the adrenergic terminals show an intense fluorescence and can be easily studied everywhere in the tissues. Depletion experiments in this laboratory have shown that the sensitivity of the method is so high that the transmitter can be demonstrated even after a reduction of more than 80%[6].

Experiments with different doses (Table I) showed that the fluorescence of the adrenergic nerves had disappeared completely 24 h after the injection of 1 and 5 mg/kg, and was markedly reduced at the dose level of 0.2 mg/kg. The highest dose was then used to examine the time course of the NA depletion. As seen in Table II, the uterine nerves were quite exceptional. They still contained a demonstrable – although reduced – NA content at 12 h, while no transmitter could be observed at 4 h in the other tissues examined.

A possible species specificity was tested by examining the uterus and the submandibular gland in different animals 4 h after a reserpine injection. Four untreated controls and 4 injected animals of each of the following species were used. The animals and doses used were: mouse, 25 mg/kg; rat, 10 mg/kg; hamster, 10 mg/kg; and guinea-pig 20 mg/kg. Among these animals only the uteri of guinea-pigs showed a weak fluorescence after the reserpine injection.

Table I. Influence of dose of reserpine on noradrenaline depletion in the rabbit, 24 h after the injection

Dose in mg/kg	Noradrenaline in uterine adrenergic nerves
5	0
1	0
0.2	++
0.02	++++

Table II. Influence of time on noradrenaline depletion after reserpine injection, 5 mg/kg rabbit

h after injection	Noradrenaline in adrenergic nerves:	
	Uterus	Other tissues
0	++++	++++
4	++	0
12	+	0
24	0	0

All the uterine nerves, which were found to be almost exclusively vasomotors (confirming unpublished observations by FALCK) seemed to react similarly: the intensity of the fluorescence of the adrenergic nerves decreased but remained 4 and 12 h after the injection of reserpine.

The results show that the adrenergic vasomotors of the rabbit and, possibly, guinea-pig uterus react to reserpine much more slowly than both the vascular and other nerves in several other tissues. The difference might be at least partly explained if the uterine nerves – in contrast to the others – had no or a very low impulse flow during the experiments (FUXE and SEDVALL[6]). A diminished impulse flow, obtained by a decentralization of the left superior cervical ganglion in 2 rabbits, did not prevent the complete depletion of the monoamine in different tissues (skin, tongue, submandibular gland) observed 4 h after the injection of 5 mg/kg. It can thus be concluded that adrenergic terminals with the same general function can differ (at least quantitatively) with respect to basic properties. This is now explored further.

Zusammenfassung. Nor-Adrenalin (NA) verschwindet nach einer Reserpininjektion im Uterus des Kaninchens langsamer als in anderen Organen: nach 12 h noch Nachweis von Aminen in den vasomotorischen Uterusnerven, nicht aber zum Beispiel in der *Gl. submandibularis*. Tendenz zu gleicher Reaktion zeigt der Uterus des Meerschweinchens, nicht aber der Ratte, der Maus oder des Hamsters. Es wird der Schluss gezogen, dass verschiedene sympathische Nervenendigungen gleicher Aufgabe variierende Grundeigenschaften zeigen.

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[1] This study was aided by grant NB 02854-04 from the United States Public Health Service.

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Reproduction of Irradiated Monkey Kidney Cells: Observations on Fixed Stained Cultures and by Phase Contrast Cinematography

Tissue culture cells are used today largely in the study of the biological effects of radiation. For reasons already expressed in a previous publication[1], we prefer to use primary monkey kidney cell cultures (*M. rhesus*). In the course of the research mentioned, we have noted that irradiation with X-rays causes the appearance of giant cells which contain one or more micronuclei. We have also shown how, under the experimental conditions which we adopted and for exposition doses comprising between 300 and 1500 R, the number of cells with micronuclei and the number of micronuclei per cell were proportional to the irradiation dose used. This phenomenon must be included among the modifications that occur in the reproduction of the irradiated cells.

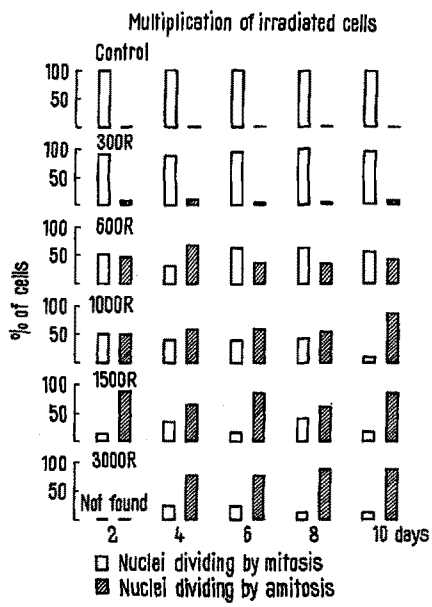
[1] P. VERANI, D. BALDUCCI, and M. CHIOZZOTTO, Exp. Cell Res. *32*, 333 (1963).

