TABLE I.

	M.P.	Neutral equivalent	% nitrogen	M.P. methyl ester	M.P. *p*-bromophenacyl ester
Acid isolated from milk	188°	185	7.7	84°	151-2°
Hippuric acid	188°	179	7.8	85°	152°

was dissolved in chloroform and chromatographed on a column of Decalso.[1]

It was found that the chloroform which passed through the columns contained appreciable amounts of a substance which separated in crystalline form when the solutions were concentrated. Recrystallization of this material from an alcohol-ether mixture yielded colorless needles of a nitrogen-containing compound, m.p. 84°, which by alkaline hydrolysis yielded an acid, m.p. 188°. This acid contained 7.7% N and possessed a neutral equivalent of 185. Additional amounts of the free acid were obtained by acidification of the ethyl acetate-extracted esterification residues. These data indicated that the substance isolated from the esterified milk concentrate was the methyl ester of hippuric acid. A mixture of the isolated free acid with hippuric acid showed no depression of the melting point. Further confirmation of the identity of the isolated compound was obtained by preparation of the *p*-bromophenacyl ester from the sodium salt of the acid. The melting point of this derivative agreed with that of *p*-bromophenacyl hippurate. The data are summarized in Table I.

Quantitatively, only a minimum value for the amount of hippuric acid in milk can be derived from this work, inasmuch as there was no way of knowing how much hippuric acid had been lost during the various procedures used for the preparation of the biotin concentrate from milk, and no attempt was made to obtain a quantitative separation of the hippuric acid present in the biotin concentrate. From 75 g of concentrate, representing approximately 1160 kg of milk, there were obtained 11 g of hippuric acid; this would indicate an original minimum concentration of 10 γ hippuric acid per gram of milk.

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Distribution of Poliomyelitis Virus in Central Nervous System of Mice Paralyzed After Intracerebral Inoculation.*

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Tests using mice inoculated with the Lansing strain of poliomyelitis virus have been handicapped by irregularities both in morbidity and in the length of incubation periods. Some of the factors related to these difficulties such as the strain of mice[1] and the pH of the inoculum[2] have been reported. Also it has been noted that serial passage of cord and brain stem material from the first mice of an inoculated group to become paralyzed resulted in shorter incubation periods while passage from the last mice to become paralyzed resulted in longer incubation periods. The age of mice was not found to be an important

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1 Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 86.

2 Hammon, W. M., and Izumi, E. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 579.

TABLE I.
Incubation Periods in Mice After Intracerebral Inoculation with Cord Suspensions in Saline of Different pH.

Intervals between inoculation and onset of paralysis	Inoculated suspension pH 7		Inoculated suspension pH 3.7
	No. of mice	% of total	No. and % of total
1-5 days	90	46	51
6-10	59	30	33
11-15	17	9	12
16-20	4	2	3
21-30	3	2	0
Survived >30	22	11	1
Total	195		100

factor in susceptibility.[3] Some workers[4] have obtained consistent results by using large pools of spinal cords from young mice which developed paralysis during the first few days after inoculation. These observations suggested that the potency of virus present in the central nervous system varied inversely with the length of incubation period. The present study was made in order to determine the amount of virus contained in the brain and spinal cord of mice which had developed paralysis at different intervals after intracerebral inoculation of the virus.

Carworth Swiss mice 3 to 5 weeks old were injected with mouse-passage virus of the Lansing strain of poliomyelitis. Each received intracerebrally 0.03 ml of a 10% suspension of infected mouse spinal cords in saline of pH 7. The numbers of mice found paralyzed or dead after inoculation during a period of observation of one month are shown in Table I. The results obtained with a smaller group of mice similarly treated, except that the inoculum was in buffered saline of pH 3.7 instead of pH 7, also are listed. It is seen that 75% of the mice which received the inoculum in saline of pH 7 developed paralysis within 10 days while 10% survived for at least one month. The respective figures for the group which was given the inoculum in saline of pH 3.7 were 85% and 1%. This effect of pH of the inoculum is in accord with results of work previously cited.[2]

The mice which were inoculated with the suspension of pH 7 and which developed paralysis after injection, were sacrificed by

TABLE II.
Virus Titer of Pooled Brains or Cords of Mice Developing Paralysis and Killed at Various Intervals After Intracerebral Inoculation.

