meprobamate does not exhibit this difference in activity in the 2 animal preparations. This suggests that at the spinal level, both drugs exert similar net effects but at supraspinal levels their actions are different. Mephenesin, another relaxant drug, does act on supraspinal reflex transmissions(7) particularly the reticular facilitatory centers influencing spinal reflexes(8). It is likely that styramate which showed other similarities with mephenesin, may affect such areas. A generalization of our conclusions would lead to the possibility that centrally acting skeletal muscle relaxants may independently affect reticular facilitatory centers without influencing the ascending arousal system. Styramate and mephenesin are examples of this type of drug. On the other hand, there are relaxants *e.g.* pentobarbital, which at same dose level, depress both facilitatory and arousal systems. Meprobamate, another relaxant drug, does not affect the facilitatory system, assuming this is the correct interpretation of our data. Its action on arousal is controversial(9,10) but whether it depresses arousal or not, it still appears to belong to a class different from styramate and mephenesin. At this time, it can only be speculated whether differences encountered in our investigation between styramate and meprobamate are in some way related to their differences in anticonvulsant activity. Styramate in mice is much more effective in preventing extensor tonus spasm of hind leg following electroshock, Metrazol and strychnine induced seizures than meprobamate(1).

Summary. Effects of styramate and meprobamate upon spinal polysynaptic (SCF) and monosynaptic (patellar) reflexes in decerebrate and spinal cats were investigated. Both drugs selectively depressed the spinal polysynaptic reflex, however styramate was much more potent in decerebrate than in spinal cats. The significance of this selective effect was discussed in relation to other actions of muscle relaxants.

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Intracerebral Passage of Sarcoma 180 in Mice and Hamsters.* (24901)

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A number of investigators have transplanted neoplasms, including those of human origin, into brains of laboratory animals. Early literature has been reviewed by Sailer (1) and Vazquez-Lopez(2). Recently other investigators(3-7) have used intracerebral (IC) route for biologic studies of various experimental neoplasms. Sarcoma 180 is an easily transplantable murine tumor, growing well in any strain of mice(8) and has also been adapted to growth in tissue culture (TC) (9). However, despite these facts it has not been previously passed intracerebrally. It seemed probable therefore that

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IC passage of this tumor might offer a means of uncovering its variants and of altering its host range. The present report describes serial IC passage of sarcoma 180 in mice and subsequently in suckling hamsters. A TC strain of sarcoma 180 cells was also inoculated into mice for comparative study.

Materials and methods. Sarcoma. Sarcoma 180 was from 2 sources: (a) A subcutaneous tumor in DBA mouse kindly supplied by Dr. Margaret Kelly of Nat. Cancer Inst., and (b) A tissue culture of tumor cells (in 18th tissue culture passage) supplied by Dr. Harry Eagle of Nat. Inst. of Allergy and Infectious Diseases. Suckling Swiss mice, less than 1 day old, and adult Swiss mice, 3-4 weeks old, were from NIH randomly bred or inbred stock. Hamsters (*Mesocricetus auratus*) usually less than 1 day old, were used. All animals were observed for 4 weeks after inoculation. *Tissue cultures.* Passages of sarcoma cells were made at weekly intervals in 32 oz. Blake bottles, using Eagle's medium with 10% horse serum. Cells were scraped off the glass, centrifuged 350 rpm 15 minutes, and transferred to fresh bottles. For animal inoculation, cells were suspended in Eagle's medium, with 2% calf serum. For cell counts, 0.5 ml of this suspension was mixed with 1 ml of 0.1% crystal violet in 0.1 M citric acid. *Passage in animals.* Tumor or brain tissue was minced with scissors and 20% suspensions by weight were made in Eagle's medium with 2% calf serum. After about 5 minutes, the supernate was inoculated into animals. For titration, the supernate of a 10% suspension usually containing about 5 x 10^6 sarcoma cells/ml was used. These cells were identifiable by their large size and dark staining characteristics (Giemsa). *Histology.* Impression smears of brains were stained by the Giemsa method. Tissues fixed in 10% buffered formalin were sectioned and stained with hematoxylin-eosin.

