more promptly and at lower concentrations of "purified" DL-homocystine in the presence of 10 millimicrograms of methionine, although some delayed growth occurred in its absence. Certainly, conclusions relative to the metabolic utilization of homocystine by microorganisms must take into account the stimulatory effect of traces of methionine.

Summary. *Brucella suis* utilized either L-cystine, L-cystathionine, or L- or D-methionine as sole sulfur source in an asparagine-lactate medium. DL-homocystine, DL-homocysteine, and DL-homocysteine thiolactone also were utilized under the same conditions, but only to a degree related to their methionine content. Purified preparations of DL-homocystine were not utilized except in the presence of small quantities of supplementary methionine. D-, L-, and DL-methionine were equally effective in promoting utilization of DL-homocystine, and their effect increased to a maximum over a range from 1 to 20 millimicrograms of methionine sulfur per ml. Oxidized casein hydrolysate and methionine sulfone inhibited the activity of D-methionine. The mode of action of methionine and the possible significance of these observations are considered from the standpoint of the intermediacy of homocyst(e)ine in sulfur transformations.

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Immunization of Mice with Inactivated Type 2 Poliomyelitis Virus Against Types 2 and 3 Viruses. (19745)

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Homotypic immunization of mice with inactivated suspensions of Type 2 (Lansing) poliomyelitis virus has been reported(1-3). The amount of vaccine needed for definite protection of a mouse was derived from about 40 mg, or more, of infected central nervous system (CNS) tissue; Schwerdt *et al.*(4) however, immunized cotton rats with less material, about 10 mg (Loring *et al.*(5)).

Shortly after the MEF1 strain, Type 2, of poliomyelitis virus had been successfully adapted to newborn mice(6,7), and after it was found that sera from persons having Type 1 (Brunhilde) virus infection but no past or concurrent infection with Type 2 virus reacted with a complement-fixing antigen prepared with the adapted MEF1 strain(8), the present studies were begun. They concerned the possibility of an increased immunizing power developed by the newborn-mouse adapted MEF1 virus and of cross-protection after vaccination with it against types other than Type 2. In the studies reported here, therefore, vaccines were prepared using both the newborn-mouse adapted MEF1 strain and the standard, unadapted, virus. The MEF1 standard (Type 2) and the Leon (Type 3) were used as challenge agents. The reason for selecting Type 3 as the heterotypic agent was that of expediency, namely, the Leon strain can be propagated in mice by the intraspinal

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TABLE I. Vaccination of Mice with Inactivated Suspension of MEF1 Strains of Poliomyelitis Virus. Challenge with Standard MEF1 Strain.

Exp. No.	Vaccine, 10% brain suspension: Strain	LD_{50}	Inact.	Test for virus	Amt inj. per mouse, mg of tissue	Challenge	Result: Log −1	−2	−3	−4	−5	LD_{50} titer log	LD_{50} control minus test. log	Protection index‖
1	Adapt.	3.2	F*	0/10§	60	I.cer	0	0	0	0		<.5	>3.2	>1600
	Stand.	3.2	F	0/12	60		3/9	0	1	2		1	2.7	500
					0		10	8	8	5	1	3.7		
2	Adapt.	—— Same as Exp. 1 ——			10	”	1/9	1	0	1		<.7	>1.9	>80
	Stand.				10		5/9	6	4	1/9		2.3	.3	2
					0		4/7	9	3	2	0	2.6		
3	Adapt.	—	F	0/10	10	”	3/8	5	0	0		1.4	1.9	80
	Stand.	—	F	0/10	10		8	7	4	4/9		2.8	.5	3
					0		10	8	6	3	1	3.3		
4			F	0/12	10	”	0	0	0	0		<.5	>2.9	>800
					1		4	2	0	1		1.1	2.3	200
	Adapt.	5.6	F†	0/12	10		2/9	2	0	0		<.8	>2.6	>400
					1		3	4	3	0		1.4	2	100
					0		6/9	8	8	4	1	3.4		
5	Adapt.	5.4	UVL‡	0/18	10	”	2	1/8	0	0		.7	3.3	>2000
					1		4	6	5	0		2.1	1.9	80
					.1		10	10	10	8		4.4	−.4	<1
					0		9	10	9	5	2	4		
6	Adapt.	—— Same as Exp. 4 ——			10	I.spinal	7/12	5/13	1/12	0/13		1.6	2.4	250
					0		9/9	9/10	6/9	5/9	3/10	4		

* F = Formalin, .4%. † F = Formalin, .4%, incubated for 17 hr at 37°C. ‡ UVL = Ultra-violet light irradiated. § No. of animals paralyzed or dead over No. inoculated. Under result, 10 mice were inoculated for each dilution unless otherwise stated; *e.g.* 1 = 1 mouse died of 10 inoculated; 1/9 = 1 died of 9 inoculated. ‖ Protection index = antilog of the difference between log LD_{50} of control minus log LD_{50} of test animals.

route of inoculation(9). Studies on Type 1 virus used for challenge are also under consideration.

