thalamus in the regulation of emotional expression is well-known. Hence, these observations may be of some assistance in understanding the changes in personality which sometimes are observed clinically following treatment with these hormones. Since the anatomical modifications induced by cortisone were more general and the rats so treated were in poor condition at the time of autopsy, further studies are being carried on to determine the specificity of the changes elicited by cortisone in the hypothalamus and in the thalamus.

Summary. Administration of ACTH caused chromatolysis in the cells of the paraventricular hypothalamic nucleus. Cortisone affected this nucleus but induced more widespread chromatolysis and vacuolation of thalamic and hypothalamic nerve cells.

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Intraspinal Inoculation of Mice in Experimental Poliomyelitis. (18489)

KARL HABEL AND C. P. LI.

From the Microbiological Institute, National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Md.

A large proportion of the experimental work accomplished in the field of poliomyelitis has been through the use of mice inoculated intracerebrally with the Lansing rodent-adapted virus(1). Low titers, prolonged incubation periods and irregular responses of individual animals have continued to present difficulties in the use of this intracerebral technic(2). The apparent hypersusceptibility of the anterior horn cells of the spinal cord has made it seem obvious that direct inoculation of virus in this location should give better results. Melnick, Horstmann and Ward(3) recognized this and used an intraspinal technic for inoculating monkeys with poliomyelitis materials. However, in spite of a consistently shorter incubation period they concluded that this technic was not practical since a large number of undesirable side reactions occurred and there was evidence that it was a less sensitive method in monkeys than intracerebral inoculation. Howe and Bodian have reported the successful intraspinal inoculation of monkeys with central nervous system virus(4). Other workers in unpublished experiments have attempted intraspinal or intracisternal inoculation of mice through the skin with negative results due to technical difficulties. Using dye solutions the following technic in mice has been developed and its possible application to poliomyelitis research evaluated.

Technic of intraspinal (I.S.) inoculation. Under ether anesthesia the middle of the back of the mouse is wetted down with alcohol and a transverse incision through the skin is made with small iris scissors. The mouse is then held in one hand in such a way that the vertebral column is slightly flexed. Using a ¼ inch 27 gauge short bevelled needle and a 0.25 ml tuberculin syringe the needle point is introduced between the vertebrae at the level of the lumbar enlargement of the cord, slightly to the right of the midline. The direction of the pathway of the needle as slight pressure is applied is at about a 45° angle toward the head and a very slight angle toward the midline. As the point of the needle enters the spinal canal a definite "giving" sensation is felt by the hand holding the syringe. A volume of 0.02 ml is then injected and the needle removed along the same pathway by which it was introduced. No appli-

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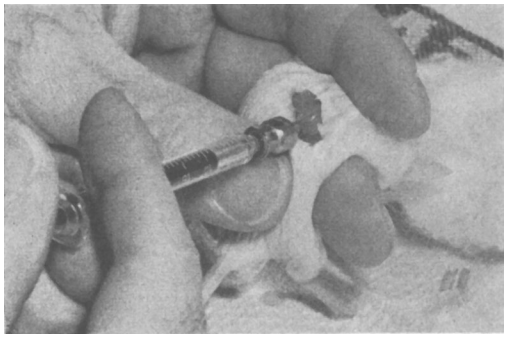

FIG. 1.

cations or dressings are applied to the skin wound which heals without any difficulty. (Fig. 1).

In the preliminary work using dilute aqueous methylene blue as the inoculum, immediately following inoculation dye was observed in the subarachnoid space and the central canal of the cord for varying distances cephalad to the point of inoculation. Once mastered this technic goes rather rapidly. With one assistant anesthetizing the mice, one technician can readily inoculate 100 mice in one hour's time. The age of mice affects the ease with which intraspinal inoculation may be accomplished. Best results seem to be obtained with 4-week-old mice but difficulty of introducing the needle between the vertebrae is not encountered until mice over 6 weeks of age are used.

