All glucose values were at least 500 mg %.

responses of hyperglycemia and decreased liver glycogen content, there were similar changes in other chemical constituents in all groups—an increased neutral fat and decreased liver potassium content, and an increased plasma potassium level. The changes in the tissue chemistries of the 4- and 24-hour groups after alloxan were discussed as signs of an hepatotoxic action of alloxan, or responses of a stress type which were adrenocortically conditioned. It was also shown that the nature of the altered chemistries 72 hours after alloxan were essentially the same in intact and adrenal-demedullated rats.

The technical assistance of Frank Szematowicz is gratefully acknowledged.

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Received June 15, 1954. P.S.E.B.M., 1954, v86.

Virulence of Strains of Herpes Simplex Virus for Mice. (21191)

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It has been our experience that in the use of neutralization tests the HF strain(1) of herpes simplex virus usually yields low and at times inconclusive neutralization indexes (2-4). In spite of the many factors involved, apparently good indexes can be obtained with some of the recently isolated strains of herpes(2-5). In subsequent reports it will be shown that the strain of virus and the method of preservation are of importance. This paper will report on the virulence of various strains of virus for mice and the ultimate selection of

TABLE I. Strains of Herpes Simplex Virus from Human Sources.

Patient	Age (yr)	Herpetic infection	Reference No.	Virus source	Mouse passages	Incubation 10% suspension (hr)
HF	—	Recurrent	1	Lip	—	30
JM	1	K.V.E.*	2	Skin	9	72+
FR	16	"	2		11	96
HT	23	"	2		4	72
EB	25	"	2		4	72
CF	1	"	3		9	72
RE	2	"	3		4	40
GN	22	"	3		10	48
AG	8	Rhinitis	4	Blood	9	40
WR	6	Stomatitis	5	Mouth	6	72
PA	5	"	8		10	48
BT	57	Recurrent	8	Skin	6	72

* Kaposi's vericelliform eruption *(Eczema herpeticum)*.

the RE strain as a suitable test agent. Virulence in mice was tested by the cerebral and peritoneal routes in young and old mice. Moreover, since it has already been shown that inapparent infection may occur after intracerebral inoculation(6), surviving mice in both age groups were tested for immunity in order to see how much of the lower titer in old mice could be attributed to inapparent infection.

Materials and methods. Strains of herpes simplex virus. Strains of virus (excluding HF) were recovered in this laboratory from patients with herpetic infection. The AG strain was isolated by direct intracerebral inoculation of patient's blood into mice. All others were first inoculated onto the scarified corneas of rabbits, and later the infected cornea was transferred to mice by intracerebral inoculation. After several rapid mouse to mouse passages freshly prepared mouse brain suspensions were titrated intracerebrally in mice. Dilutions of virus were prepared in 20% skim milk in saline. The amounts injected were 0.03 cc intracerebrally and 0.2 cc intraperitoneally into groups of 4 or 5 mice. LD_{50} titers were calculated according to the method of Reed and Muench(7). *Tests for immunity.* At the completion of each titration the surviving mice were tested for immunity to the HF strain of virus. This virus was maintained as a 20% infected mouse brain suspension in undiluted skim milk and stored in dry ice. An ampule was thawed, diluted to 10% with physiological saline and 0.03 cc inoculated intracerebrally. Unused portions of the ampule were discarded. *Mice.* The mice were albinos bred in our own laboratory. Young animals of either sex weighing between 9 and 11 g were used when 18 to 21 days old. The older mice were at least 6 weeks of age and weighed over 20 g. Although both males and females were used for the older animals only one of the sexes was used for each experiment. Control animals for the immunity tests were approximately the same age, weight, and where possible, the same sex as the test mice.

Results. The histories of the 12 strains of herpes which were used are listed in Table I. Included in the table is the age of each patient, the clinical type of infection and the source material which yielded the virus. The recovered agents were passed several times by intracerebral inoculation of mice until the incubation period had been shortened. The number of these mouse passages required to stabilize the virus is also listed. It was found that the FR strain even after 11 passages still required 4 days to kill mice. The incubation period for 6 of the strains was approximately 72 hours. The PA and GN strains killed the animals in 2 days while the RE required a period of 36 to 40 hours. The laboratory-adapted HF strain required only about 30 to 36 hours to kill mice.

Intraperitoneal titrations in mice. Titrations in intraperitoneally inoculated mice revealed LD_{50} titers in the old mice which were low; in most cases an end point could not be reached even with a 10% suspension. The results are shown in Table II. On the other

TABLE II. Intraperitoneal Inoculation of Strains of Herpes Simplex Virus into Young and Old Mice.

Strain	LD_{50} titer in mice,* 0.2 cc intraperitoneally		Difference, young/old	
	Young†	Old‡	Logs	Fold
HF	3.5	2.0	1.5	32
JM	3.0	.6⁻	2.4⁺	>300
FR	3.0	1.0	2.0	100
HT	3.3	2.2	1.1	13
EB	2.7	.7⁻	2.0⁺	>100
CF	2.0	.6⁻	1.4⁺	> 25
RE	3.5⁺	1.5	2.0⁺	>100
GN	3.3	.5⁻	2.8⁺	>600
AG	—	—	—	—
WR	4.2⁺	1.5⁻	2.7⁺	>500
PA	3.5	.5⁻	3.0⁺	>1000
BT	3.1	.6⁻	2.5⁺	>300

* Log of reciprocal of LD_{50}.
† Mice 18-21 days old.
‡ Mice 6 weeks of age or older.

hand, as was expected, the titers in the young mice were higher, ranging between $10^{-2.0}$ and more than $10^{-4.2}$; most of them being in the vicinity of $10^{-3.5}$. The figures in the last column demonstrate the increased susceptibility of the young mice. In a majority of the cases there was a difference of at least 2 logs between the 2 age groups. Animals surviving the intraperitoneal titrations were subsequently tested for immunity by intracerebral inoculation with the HF virus. The results are not recorded since only a few mice survived. This can only be interpreted to mean that intraperitoneal inoculation with living virus was effective in protecting only an insignificant number of mice against cerebral challenge.