The properties and general manner of handling the newborn-mouse adapted variant of MEF1 virus have been described(6,7).

Vaccines. Suspensions used for vaccination were prepared as a 10% suspension of infected brain tissue (at first cord was also used) in physiological saline solution. A Waring Blendor homogenized the suspension and inactivation was carried out usually by addition of commercial formalin in final concentrations of 0.2 to 0.4%, and in a few instances by irradiation with ultraviolet light.† The formalinized vaccines were held at 4°C (in one instance after incubation at 37°C for 17 hours) (Table I) and were tested for activity usually at weekly intervals by intracerebral inoculation‡ into 8-15 mice. The mice were held under observation for at least 28 days. Failure of any mice to show neurological signs or to succumb was taken as an indication of inactivation. It is to be noted that this time (28 days) of observation was therefore added to the time required for inactivation by formalin before the vaccine was used in preventive inoculations. As will be seen later, on 2 occasions only, Exp. 7 and 8 (Table II), traces of active virus were found. In Tables I and II are shown the pertinent data for each vaccine, including the LD_{50} titer of the virus before inactivation, concentration of formalin used, time elapsed between the preparation of the vaccine and its preventive inoculation, and the number of mice that showed CNS signs or died following the intracerebral injection of the undiluted (10% CNS tissue) vaccine in the test for the presence in it of active virus.

Immunization. Mice were chosen when 30 to 40 days old and collected in 2 or more groups: control, and vaccination. The vaccine, adequately diluted, was given intraperitoneally in 0.1 or 0.2 ml twice or thrice at 7-day intervals. The animals were challenged 8 to 10 days after the last injection. The total amount of vaccine given to each mouse is

TABLE II. Vaccination of Mice with Type 2 Newborn-Mouse Adapted MEF1 Strain and Intraspinal Challenge with Type 3 (Leon) Virus.

Exp. No.	Vaccine, 10% brain suspension: Passage No.	LD_{50} titer log	Inactivation	Days after prep.	Test for virus	Amt inj per mouse, mg of tissue	Result: No. mice	% dead or paral. at day 7	14	21	28	35	At end of exp.: No. dead	No. paral.	No reaction	Mortality: x^2	p
7	110	5.7	F. .2%	8	3/7	60	71	39	39	30	27	25	1	17	53	44.6	p = <.001
						0	77	77	88	90	88	88	40	28	9		
8	113	5.9	F. .2%	33	7/28	60	59	42	44	29	24	25	1	14	44	6.2	.05>p>.01
						10	63	40	30	22	21	18	0	11	52	9.2	.01>p>.001
						0	60	67	75	73	70	65	10	29	21		
9*	76	5.6	F. .4%	180	0/12	20	23	30	35	30	26	26	0	6	17	6.3	.05>p>.01
	89	5.4	U.V.L.	60	0/18	20	30	33	43	33	20	20	2	4	24	4	p = .05 app.
						0	26	46	65	65	65	65	8	9	9		
10	114	5.5	F. .3%	66	0/30	10	30	53	57	57	57	37	0	11	19	12.1	p = <.001
						0	34	73	85	85	85	85	13	16	5		
11	115	5.6	F. .4%	71	0/14	20	33	43	33	33	27	27	0	9	24	11.5	p = <.001
						0	28	71	79	75	71	75	10	11	7		
12	123	5.6	F. .3%	43	0/15	10	25	8	8	8	8	8	0	2	23	5.6	.05>p>.01
						0	26	38	42	54	54	54	7	7	12		

* Exp. 9 35-42 days under observation; abbreviations as in Table I.

† We are grateful to Dr. George I. Lavin of the Rockefeller Institute for his generous cooperation.

‡ All such experimental procedures were performed with the aid of ether anesthesia.

shown in the tables as the number of mg of infected tissue contained in the total inoculum, 1 ml of 10% vaccine containing 100 mg.