This is a relatively traumatizing procedure compared with the intracerebral (I.C.) technic. In 2 groups of 200 mice each the mortality during the first 24 hours after intracerebral inoculation was 2% while the same material intraspinally gave 8% traumatic deaths. Occasionally hind leg paralysis will develop as the result of trauma. This almost invariably develops in the first 24 hours and if the animal survives beyond that time the paralysis persists but does not increase unless the animal develops poliomyelitis infection.

Results with Lansing virus. Incubation period and time of death. Lansing infected mouse cords (I.C. passage No. 368) were used to initiate a series of I.S. passages. When relatively heavy inoculum of virus (10^{-1} or 10^{-2} dilutions) were used incubation periods were usually 2 days following intraspinal inoculation. A direct comparison of serial dilutions of virus from the 28th I.S. passage was made I.C. and I.S. using approximately 25 mice per dilution. The results in Table I show the average day of symptoms and day of death at each dilution in the two groups when observed for 35 days after inoculation. The differences in incubation periods are striking. Of a total of 78 mice dying after I.S. inoculation only 6 developed symptoms more than 10 days post-inoculation. In the I.C. group 6 of 92 had incubation periods over 30 days. Deaths in both groups usually occurred within 24 to 48 hours from the onset of paralysis.

Site of paralysis. In Table II a comparison of the location of the paralysis following I.S. and I.C. inoculation of large groups of mice with I.S. Passage No. 28 shows that the proportion of mice dying with preceding paralysis when checked only once daily is about the same in both groups. However, there is a definite reversal in the predilection of site of early paralysis, the I.S. inoculated virus most frequently causing hind leg paralysis while I.C. virus usually involving the front legs first. This difference in the predilection of the site of paralysis by the two methods of inoculation would of course be expected since by I.S. inoculation the virus is deposited in the lumbar section of the cord where innervation for hind leg muscles arises.

Titrations of virus content. A number of comparative titrations of various Lansing virus suspensions (all mouse CNS materials) have been made by I.S. and I.C. technics. In Table III, 14 of these comparisons show that I.S. titers were higher in 13 instances and the average results were 0.7 of a log higher in the I.S. series. All titrations were done with 10 mice per dilution using half log differences in the serial dilutions. In an attempt to evaluate the reproducibility of results of titrations by the I.S. technic the experiment summarized in Table IV was set up. Here serial half log dilutions of I.S. Passage No. 28 Lansing virus were inoculated into 25 mice by I.S. and 25 by I.C. technics but for each dilution were divided into 5 groups of 5 mice each and observed for 35 days separately. This resulted in what was really a titration using 5 mice per dilution but re-

TABLE I. Incubation Period IS Versus IC Inoculation with Lansing Virus.

	Route inoculated	Virus dilutions† 2	2.5	3	3.5	4	4.5	5	5.5	6
Mortality*	IS	—	—	22/24	18/22	12/22	9/25	4/22	7/24	6/22
	IC	24/25	24/24	17/24	10/25	7/25	2/24	6/24	2/27	—
Avg incubation time‡	IS	—	—	2.4	3.8	4.3	5.1	4.7	7.0	18.0
	IC	14.4	15.5	16.7	19.1	17.5	13.0	6.4	8.5	—
Avg death time‡	IS	—	—	4.2	6.0	6.0	7.0	6.0	7.5	18.8
	IC	15.5	17.6	18.9	19.9	18.7	15.5	10.8	9.5	—

* Deaths over total mice inoculated.
† IS mouse passage No. 28 Lansing virus.
‡ Days from inoculation.

TABLE II. Location of Paralysis in IS and IC Inoculated Mice—Lansing Virus.

Route of inoculation*	No. mice	Total dead	Dead with paralysis No.	%	Paralyzed in: Front leg first	Hind leg first	Ratio Front leg/hind leg
IS	161	82	46	57	19	33	0.6
IC	200	96	47	49	41	12	3.4

* Inoculated with various dilutions from 10^{-3} to 10^{-6} of IS mouse passage No. 28.

TABLE III. Comparison of Titers of Lansing Virus by IS and IC Technics.