Results. Mice. Sarcoma 180 from DBA mouse was passed intracerebrally (IC) to an adult C_3H mouse. The brain tissue from this mouse was used to establish 2 passage lines: (a) IC in suckling Swiss mice. (b) IC in adult Swiss mice. A third passage line was initiated directly from the DBA mouse and carried in adult Swiss mice by subcutaneous (SC) route. These have been maintained for 30, 25, and 12 passages respectively.

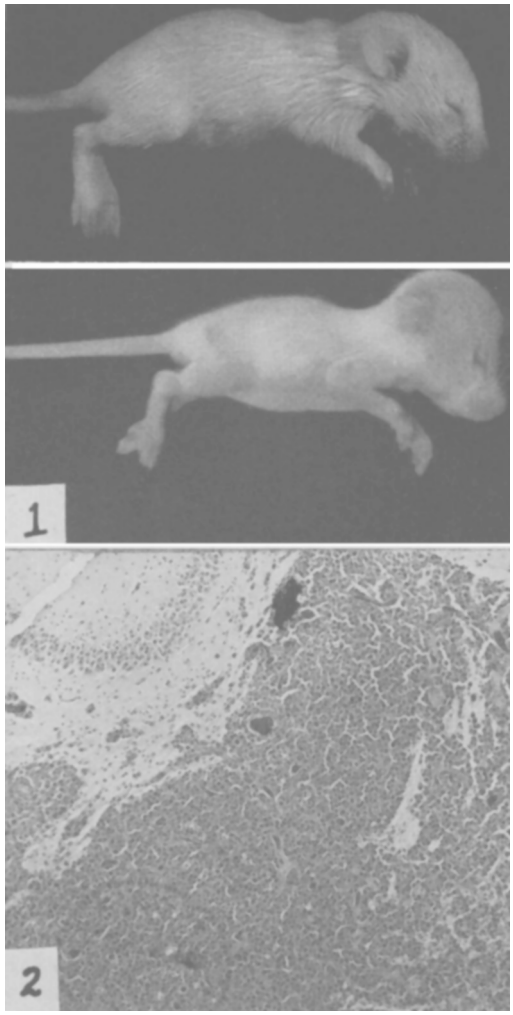

FIG. 1. Normal mouse (above) and mouse with brain sarcoma (below) showing enlargement of head.

FIG. 2. Section of brain of a suckling mouse inoculated IC with sarcoma 180 and sacrificed on 7th day after inoculation, showing sarcoma cells filling lateral ventricle and invading cerebral tissue. Hematoxylin and eosin, ×73.

Suckling mice inoculated IC showed swelling of head in 5 to 6 days (Fig. 1), and failed to gain weight normally. Occasionally, a tumor protruded through the skull. Death usually occurred in 9-11 days. Adult mice inoculated IC did not show cranial enlargement but occasionally developed leg paralysis; they usually died in 9-13 days. All IC passage

TABLE I. Results of Injection of a Subcutaneous Sarcoma Suspension into Adult and Suckling Mice by Different Routes.

Source of suspension	Inoculum (ml)	Route	Mice		No. with tumors	No. died	Avg time (days) until death
			Age	No.			
From 7th subcut. passage	.05	Subcut.	Adult	30	4	0	
	.01	Intracer.	Suckling	10	10	10	12.6
	"	Intraper.	"	11	10	10	20.3
	"	Subcut.	"	12	4	1	17
From 8th subcut. passage	.1	Subcut.	Adult	30	22	3	26
	.01	Intracer.	Suckling	10	10	10	13
	"	Intraper.	"	10	10	10	13.7
	"	Subcut.	"	10	9	4	17.5

mice, suckling and adult, inoculated with the 20% suspension, died.

Brains of mice inoculated IC were unusually soft, with whitish surface areas. Impression smears of cut surface of brain showed numerous round or oval cells with blue cytoplasm and large purple nuclei. Some cells were in bundles. There was great variation in size and shape. These cells, not found in uninoculated mice, were considered to be from the sarcoma.