Virus used for challenge and route of inoculation. The MEF1 standard strain was given by the intracerebral route in the usual manner. The Leon strain adapted to mice by Habel and Li(9) was inoculated intraspinally as a 10 or 20% suspension of infected cord tissue. This strain in its 50th passage in mice was sent to us by Drs. Habel and Li, to whom we express our thanks. It has undergone 6 additional passages in this laboratory. In our hands the strain showed the properties described by Habel and Li(9): it was pathogenic only after intraspinal inoculation; virus could be recovered from the spinal cord but not from the brain in affected animals; at dilutions of cord greater than 10^{-1}, mice were rarely affected; the incubation period was 3 days or more, usually from 4 to 10; finally, following inoculation of 10 or 20% suspension, the morbidity in the present tests was 50 to 90% and mortality, 15 to 55%. When the Lansing virus was injected intraspinally into mice, its properties differed wholly from those of the Habel-Li Leon strain(9): the incubation period was shorter, 2-4 days; death almost invariably followed paralysis; and the LD_{50} titer was $10^{-4.0}$ or $10^{-4.5}$. It is clear, therefore, that there was no admixture of viruses in the Leon strain here used and that it retained its characters as defined by Habel and Li.

Results. Table I is illustrative of experiments in which mice vaccinated with the newborn-mouse adapted or with the standard MEF1 virus were challenged with MEF1 standard strain by the intracerebral route, except in one instance of challenge by the intraspinal route. It is plain that a vaccine prepared with the standard MEF1 strain protected mice when the total amount of vaccine given each mouse was equivalent to 60 mg of infected tissue, but no protection was elicited when 10 mg of tissue were given. In contrast, when vaccines were prepared with the newborn-mouse adapted variant of MEF1 strain, as little as 1 mg of infected brain tissue, equivalent to 0.01 ml of a 10% suspension, gave protection against 80 to 200 LD_{50} of virus; 0.1 mg of tissue, on the other hand, failed. Protection could also be achieved against challenge inoculation given by intraspinal route, as shown in Exp. 6 (Table I).

Table II is a summary of tests in which vaccines derived from the MEF1 variant virus only were used, and the challenge was carried out by intraspinal inoculation of mouse-adapted Leon virus. As indicated above, the low degree of pathogenicity of the Leon strain for mice prevented inoculation of serial dilutions of virus; hence the challenge in the experiments reported in Table II comprised 10 or 20% dilutions of virus in a volume of 0.02 ml. Following this intraspinal inoculation, approximately 10% of the mice died immediately, and an additional 10 to 20% of the animals showed paralysis of one or more limbs or marked scoliosis immediately after inoculation, which at times persisted for several days, or even indefinitely. In order to distinguish this type of paralysis induced by technic from that following virus activity, all mice that were paralyzed 24 hours following intraspinal challenge were discarded. Specific paralysis first appeared on the 4th day, rarely on the 3rd, as already indicated. The paralysis in the control animals was of a progressive type, beginning usually in one limb and extending to all extremities within the next 5 or 6 days. The paralysis was flaccid, and was accompanied by considerable atrophy of tissue and loss of weight. Control animals that survived after being paralyzed rarely recovered mobility of the affected limbs. In contrast, vaccinated mice that became paralyzed showed a limited extent of paralysis involving usually only one or 2 limbs, rarely more, and within 8 to 10 days developed recognizable recovery of function. With time more and more animals recovered completely, often as many as half the number paralyzed within the first 7 days. Little or no loss of body weight was noted.

Exp. 7 and 8 (Table II) were carried out with formalinized suspensions which were not ripened long enough for the virus to become completely inactivated. Thus, in Exp. 7 the formalinized vaccine, undiluted and also in dilutions of 10^{-1} and 10^{-2}, was tested for viral activity. Three of 7 mice died when given

undiluted vaccine intracerebrally but none in the groups of 10 mice receiving diluted vaccine. It is of interest that 8 days after preparation of this vaccine, the first dose contained less than one infective unit of virus; when the second and third injections were given 7 and 14 days later, no active virus was present in the undiluted vaccine. Similarly, the vaccine used in Exp. 8, tested undiluted 33 days after preparation, killed 7 of 28 mice. None of all the other vaccines had any recoverable virus at the time when used for the immunization test. There was no significant difference in results, therefore, when a minute amount of active virus was by chance present in the vaccine.

The type of immunity induced by formalinized vaccine is well exemplified by the data in one of the typical experiments, Exp. 7 (Table II). The control animals, once paralyzed, either died or remained paralyzed during the period of observation. In contrast, in vaccinated mice morbidity reached a peak of 50% at 8 days, then diminished gradually, so that at 35 days only 25% of the mice were still paralyzed, the others having recovered. In the same experiment, furthermore, the control animals showed an average loss of 13% of their body weight by the 21st day after challenge, with no subsequent gain or loss. The vaccinated mice, on the other hand, showed no loss of weight by the 21st day and an average gain of 5% by the 35th day. The χ-square test, with Yates correction, applied to the figures of mortality in control and vaccinated animals, exhibited a significant difference in all experiments (Table II).