With the aim of documenting this phenomenon better, we have sought to study the reproduction of irradiated monkey kidney cells, using both fixed stained preparations and phase contrast cinematography.

Materials and methods. For each test the cells are obtained by trypsinization of the kidneys of several monkeys in order to reduce the variability of the single animal. The cells suspended in Hanks saline solution to a concentration of 500,000/ml are irradiated in covered Petri dishes at room temperature. The dishes are exposed for differing time intervals to an X-ray apparatus (Picker 260 KVP type, operating at 230 KVP and 14 ma, filtered with 1 mm Al and 0.5 mm Cu). The exposure rate employed is of 57.8 R/min. A total of 300, 600, 1000, 1500 and 3000 R, respectively, have been administered. After the irradiation, the cells are diluted in growth medium so as to obtain a final concentration of 100,000 cells/ml. 200,000 cells are distributed in each Barski-Leighton type tube containing a coverslip. The cultures are then incubated at 37°C. At various intervals, the cultures are fixed in Bouin fixative and stained with Hematoxylin-eosin. In order to make cinematographic recordings, a small perfusion chamber derived from the thickness of a plate of atoxic plastic material (Teflon) was prepared as described[2].

Observation on fixed stained cultures. In irradiated monkey kidney cells, we have observed that two or more days after irradiation some cells appear with the nuclei dividing directly by amitosis (Figure 1). Sometimes the division by amitosis is irregular, which means that the nucleus divides into a nucleus of almost normal size and a micronucleus. In the course of 10 days' observation, in the control cultures the nuclear divisions have only passed through the mechanism of mitosis. In the corresponding irradiated cultures a number of cells have the nuclei dividing by amitosis; this number, within certain limits, increases with increase of the irradiation dose (Table). It should be added that in the irradiated cultures some mitoses are clearly atypic, because in metaphase some broken and irregularly dislocated chromosomes are present with respect to the equatorial plate (Figure 2). A small percentage of mitosis, with irregularly dislocated chromosomes, is present also in the normal cultures, but this percentage does not exceed 8% after 10 days' cultivation. In the irradiated cultures, the percentage of irregular mitosis increases with increase of the irradiation dose and exceeds 90% after administration of 3000 R and after 10 days' cultivation.

Observation by phase contrast. Cinematographic phase contrast recordings have given less satisfactory results than those which we expected. The reasons which limit the possibility of this type of investigation are: the need to use a very high magnification, the difficulty in following the formation and disposition of the chromosomes of living unstained cells of a superior mammal, and the indistinctness of the smaller micronuclei hidden by the mitochondria. Cinematography, on the other hand, allows us to study the evolution of a given cell as a function of time, while observations on fixed stained cultures permit examination of a cell at only one point in time. We have followed the division of two cells by mitosis and the divi-



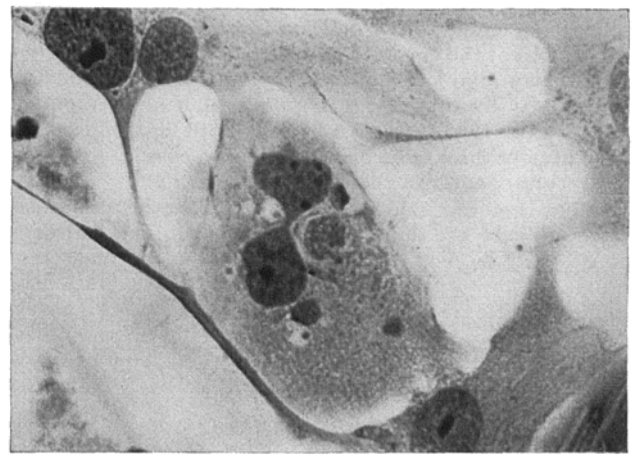


Fig. 1. Culture on 6th day after irradiation with 1500 R. Nucleus dividing by amitosis (× 800).

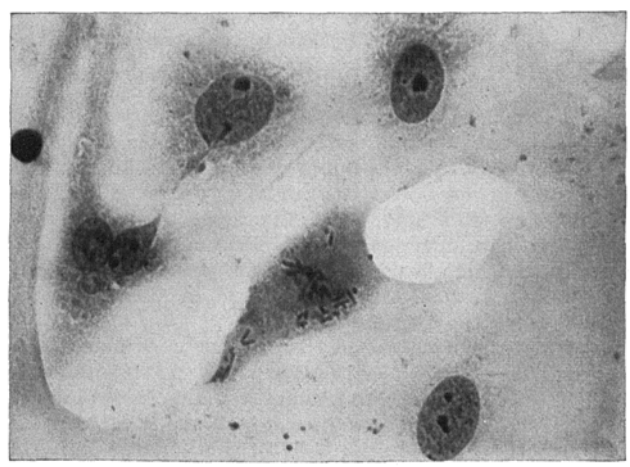


Fig. 2. Culture on 4th day after irradiation with 1500 R. Atypical mitosis and nucleus dividing by amitosis (× 800).