Test	IS	IC
1	5.4	4.5
2	5.0	4.4
3	4.6	3.8
4	4.5	5.0
5	5.1	4.9
6	4.7	4.2
7	4.2	3.7
8	4.0	3.4
9	4.4	3.6
10	4.4	3.3
11	5.1	4.1
12	5.0	4.0
13	4.9	4.2
14	4.4	3.6
Avg	4.7	4.0

peated 5 times. By combining the results of 1, 2, 3, 4 or all 5 of these groups of mice by all possible combinations the results for repeated tests using 5, 10, 15, 20 or 25 mice per dilution could be obtained. In Table IV it is seen that the greatest variation for I.S. inoculation was 0.4 of a log in the 5, 10 and 15 mice per dilution tests and was only 0.1 of a log when 20 mice were used. After I.C. inoculation the greatest variation was 0.5 of a log when only 5 mice per dilution were used but was still 0.2 of a log when 20 mice were employed. As a further check on the consistency of the results by the I.S. technic, two simultaneous titrations by two technicians were performed by both technics. Table V shows the results in two tests to be perhaps slightly more consistent with the I.S. technic.

Distribution of virus between brain and

TABLE V. Comparisons of Titrations by Two Technicians.

Operator	Test No. 1 IS	IC	Test No. 2 IS	IC
A	4.2	3.7	4.4	3.6
B	4.0	3.4	4.4	3.3

TABLE IV. Repeatability of End-points in Titrations of Lansing Virus by IS and IC Technics.

		Combination of groups 1	2	3	4	5
No. mice per dilution		5	10	15	20	25
No. titrations*		5	10	10	5	1
Avg 50% end-points	IS	4.4	4.4	4.4	4.4	4.4
	IC	3.7	3.6	3.6	3.6	3.6
Extreme 50% end-points	IS	4.2-4.6	4.2-4.6	4.2-4.6	4.4-4.5	—
	IC	3.4-3.9	3.4-3.8	3.5-3.8	3.5-3.7	—

* Lansing IS mouse passage No. 28.

TABLE VI. Virus Content of Brain and Cord from I.S. and I.C. Lansing Passage Mice.

		I.S. inoc.				I.C. inoc.			
		Cord titer		Brain titer		Cord titer		Brain titer	
		I.S.	I.C.	I.S.	I.C.	I.S.	I.C.	I.S.	I.C.
I.S.	P30	4.17	4.21	>1.5	>1.5	3.76	3.59	3.28	2.43
I.C.	P379	4.81	4.62	>1.5	>1.5	4.29	4.0	3.15	3.43

cord. Preliminary experiments had indicated that the brains of mice paralyzed after I.S. inoculation of Lansing virus were very low in virus content. In order to determine if this was a characteristic of the I.S. passage virus the experiment summarized in Table VI was performed. Here the 30th I.S. passage virus was inoculated by the I.S. route at a 10^{-3} dilution into one group of mice and I.C. at 10^{-2} dilution in a second group. At 48 hours after inoculation paralyzed mice in each group were killed, the brains and cords being harvested separately. Each pool of brains and cords were then titrated in mice by both the I.S. and I.C. routes. Similarly the 379th I.C. passage Lansing virus was inoculated I.S. at 10^{-2} and I.C. at 10^{-1} and the brains and cords harvested from mice paralyzed at 48 hours, with subsequent I.S. and I.C. titrations. As seen in Table VI with both original passage viruses the brains of mice inoculated I.S. had practically no demonstrable virus whereas the brains of those inoculated I.C. had good titers although not as high as the corresponding cords. There did not appear to be consistent or significant differences in the titers obtained with the I.S. strain and the I.C. strain of virus.

Virus neutralization tests. Since one of the chief applications of a new technic which would shorten the observation time required in the use of Lansing virus in mice would be in routine neutralization tests of serum samples, the I.S. and I.C. technics were compared against two serums. Lansing I.S. Passage No. 28 virus diluted 1/50 was mixed with equal parts of the two serums undiluted and in four-fold dilutions to 1/1024 for I.C. inoculation. The same virus diluted 1/500 was mixed with a second set of similar serum dilutions for I.S. inoculation. One serum specimen was from a monkey hyperimmunized with Lansing monkey cord virus and the other was human serum globulin obtained from a commercial biological laboratory. The two serum samples were inactivated at 56°C for 30 minutes before use. Serum-virus dilutions were held at 4°C for 4 hours then inoculated into groups of 10 mice for each dilution. Virus of each series was also mixed with normal monkey serum (¼ dilution) and after ice-box incubation serial dilutions in this serum were made to determine the titer of the virus used in the test. For the I.C. test 1.4 logs of virus had been used but only 0.9 of a log in the I.S. test. Table VII shows good correlation between the results of neutralization by the two technics.

