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**.

doses of 2,500, 5,000, 12,500, 25,000, and 50,000 r units. It was shown that treatment with 2,500 r units causes a depression in the growth rate of the irradiated culture. This depression is transient and is gradually overcome in the course of subsequent passages. The effect of treatment of cell cultures with doses of from 5,000 r units to 50,000 r units is entirely different. Doses of this magnitude inhibit cell proliferation irreversibly. Cultures treated with these doses, although they develop well after irradiation and differ little from cultures irradiated with 2,500 r units in both size and appearance, are destined to die. They can be cultivated for some time, but succumb after a number of passages. Cultures irradiated with doses between 5,000 to 25,000 r units have an average survival of 5.5 passages; cultures irradiated with 50,000 r units survive 3.1 passages.

In view of the particular biological and clinical significance of irradiations causing a delayed lethal effect on cells, an attempt was made to determine, exactly, the minimal X-ray dose exerting this effect.

Material and Methods. (a) *Culture technic.* Our experiments were carried out on standardized cultures of chicken fibroblasts derived from heart of 7-day-old embryos. The cell colonies were cultivated in hanging drops according to the usual procedure, using a mixture of fowl plasma and embryo extract as the medium. Growth curves of the cultures were constructed by the planimetric measurements of outline drawings of the surface area made every 24 hours.

(b) *Radiological technic.* Irradiations were carried out using a demountable X-ray tube which worked at a tension of 35 KV on currents of 2 MA. The tube had a copper-anticathode and a window of aluminum foil, 30 μ in thickness. Absorption analysis showed that the rays penetrating through the window foil and the 0.03 mm thick mica-coverglass of the cultures were mainly copper K-rays. The X-ray intensity at the distance of the irradiated subject was about 8,500 r/min for a tube current of 2 MA.

(c) *Experimental procedure.* Fibroblast cultures were irradiated, immediately after transfer, with the following X-ray doses: 3,000, 4,000, and 5,000 r units. The irradiated cell colonies were cultivated throughout successive passages. Sub-cultures were made at intervals of 3 days, and this routine was continued as long as the cultures survived, or until full recovery had been attained.

Results. The results of the experiments

TABLE I.

Irradiation with 3,000 r units		Irradiation with 4,000 r units		Irradiation with 5,000 r units	
Exp. No.	Results	Exp. No.	Results	Exp. No.	Results
23199	+	23198	—(6)	19893	—(7)
23201	—(4)	23200	—(4)	19894	—(6)
23782	+	23202	—(4)	20141	—(7)
23788	—(2)	23203	+	20142	—(7)
23789	+	23785	—(5)	20143	—(7)
23790	+	23786	—(5)	20300	—(5)
23998	+	23787	—(5)	20662	—(3)
23999	+	23793	—(7)	20808	—(3)
24000	+	24003	+	20809	—(4)
24001	+	24004	+	20810	—(5)
24002	+	24005	+	20811	—(4)
		24006	—(5)	20812	—(5)
		24480	+	24481	—(5)
		24482	+	24483	—(5)
		24486	—(5)	24485	—(5)
		24488	—(5)	24487	—(6)
		24490	—(6)	24489	—(3)
				24491	—(6)

\+ Prolonged cultivation possible.
— Prolonged cultivation impossible.
Bracketed figures represent the number of passages through which cultivation of the irradiated cell culture could be carried.

(Table I) may be summarized as follows: Irradiation with 3,000 r units does not produce permanent damage of the cell culture; with few exceptions, cultures exposed to this dose survive and after several passages show a quite normal rate of growth. Irradiation with 4,000 r units produces variable results; out of 17 cultures treated with 4,000 r units, 11 cultures succumbed in the course of the succeeding transfers, while 6 cultures survived. Irradiation with 5,000 r units renders prolonged cultivation of the cell colonies impossible. A dose of 5,000 r units is, therefore, to be regarded as the minimal "delayed lethal dose" for chicken fibroblasts.

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Effect of Biotin Deficiency on Duration of Infection with *Trypanosoma lewisi* in the Rat.*

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Before August, 1942, when this study was undertaken, there had been few reports on the effect of a vitamin deficiency on the course of a parasitic protozoan infection. Since much work on biotin deficiency had already been done in this laboratory this vitamin was thought suitable for the purpose. It was planned, therefore, to determine the effect of biotin deficiency on the course of infection with a blood protozoan.

Trypanosoma lewisi was chosen because it was considered advisable to use an organism whose course of infection had already been well studied.[1]

The rats used in these experiments were white albinos bred for the most part in this laboratory. The experimental rats were kept on a modified egg white diet consisting of:

	g
Cornstarch	185
Salt mixture	50
Peanut oil	150
Cod liver oil	30
Water	25
Egg albumen†	300
Sugar	185

They were also given the following vitamin supplement daily: thiamine, 20 γ; pyridoxine, 20 γ; riboflavin, 25 γ; calcium pantothenate, 100 γ. In addition the experimental rats were given 50 mg of choline chloride thrice weekly. The food intake was high in all cases until the terminal phases of the biotin deficiency. The control rats were kept on a modified Sherman diet,[2] with liver or beef twice a week and cabbage once a week in addition.

All rats in a specific group were inoculated intraperitoneally with the same number of *Trypanosoma lewisi.*‡ However, the numbers of parasites in these inocula were only roughly approximate from experiment to experiment. Examination of the blood, tail blood or if necessary, heart blood, was carried out every other day until the end of the infection. After the first disappearance of the parasites from the blood the rats were followed for 6 days and then observations were discontinued. It should be noted that these rats were not *Bartonella* free, but since this was true of both the experimentals and the controls it was felt that the differences in the 2 groups can be safely ascribed to the differences in their diet.

* Under a grant from the Rockefeller Foundation.

1 Taliaferro, W. H., "The Immunology of Parasitic Protozoa" in *Protozoa in Biological Research*, edited by G. N. Calkins and F. M. Summers, Columbia University Press, 1941.

† Purchased from the Weideman Co., Cleveland, Ohio.

2 Smith, A. H., and Bing, F. C., *J. Nutrition*, 1928, **1**.

‡ Our strain of *Trypanosoma lewisi* was supplied us through the courtesy of Dr. W. H. Taliaferro of the University of Chicago.