[2] D. Balducci and L. Castelli, Rend. 1st Sup. Sanità *20*, 865 (1957).

sion of a nucleus of a third cell by amitosis. The times of total performance of the mitosis were 30 and 60 min; the division of the nucleus by amitosis takes about 2 h, but, at least during the time of our observation, it was not followed by division of the cytoplasm.

Discussion and conclusions. The reproduction of irradiated cells can be studied by integrating the observations that we obtained on fixed stained cultures and on living cultures observed by phase contrast with time cinematography. The relation between the number of cells that begin mitosis and the number that have nuclei dividing by amitosis (Table), established by observations on fixed stained cultures, was revised according to the value of the data provided by cinematography. The mechanism by which the nuclei divide by amitosis required, in some cases, more than twice the time necessary for a cell dividing by mitosis. Therefore the reported percentages can have only an indicative function, because they would be modified as a function of the time of evolution of each process.

Another consideration is that a notable percentage of cells which enter mitosis develop in irregular mitosis. The dislocation of the chromosomes is so atypical that it is not easy to say (and cinematography has not proved beneficial to us in this respect) if these irregular mitoses remain abortive, or if they evolve in cells with micronuclei by formation of these from dislocated chromosomes which do not return again into the common mass.

It has been reported[3] that giant cells can begin mitosis but, owing to the damaged chromosomes, atypical anaphases may be derived, showing numerous chromosomal bridges. The authors do not say what the evolution of such cells attempting mitosis may be, and if, as a result of the chromosomal bridges, cells may be present in irradiated cultures, with the daughter nuclei joined together. We do not think that the cells showing the nuclei dividing by amitosis, present in our preparations, are postmitotic figures of this type because of three considerations: (1) the percentage of mitosis is inversely proportional to the dose used, although irradiation causes an increase of the time of evolution of mitosis[4–6]; (2) very often we observed a direct division of the nucleus without any shape that reflects an attempt at mitosis; (3) at every dose level used, we have observed that nuclei are joined together or detached by one bridge only, and we have never observed images that could be the result of numerous chromosomal bridges[7].

Riassunto. Colture primarie di rene di scimmia sono irradiate con 300, 600, 1000, 1500 e 3000 R. La riproduzione delle cellule irradiate è studiata con osservazioni su colture fissate e colorate e con cinematografia a contrasto di fase. In un certo numero di cellule irradiate i nuclei si dividono direttamente per amitosi; tale numero aumenta con l'aumentare della dose di irradiazione. La cinematografia a contrasto di fase ci permette di seguire i tempi di evoluzione della riproduzione cellulare.

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Probable Induction of Chondrogenesis by the Spinal Cord Tissue in the Ammocoetes

The present study was undertaken primarily to inquire if ablation of the spinal cord in the base of the tail in the ammocoete larvae of the lamprey would have any adverse effect on regeneration of the tail when the latter is amputated at the same time. While the results gave a negative reply to this inquiry, certain developments were noted in some cases which indicate the capacity of the spinal cord tissue to induce cartilage formation in these animals. These observations are reported below.

The material consisted of 15 ammocoetes of *Petromyzon marinus* and *Entosphenus lamottenii*, 72–105 mm in length. In each case, after anaesthetizing the animal in 1:4000 MS222 solution, an incision was made through the tail muscles alongside the base of the dorsal fin on the right side near the cloaca. The incision exposed the spinal cord, a small piece of which was cut out and removed. The caudal part of the spinal cord was thus separated from the same organ in the rest of the body. The distal portion of the tail was amputated immediately after this operation. All the animals survived; they were killed at various intervals and their tails sectioned for microscopic study.

Although attempts had been made to remove the severed segment of the spinal cord completely from the operated region, the sections showed that it was not successful in three cases, in which several small fragments of the spinal cord tissue were left behind in the area near the base of the dorsal fin. One of the three animals was killed and its tail sectioned 26 days after the operation. In this case many mesenchymal cells, together with numerous leucocytes, were found disposed around these fragments of the neural tissue.

The other two animals were killed and their tails sectioned 90 days after the operation. In both of them definite cartilaginous structures were found to have developed around the spinal cord fragments left behind in the area near the base of the dorsal fin. These structures were tubular with the spinal cord fragment occupying the lumen in each case. In one of the two animals there were two such tubes, more than 250 μ in length, situated parallel to the long axis of the tail. These cartilages had developed in entirely abnormal positions. The operation had caused quite extensive damage to the muscles in this region and much dedifferentiation of this tissue had occurred in this area. It is probable that the cells for the abnormal cartilages may have been derived from the dedifferentiating muscles in the operated region. That chon-