As the quantity of inactivated vaccine given was small, it became of interest to find out whether circulating neutralizing antibody was produced. To this end, 6 mice from each of the vaccinated and control groups (Exp. 11) were bled under ether anesthesia, on the day of challenge. The sera were tested for neutralizing antibody against the standard MEF1 virus both by means of virus and of serum dilutions. It was found that the undiluted serum from vaccinated mice had a neutralization index of 160, or greater, and that the titer of this serum was $10^{-0.8}$, when tested against 40 LD_{50} virus. In tests for neutralizing antibody against the mouse-adapted Leon strain, no satisfactory results could be secured because of the failure of controls to react regularly even with low dilutions of the virus. A test using the monkey or tissue culture is therefore indicated.

Discussion. It is apparent from the data reported here that the immunogenic potency of the newborn-mouse adapted MEF1 strain of poliomyelitis virus is far greater than that of the standard, nonadapted parent strain, when tested under similar conditions. Thus it was found that an amount of inactivated vaccine deriving from 60 mg of CNS tissue infected with the standard MEF1 strain was needed to induce significant protection; on repeated occasions, 10 mg of this strain failed to protect even against 3 or 4 LD_{50} of virus. In contrast, as little as 1 mg of tissue infected with the adapted strain was sufficient to give a significant, reproducible protection against a homotypic challenge with Type 2 (MEF1) strain of poliomyelitis virus.

The low degree of pathogenicity of the Leon strain (Type 3) for mice precluded quantitative studies. It was evident, however, that there was a certain degree of cross-protection against this Type 3 virus when mice were vaccinated with relatively small amounts of inactivated MEF1 newborn-mouse adapted virus. Thus, of a total of 141 mice vaccinated with 10 or 20 mg of completely inactivated virus (Exp. 9, 10, 11, 12, Table II) only 2 died following intraspinal inoculation of the Leon strain; while in the same experiments 38 of 114 unvaccinated controls died after the challenge (χ-square $= 46.1$; $p < 0.001$).

The fact that large numbers of vaccinated mice developed only transient paralysis when challenged intraspinally with the Leon strain might be interpreted to indicate that there was a degree of virus multiplication which, however, was inhibited at a certain point with result that the extent of paralysis was much less and its duration shorter than in control mice. In controls, on the contrary, the infection was infrequently self-limited and often progressed to cause death. It is not possible at the moment to declare whether this type of resistance in the vaccinated is based on tissue immunity or on circulating neutralizing an-

tibody. Homotypic neutralizing antibody was found in the serum deriving from vaccinated mice; as for heterotypic antibody, experimental limitations prevented a clear-cut result indicating the significance of neutralizing antibody against Type 3 virus. It is of interest to recall in this connection that Sabin (10) found cross-neutralizing antibody to arise in man against Type 2 virus during the early stage after infection with Type 1 poliomyelitis virus. Whether the cross-protection induced with the inactivated MEF1 newborn-mouse variant would extend to include Type 1 has not as yet been investigated. Studies similar to the ones reported here would be indicated in monkeys with Type 1 virus; in these animals the oral route of infection rather than the intracerebral may possibly be used quantitatively(11). In this case more proper conditions for detecting any cross-protection between Types 1 and 2 might be available since it is known that with several other neurotropic viruses a low or even negligible degree of resistance against an intracerebral challenge proves to be considerable on challenge by peripheral routes.

Finally, in view of the greater degree of multiplication of the newborn-mouse adapted MEF1 virus in monkey testicular tissue culture(12), it is suggested that use of the adapted strain in such cultures might prove advantageous for studies similar to those reported here.

Conclusions. Experiments were carried out in mice in which an inactivated suspension of the MEF1 newborn-mouse adapted strain of Type 2 poliomyelitis virus was used as an immunizing agent. It was found that 1) the adapted strain had a far greater immunogenic potency than the standard when the animals were challenged with the homotypic strain; and 2) a significant and reproducible cross-protection was detected when the mice were challenged intraspinally with a heterotypic, Type 3 (Leon) strain.

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Statistical Analysis of Renal Clearance by the Dog.* (19746)

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A lack of adequate references in the literature to the values of normal renal function in the dog made it apparent that a statistical analysis on a large number of data obtained in this laboratory might be of general use. Therefore, a statistical analysis of "normal" renal clearance was performed on the data accumulated over a period of 6 years using 31 female dogs. The measurements of renal function included glomerular filtration (creatinine clearance)(1), p-aminohippurate (PAH) renal plasma flow and Tm(2), arginine Tm(3), and "filtration fraction" of penicillin-G clearance(4,5) compared with filtration fraction of para-aminohippurate clearance. No attempt was made to replicate