Intracerebral titrations in mice. The results of the titrations with the various strains of virus in young and old mice are listed in Table III. Comparisons of results between the 2 groups of animals indicated that the higher LD_{50} titers were obtained in younger mice. In fact, except for 2 strains, HF and CF, in which the titers were similar, the old mice were consistently more resistant. In 2 instances the differences in resistance were considerable. More than 1000-fold difference was seen with the GN strain and more than 80-fold difference with the PA and RE strains. All of the remaining strains ranged in the vicinity of a 10-fold difference.

The difference in LD_{50} titers was somewhat narrowed by the subsequent immunity tests. The survivors of each titration were tested intracerebrally with the HF virus and those found resistant were regarded as having had a previous non-fatal infection. When the number of animals with subclinical infections was added to the number of fatal infections, it was found that, although there was little change in the corrected figures in the young mice, there was a considerable upward revision of LD_{50} titers (now infective Dose or ID_{50}) in the old mice. It also suggested that the virus generally continued to multiply in the brains of young mice until death occurred, but that in some of the older mice inoculated with comparable dilutions, resistance was sufficient to overcome the infection and render them immune to later cerebral challenge. The differences in intracerebral resistance between young and old mice which previously were marked when mortality was used as a criterion, were now found to be small. Only the BT and RE strains exhibited a difference of one or more logs.

Discussion. Recovery of an infectious agent and demonstration of an antibody rise during convalescence were the means used in this laboratory to establish a diagnosis of herpes simplex infection. Although these objectives were achieved with current procedures, it soon became apparent that there was a great deal of variation among recently isolated strains. Moreover, it also became obvious to us that better neutralization indexes could be obtained with strains of virus other than the HF strain. The RE virus was chosen not only because of its high titer but also because it yielded good neutralization indexes (8). In a later paper it will be shown that various factors come into play to yield good neutralization indexes. Here are presented the results of inoculation into mice of the recently isolated viruses.

Generally, after intraperitoneal inoculation, young mice exhibited higher LD_{50} titers than old mice. This is in keeping with the observation that resistance to peripheral injection increases with age(9-12). After intracerebral inoculation contrary to expectation young and old mice were not equally susceptible to fatal infection. Higher titers were usually obtained in the young mice and it was some-

TABLE III.
Intracerebral Inoculation of Strains of Herpes Simplex Virus into Young and Old Mice.

Strain	LD_{50} titer in mice, 0.03 cc intracerebrally		Fold difference, young/old	ID_{50}* intracerebral titers in mice		Fold difference, young/old
	Young	Old		Young	Old	
HF	5.2	5.0	2	5.2	5.2	1
JM	5.4	4.3	13	5.4	4.8	4
FR	4.8	3.8	10	5.0	4.5	3
HT	5.4	4.7	5	5.5	4.8	5
EB	4.5	3.2	20	5.0	4.5^+	< 3
CF	5.2^+	5.3^+	1	5.4^+	5.4^+	1
RE	6.4^+	4.5	> 80	6.4^+	4.8	>40
GN	5.0	2.0^-	>1000	5.0	4.5	3
AG	6.0	5.0	10	—	—	—
WR	5.5	4.5	10	5.7	5.2	3
PA	3.7	1.7^-	> 100	4.0	3.5	3
BT	5.0	3.3	50	5.0	4.0	10

* Infective $dose_{50}$.

what surprising to find that in a majority of instances there were significant differences between the 2 groups of mice. Kilbourne and Horsfall(13) reported on a strain of herpes in which suckling mice were more susceptible than 3-week-old weanlings. However, their titers in suckling mice were no higher than some of the titers in weanling mice reported here. In our experiments an occasional animal developed obvious signs of central nervous system involvement but did not die. This differs from the observation of Andervont (14) that once signs appeared the animal died. Florman and Trader(15) reported titers of 10^{-4} in 25 g mice and undoubtedly use of younger mice would have yielded higher titers.

Summary. Twelve strains of herpes simplex virus, 11 recently isolated, and one, the laboratory HF strain were studied in mice. The incubation periods after intracerebral inoculation of a 10% suspension ranged from 30 to 96 hours. After intraperitoneal inoculation old mice resisted between 20-fold and at least 3000-fold more virus than did the young mice. Comparison of the results of intracerebral inoculation of young and old mice indicated that for 3 of the strains there was no difference; for 9 of them the old mice showed at least a 10-fold greater resistance of which 3 showed a 50-fold or greater difference and one more than 1000-fold difference. The differences in intracerebral susceptibility to fatal infection were caused by sublethal infection in older mice. When these were taken into account the differences were negligible.

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Received June 15, 1954. P.S.E.B.M., 1954, v86.