Intervals between inoculation and onset of paralysis	Pooled material tested	No. of mice paralyzed after inoculation of 6 mice with each dilution.* Dilutions				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1-5 days	Brains	6	4	3	0	0
	Cords	5	6	5	2	0
6-10	Brains	2†	0	0	0	0
	Cords	6	5	4	2†	0
11-20	Brains	0	0	0	0	0
	Cords	6	5	4	2	0
21-30	Brains	2†	0	0	0	0
	Cords	6	5	4	2	0

* Observed for 60 days.
† Found dead.

[3] Young, L. E., and Merrell, M., *Am. J. Hyg.*, 1943, **37**, 80.

[4] Kramer, S. D., Personal communication.

etherization. First the brain, then the spinal cord, the latter including the medulla, were removed separately and stored whole in dry ice until used. Incidental results of titrations of infected mouse spinal cords after various conditions of storage indicated that virus might remain at its original titer for at least 3 weeks when kept as a 10% suspension in saline at 4° or −70°C.

Material from the mice killed during each successive interval of 5 or 10 days was pooled. These pools of brains and spinal cords, respectively, were tested at one time by intracerebral injection of groups of 6 mice with serial dilutions in saline of pH 7. The results are shown in Table II. It is evident that mice which became paralyzed during the first 5 days after inoculation had essentially the same amount of virus in the brain as in the spinal cord-medulla. After 5 days, however, little or no virus was recovered from the brain although the various pools of cord and medulla collected at different intervals up to 30 days contained essentially the same amount of virus at all times.

Summary. Mice which developed paralysis within 5 days after an intracerebral inoculation of Lansing strain of poliomyelitis virus had approximately as much virus in the brain as in the spinal cord-medulla. After 5 days little or no virus was recovered from the brain although pools of cord-medulla of mice which became paralyzed up to 4 weeks after injection were found to contain as much virus as did the pool of the first 5 days.

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Delayed Lethal Effect of X-rays on Fibroblasts Cultivated *in vitro*.

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Studies on the effect of X-rays upon cells growing *in vitro* have shown that with sufficiently high X-ray doses the outgrowth of the irradiated cell culture can be checked immediately and completely. For colonies of chicken fibroblasts, 250,000 r units are the minimum dose which produces this effect ("the immediate lethal dose").[1]

It is also possible to produce a lethal effect on cells *in vitro* by exposure to considerably smaller X-ray quantities. X-ray doses, which are themselves too low to cause immediate cessation of growth of the irradiated culture, are capable of exerting a lethal action after a latent period (Fischer and Baastrup,[2] Santesson,[3] Spear,[4] Cox[5]). Cultures treated with such X-ray doses continue to grow for some time; but after a certain number of passages, cell growth ceases.

The mechanisms of action of the immediate and the delayed lethal doses, respectively, are, in all probability, fundamentally different. The immediate lethal doses stop the outgrowth in the irradiated culture by altering the motility of the cells. The delayed lethal doses act, on the other hand, by affecting a more susceptible cell function, namely, cell division. Cultures treated with these doses die in the course of subsequent transfers because the cells become incapable of multiplication.

In a previous communication[6] we reported on the delayed effect on cell cultures of X-ray

[1] Doljanski, L., and Goldhaber, G., *Growth*, 1942, **6**, 235.

[2] Fischer, A., *Gewebezüchtung*, München, Müller & Steinicke, 1930.

[3] Santesson, L., *Upsala Läkareförenings Forhandlingar*, 1928, **34**, 591.

[4] Spear, F. G., *Proc. Roy. Soc., B* (London), 1930, **106**, 44.

[5] Cox, S. F., *Brit. J. Radiol.*, 1931, **4**, 111. 1942, **6**.

[6] Goldhaber, G., and Doljanski, L., *Growth*, 1942, **6**.