All mice of the SC passage series developed tumors at site of injection but only 25% of these mice died during period of 4 weeks. In some mice the tumor regressed and consequently the mice recovered. The 20% brain suspensions from the IC series in suckling mice also caused tumors in all mice by the SC route.

Suspensions of tumor tissue from 7th and 8th subcutaneous passages were inoculated into suckling mice by IC, SC, and intraperitoneal (IP) routes. Results (Table I) indicated that the IC and IP injection of suckling mice caused rapidly fatal tumors, and that suckling mice were more sensitive than adults when injected SC.

Brain suspensions from 22nd, 26th, and 31st IC passages in suckling mice produced tumors in all adult mice inoculated SC (0.1 ml) at a dilution of 10^{-1} (Table II) but did not give 100% "takes" at higher dilutions. The same suspensions given IC to suckling mice (0.01 ml) killed all mice at dilutions as high as 10^{-3}, and once at 10^{-4} (Table II). The IC injection of suckling mice is thus about 1,000 times more sensitive for detecting tumor cells than SC injection of adults.

Hamsters. A brain suspension from 16th IC passage of suckling mice was inoculated intracerebrally into suckling hamsters, less than 1 day old. A number developed neurologic signs including paralysis of legs. Some died in 2 or 3 weeks while others recovered. Second passage was not successful. However, after 6 alternate IC passages in suckling mice and hamsters, the sarcoma cells became adapted to continuous IC passage in hamsters, killing more animals in a shorter time. To date, it is in its 12th consecutive hamster passage, in which all inoculated hamsters succumbed except a few which were killed. Inoculation of brain suspensions of these hamsters caused sarcoma in mice.

In earlier IC passages, some hamsters developed swollen heads in second or third week when the animals were already of good size. Some of these were sacrificed and at autopsy a large cavity filled with bloody fluid was found in the brain which was reduced to less than one-half its normal size. Sarcoma cells were found in brain tissue and bloody fluid.

Histopathology. In IC inoculated adult mice there was proliferation of undifferentiated cells which completely filled the meningeal spaces. Proliferation of tumor cells occurred around and in the arachnoid membrane, and around penetrating blood vessels, but no invasion of brain tissue was seen. Similar lesions were found in IC inoculated suckling mice but proliferation of tumor cells was much greater and there was invasion of brain tissue (Fig. 2). In 2 suckling mice there was almost complete replacement of brain by tumor tissue. There were areas of destruction and hemorrhage in the remaining brain tissue surrounding tumor masses. In IP inoculated suckling mice there were large tumor masses

TABLE II. Titrations of Sarcoma 180 (Brain Suspension) in Suckling and Adult Mice by Different Routes.

Source of brain suspension	Dilution	Inoculum (ml)	Route	Mice		No. with tumors	No. died	Avg time (days) until death
				Age	No.			
22nd intracer. passage in suckling mice	10^{-1}	.1	Subcut.	Adult	30	30	11	21.1
	10^{-2}	"	"		30	24	5	22.2
	10^{-3}	"	"		30	6	0	
26th intracer. passage in suckling mice	10^{-1}	.01	Intracer.	Suckling	11	*	11	11
	10^{-2}	"	"		7	"	7	12
	10^{-3}	"	"		8	"	8	14.5
	10^{-4}	"	"		11	"	8	16.4
	10^{-5}	"	"		11	"	2	20
31st intracer. passage in suckling mice	10^{-1}	.1	Subcut.	Adult	20	20	4	26
	10^{-2}	"	"		20	16	0	
	10^{-3}	"	"		0	0	0	
	10^{-1}	.01	Intracer.	Suckling	9	*	9	9.3
	10^{-2}	"	"		9	"	9	10.6
	10^{-3}	"	"		9	"	9	11.4
	10^{-4}	"	"		9	"	9	15.3
	10^{-5}	"	"		9	"	7	16.3
22nd intracer. passage in adult mice	10^{-1}	.03	Intracer.	Adult	10	"	10	11
	10^{-2}	"	"		10	"	10	14.3
	10^{-3}	"	"		10	"	10	15.5
	10^{-4}	"	"		10	"	9	16.6
	10^{-5}	"	"		10	"	0	

* Those mice which died after intracer. inoculation all developed swollen heads.

throughout the abdomen, surrounding the liver, spleen, adrenals and gastrointestinal tract. There was invasion of liver tissue and lungs on one occasion with many small tumor nodules throughout each organ. In some young hamsters, tumor cells appeared to have a predilection for the lateral ventricles. They proliferated there, undergoing necrosis and causing dilatation of ventricles. The proliferation of cells seemed limited, necrosis occurred and the end result was hydrocephalus.

