ml) were taken at varying intervals after ligation and injection from irradiated and non-irradiated animals and were analyzed for free and total compound by the method of Bratton and Marshall(7).

Irradiated rats received 1000 r of X-irradiation at a rate of 225 r/min from a machine operated at 250 kv, 15 ma, 0.5 mm Cu and 3.0 mm bakelite filters, 27 cm distance. At 1, 24, 48, and 72 hours after exposure, groups of 4 rats were treated as above.

Results. Fig. 1 shows the percent acetylation of PABA at various times after injection. It is apparent that during the first 3 days after exposure the irradiated animals did not differ significantly from the controls in their ability to acetylate PABA, an indication that this particular reaction, requiring CoA, was not affected by irradiation. Similar results were obtained with sulfanilamide on a small number of animals.

Discussion. Acetylation of PABA and its analogues is considered to be a function of the liver, an organ generally believed to be radio-resistant. The fate of CoA in the sensitive hematopoietic tissues, the gonads, and the intestinal mucosa is not known; however, it appears that CoA, measured in terms of an *in vivo* function, is not indiscriminately destroyed. Van Heyningen *et al.*(8) have measured CoA levels in the lens after irradiation, and have found that the concentration decreased as opacities developed; however, the decrease is a delayed effect, and not an immediate action of X-radiation.

Summary. Rats exposed to 1000 r X-radiation retained their capacity to acetylate p-aminobenzoic acid and sulfanilamide for at least 3 days after exposure, an indication that Coenzyme A is not indiscriminately destroyed by whole-body irradiation.

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Received June 16, 1954. P.S.E.B.M., 1954, v86.

Intravenous Infectivity of Type 2 Poliomyelitis Virus in Mice.* (21143)

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After Armstrong(1) had demonstrated the susceptibility of mice to intracerebral infection with Type 2 poliomyelitis virus (Lansing strain), Casals, Olitzky and Anslow(2) were successful in adapting the MEF-1 strain of the same type intracerebrally in suckling mice. It was noted that the concentration of virus in the CNS increased with progressive serial passages in suckling mice. Similar observations have been reported by others(3,4) working with the MEF-1 strain of poliomyelitis virus in young hamsters as well as in young mice. Using the suckling mouse adapted line of the MEF-1 strain, Hammon, Cheever and Sather(5) have demonstrated the susceptibility of 4 to 15 days old mice to intraperitoneal infection. Similar results were observed by Moyer, Accorti, and Cox who inoculated their strain adapted to suckling hamsters intramuscularly or intraperitoneally into 21 to 28 day old mice(3). Though the precise portal of entry of poliomyelitis virus in

* Aided by a grant from The National Foundation for Infantile Paralysis.

man is still uncertain, it is most unlikely that it invades the CNS directly. However, experimental work with small animals has been limited to direct intraneural injection, for the most part, because of lesser susceptibility when the virus is given by other than neural routes. In experimental work that aims to reproduce natural condition of infection as closely as possible, a poliomyelitis strain inducing measurable response in small animals following peripheral infection, might be of considerable value. This report contains the details of a technic that has produced a line of the MEF-1 strain pathogenic for 4 to 6 weeks old mice when administered via the intravenous route; there is also described some of the characteristics of this virus.