TABLE VII. Serum Neutralization Tests by IS and IC Inoculation.

	IC	IS
Serum A	1/461†	1/466
Serum B	1/525	1/256
Virus*	$10^{1.4}LD_{50}$	$10^{0.9}LD_{50}$

* Mouse passage No. 28 Lansing virus.
† End-points of serum dilutions giving 50% neutralization of virus.

I.S. inoculation of other laboratory animals. This technic has been relatively easily applied to rabbits, guinea pigs, hamsters and cotton rats and is made less difficult in these species if younger animals are used.

Results with other neurotropic viruses. Titrations by ten-fold dilutions were made by I.S. and I.C. inoculation in parallel series of mice using mouse adapted strains of fixed rabies, eastern and western equine encephalomyelitis, St. Louis encephalitis and choriomeningitis viruses. No differences were noted in titer or incubation period for eastern equine encephalomyelitis or choriomeningitis viruses. Both western equine and St. Louis encephalitis viruses had slightly higher titers and shorter incubation periods by the I.S. route. Rabies virus titered to the same endpoint by both technics but the I.S. inoculation resulted

in a shorter incubation period.

Discussion and summary. A relatively simple technic for the intraspinal inoculation of mice which lends itself to routine laboratory use has been described. For Lansing poliomyelitis virus, it seems to be a more sensitive method of detecting virus than the intracerebral technic. The quantitation of virus titers by this method is consistent between two different technicians and on repeated tests by one technician from any one set of virus dilutions. It can be applied to the routine testing of serums for Lansing antibodies. The chief advantage of the technic is the marked shortening of the incubation period of Lansing infection in mice as compared to that following intracerebral inoculation.

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Effects of Ultrasonic Vibrations on Nerve Tissues.* (18490)

V. J. WULFF, W. J. FRY, DON TUCKER, FRANK J. FRY, AND CARLTON MELTON.
From the Departments of Physiology and Electrical Engineering, University of Illinois

Numerous studies have already been published concerning the effects of ultrasound on living systems [Gregg(1), Harvey(2,3)]. Among these there are several kinds of experiments on the effects of ultrasound on nerve tissues, both excised(2) and *in situ* (4,5). Further, Lynn and Putnam(6) determined the effect of ultrasound on the brains of mammals, and demonstrated functional disturbances, neurone damage and even death of organisms.

The propagation of ultrasonic vibrations through living tissues is accompanied by a variety of physical factors such as: (1) heating caused by absorption of acoustic energy; (2) periodic pressure changes; (3) radiation pressure; (4) streaming or flow in viscous media; and, (5) high temperatures and pressures associated with cavitation, defined as the formation of holes in liquid media. Any one or all of these factors may produce significant and measurable changes in the state of a living system. The experiments described above do not afford the opportunity to determine how ultrasound produces its effect. The experiments to be described were designed to determine whether tissue heating is a major factor contributing to the effect of ultrasound on nerve tissues.

Materials and methods. The following types of preparations were employed:

(1) The ventral abdominal nerve cord of the crayfish was dissected out in its entirety and immersed in van Harreveld's solution. The sixth abdominal ganglion was always removed and either part or all of the remaining cord was used. The excised nerve cord was mounted in an electrode chamber in contact with 2 glass tubes filled with a salt solution. These salt bridges made contact with small calomel half cells which were, in turn, connected to the amplifier. The spike potentials were amplified by a condenser coupled amplifier and recorded by photographing the trace of a cathode ray tube.

(2) Intact frogs were placed vertically in the path of the sound beam by mounting the animal (dorsal surface down) as firmly as possible on a piece of plywood cut to fit the sound tank. A 2.0 cm hole, centered so

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