Inoculation of tissue culture sarcoma 180 cells into mice. The strain of TC sarcoma cells was inoculated at its various passage levels into suckling mice by the IC route and into adult mice by the subcutaneous route. A typical titration experiment is represented in Table III. About 500,000 sarcoma cells were required to give 100% "take" by subcutaneous route in adult mice. This finding is in agreement with that of Zahl(10) who used cell suspensions made from subcutaneous tumors. After IC inoculation of suckling mice with comparable doses, the TC sarcoma cells behaved differently from those of mouse origin. The death rate was much lower and those mice which eventually died survived much longer when TC sarcoma cells were used than when brain tumor tissue was used. Protruding tumors on head were often found when TC sarcoma cells were inoculated IC in suckling mice. In TC, sarcoma cells were of

TABLE III. Titration of 25th T. C. Passage of Sarcoma 180 Cells in Adult and Suckling Mice by Different Routes.

Inoculum (No. of cells)	Route	Mice		No. with tumors	No. died	Avg time (days) until death
		Age	No.			
500,000	Subcut.	Adult	10	10	0	
50,000			10	4	0	
5,000			10	0	0	
50,000	Intracer.	Suckling	11	11	6	20.5
5,000			11	11	0	
500			11	7	2	17.5
50			10	6	0	
5			9	4	1	18

2 types, rounded or spindle-shaped as observed previously by Abercrombie *et al.*(9). This was in contrast to impression smears from brains of mice or hamsters inoculated with either TC or animal passage sarcoma, where only the rounded forms were found.

Discussion. This study, in which suspensions of tumor cells were injected IC with syringe and needle (as distinguished from earlier use of a trocar) gave clear cut results. About 5 x 10^5 sarcoma cells from mouse brain gave 100% "take" by subcutaneous route in adult mice, but only 1/1,000 of that quantity caused 100% mortality by IC route in suckling mice.

Although sarcoma 180 is traditionally regarded as strictly species specific(8), in our study it has been adapted to hamsters, and more than 12 consecutive passages have been carried out in these animals. In early passages some hamsters developed hydrocephalus. According to Murphy and Sturm(11) positive results of heterologous transplantation were due to absence of a lymphocytic defensive reaction in the nervous system, but tumors were not produced when the implanted fragment touched the ventricular surfaces, which reacted like subcutaneous tissue. The question of the exact mechanism underlying development of hydrocephalus in hamsters cannot be answered at present. Further studies are now in progress.

Sarcoma cells after more than 25 passages in tissue culture produced sarcoma in adult mice regularly after subcutaneous injection. Suckling mice inoculated with TC sarcoma cells by the IC route showed a much lower mortality rate and longer survival time than those inoculated with sarcoma cells from mouse brain. Whether these 2 lines of sarcoma cells are true variants or not remains to be determined.

Summary. Mouse sarcoma 180 has been through a total of more than 25 IC passages in suckling and adult mice. Sarcomatous brain suspensions also produced sarcomas in adult mice by subcutaneous route. The IC route in suckling mice was about 1,000 times as effective as subcutaneous route in adult mice. After serial IC passages in suckling mice the sarcoma was adapted to suckling hamsters by IC injection and to date more than 12 serial passages have been carried out in this animal. A strain of sarcoma 180 cells which has undergone more than 25 TC passages produced sarcomas regularly in adult mice after subcutaneous injection and required about 500,000 cells to give 100% "takes." However, when inoculated IC into suckling mice these cells seemed less neurotropic than mouse (IC) passage sarcoma cells.

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