Materials and methods. Criss-Cross Passages. The method applied to increase the infectivity of the MEF-1 strain when given intravenously consisted of serial criss-cross passages using tissue culture fluids for the intravenous inoculation in mice and passing the virus recovered from the cord of paralyzed mice into tissue cultures as described earlier (6). *Virus.* The experiment was started with cord material from one mouse that was found paralyzed in a group of 20 mice, 8 days after intraperitoneal inoculation with MEF-1 virus, which had undergone 6 tissue culture passages in this laboratory. The MEF-1 strain was identified as Type 2 poliomyelitis virus; (a) in tissue cultures, using immune sera representing each of the 3 types of poliomyelitis virus; (b) mice immunized with living or with inactivated Type 2 virus were resistant to intravenous challenge with this line. *Animals.* CFW mice of the same source with an average age of 4 to 6 weeks were used for intravenous, intraperitoneal and intracerebral inoculations. The inoculum consisted of tissue culture fluid in a volume of 0.25 ml for the peripheral routes and 0.025 ml for the intracerebral route unless otherwise stated. The response (paralysis or death) as well as the site of paralysis was recorded daily. *Tissue Cultures.* The technic employed for the preparation of tissue cultures was essentially the same as described by Dulbecco and Vogt(7). The cultures were incubated stationary in small bottles at 36°C and inoculated when a complete tissue sheet covered the bottom of the culture vessels. The fluids were harvested as soon as all tissue was degenerated which usually occurred after 3 to 4 days at 32° or 36°C. Tissue culture fluid from the 4th criss-cross passage induced paralysis in 80 to 100% of mice within 10 days when 0.25 ml was injected into a tailvein. A group of 40 mice infected in this way were used to study the distribution of the inoculated virus in serum, brain, cord and feces. Material from 2 mice was pooled for each determination made at 24 hour intervals after infection. Histological examinations were done on cord and brain specimens. The *serum* was obtained from mice by amputation of right fore-leg. The blood was collected in isotonic saline giving a final serum dilution of approximately 10^{-1}. The blood cells were removed by centrifugation and the supernatant fluid containing the serum was titrated for antibody in tissue cultures. *Brains and spinal cords* were removed aseptically and a 1% tissue suspension in distilled water prepared, centrifuged at 2000 RPM for 30 minutes and the supernate considered a 10^{-2} dilution of the original material. *Feces* were ground in a mortar. A 10% suspension by weight was made up in buffered saline (pH 7.2). The large particles were removed by centrifugation at 2000 RPM for 15 minutes. The supernatant fluid was separated and streptomycin and penicillin were added giving a final concentration per milliliter of 500 units and 0.1 mg, respectively. This material was titrated in 10-fold dilution steps in tissue cultures.

Results. Fig. 1 contains the response-time distribution data for mice infected intravenously with undiluted, and with a 10-fold dilution of tissue culture fluid from the 6th criss-cross passage. Forty mice were used for

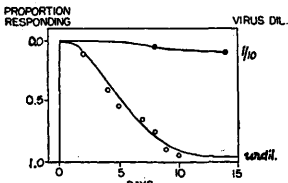

FIG. 1. Response time distribution of mice infected with MEFl intravenously.

TABLE I. Proportion of Mice Responding during Observation Period of 2 Weeks, Inoculated by Different Routes with Different Pools and Doses as Compared to Proportion of Tissue Cultures Responding to Inoculation of Various Virus Dilutions. 50% endpoints are estimated graphically from plots on log probability paper.

Route and dose (ml)	No. criss-cross passages	Negative logarithms of the virus dilutions inoculated 0	1	2	3	4	5	6	7	8	ID_{50}/ml	Geometric mean of ID_{50}/ml values
i.c., .025	6	—	10/10	10/10	9/9	4/10	—	—	—	—	$10^{5.4}$	$10^{4.9}$
	8	—	10/10	10/10	5/10	0/10	—	—	—	—	$10^{4.4}$	
i.v., .25	6	10/10	2/10	0/10	—	—	—	—	—	—	$10^{.9}$	$10^{1.0}$
	8	13/19	6/20	0/20	—	—	—	—	—	—	$10^{1.1}$	
i.p., .25	6	3/10	0/10	—	—	—	—	—	—	—	$10^{.1}$	$10^{.3}$
	8	4/10	1/10	—	—	—	—	—	—	—	$10^{.4}$	
Tissue cultures, .5	0	—	—	—	—	6/6	6/6	5/6	2/6	0/6	$10^{7.0}$	$10^{7.9}$
	6	—	—	—	—	6/6	6/6	6/6	6/6	0/6	$10^{7.8}$	
	8	—	—	—	—	6/6	6/6	6/6	6/6	1/6	$10^{7.9}$	

each dilution and 0.5 ml injected. The 50% response-time end-point of paralysis or death, estimated from the figure, is approximately 5 days. The distribution of the site of paralysis is as follows: 24% showed flaccid paralysis beginning in one or both hind-limbs, 43% in one or both fore-limbs, 25% were found dead or paralysis was so far advanced that the beginning site could not be determined, and 6% remained without any abnormal signs during an observation period of 15 days.

The proportion of survivors in the titration of tissue culture material from the 6th and 8th criss-cross passage in mice inoculated by different routes as well as titrations in tissue cultures are recorded in Table I. The ID_{50} values can be used to estimate roughly the ratio of equally effective doses, *i.e.*, the potency by the different routes relative to the tissue culture method. The relationship may be represented as follows:

ID_{50} in tissue cultures:	Intracer.	Intrav.	Intraper. route
1	10^3	10^7	$10^{7.4}$

Fig. 2 shows the distribution of virus in serum, brain, cord and feces at various intervals after intravenous inoculation. It can be seen that the virus disappeared rapidly from the serum within the first 24 hours. While the virus content of the cord increased after a 24 hour lag period, an even more prolonged lag period was observed when brain material was titrated. No virus was recovered from the feces during a 48 hour period following infection but material collected between 48 hours and 72 hours showed the presence of low concentrations of virus.

On histological examinations† of cord and brain from animals paralyzed at various intervals after infection no histological lesions could be detected in cortical areas of brain specimens collected up to 7 days after infection. But typical lesions were observed in the anterior horns of the spinal cords of animals found paralyzed on the 5th day or later.

Discussion. The results presented demonstrate the susceptibility of 4 to 6 weeks old CFW mice to peripheral infection with a line of the MEF-1 strain of Type 2 poliomyelitis

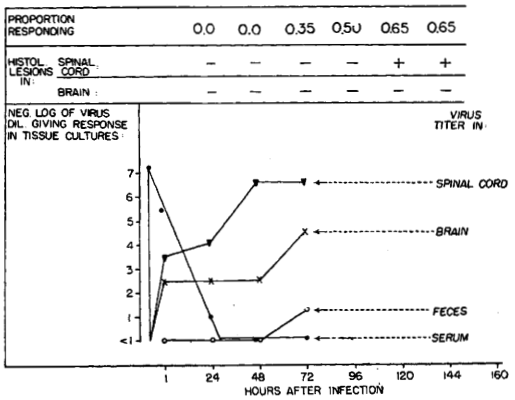

FIG. 2. Distribution of virus in spinal cord-brain-serum and feces of non-paralyzed mice at various intervals after intravenous infection.

† Dr. L. J. Lewis' help in the examination of the histological preparations is gratefully acknowledged.

virus. In order to produce this effect in mice, virus of relatively high infectivity was used. Following criss-cross passages between tissue cultures and mice the infectivity of this strain was increased almost 10-fold when titrated in tissue cultures.

The dissociation between ID_{50} inducing paralysis or death when given peripherally or centrally is striking. From this it might seem that only small amounts of virus injected intravenously will reach the CNS. Poliomyelitis virus has been demonstrated in other than nervous tissues following infection in man as well as in animals (blood, lymphnodes and spleen). Though it is questionable whether poliomyelitis virus multiplies in non-neural tissues *in vivo* the presence of virus in these organs following intravenous inoculation may result in a rapidly developing immunity which might interfere with the occurrence of the paralytic disease when virus doses are given that result in an incubation period of more than 10 days.

Summary. Poliomyelitis virus Type 2 has been successfully propagated in mice following intravenous inoculation. 10^7 ID_{50} tissue culture doses were required to produce 50% response (paralysis or death) after intravenous inoculation in mice. The intravenously inoculated virus disappeared from the serum after 24 hours but was recoverable from spinal cord, brain and feces.

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

Influence of Bean Trypsin Inhibitors on Thromboplastic Properties of Trypsin and Hypochlorite Treated Trypsin.* (21144)

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That trypsin is capable of accelerating the coagulation of blood is well known(1,2) and it has been repeatedly observed that bean trypsin inhibitors retard coagulation(3-6). The present report deals mainly with the thromboplastic properties of trypsin or hypochloric treated trypsin combined with bean trypsin inhibitors. The differentiation of the inhibitors used is briefly considered.

Methods. Coagulation time was determined as described earlier(7). Proteolytic activity was estimated according to the method of Kunitz(8). The inhibition of proteolytic activity was measured by determining the amount of inhibited enzyme required to accomplish the same degree of digestion of a given solution of casein as did a known amount of the untreated enzyme. Inhibitor solutions were added to enzyme solutions at pH 7.4 ten minutes before use. The globulin soy bean trypsin inhibitor, GSTI, was prepared according to the method of Kunitz(9) who has described the properties of the crystalline material(8). The navy bean trypsin inhibitor, NTI, was prepared as follows: 800 g of finely ground navy beans were extracted with 4 liters of water at 50°C for 18 hours. After centrifuging, trichloroacetic acid was added to the supernate to 2.5% concentration. The preparation was heated to 80 to 85°C for 5 minutes and centrifuged while hot. The

* These studies were aided by a contract with the Office of Naval Research and by the Alexander and Margaret Stewart Trust.