similar age from the control rats, were examined and are reported in this study.

The size and appearance of the 'tellurium' and control fetuses were similar. No anomalies were noted in sections of the brains of the tellurium fetuses, stained with hematoxylin-eosin. The tissues destined for electron-microscopic studies, were fixed in 2% gluteraldehyde, buffered with 0.1 *M* sodium cacodylate, to pH 7.2. The tissues were fixed for 1 h, postfixed for 30 min in 1% osmic acid, buffered with 0.1 *M* sodium phosphate to pH 7.2, washed dehydrated, embedded in Epon Resin 812 (Fisher Scientific Co.).

There were morphological anomalies in the cells in the ependymal layer of the tellurium fetuses, 13 and 15 intrauterine days old. The ependymal layer of the normal fetal rat resembled that described in the human fetus[11], fetal rabbit[12] and chick[13]. In the normal fetal rat (Figure 1) microvilli were abundant on the ventricular surface of the ependymal cells. The mitochondria were grouped in the apical portion of the cytoplasm surrounded by small, presumably pinocytotic, vesicles. The nucleus was in the basal portion of the cytoplasm. In the ependymal cells of all 'tellurium' fetuses (Figure 2) the ventricular plasmalemma was without microvilli and the number of mitochondria was greatly diminished. The mitochondria were often abnormal, smaller and darker than normal and showed distortion of cristae (Figure 3). The cells in the rest of the telencephalon appeared to be normal.

There is evidence that tellurium crosses the placenta and reaches the cerebrospinal fluid (CSF) and the fetal brain after it is injected into the mother[2]. Since the telencephalic wall is so poorly vascularized during fetal life[14] and the choroid plexuses are well vascularized, it appears that the tellurium reaches the ependyma of the lateral ventricles by way of the CSF, where the lesions were found[15].

*Résumé.* L'ingestion de tellure, associé à la diète normale, par une rate en gestation, peut donner des ratons hydrocéphaliques. Des anomalies cellulaires s'observent dans le cerveau du foetus de 13 à 15 jours.

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[11] S. Duckett, Anat. Rec. *161*, 231 (1968).
[12] V. A. Tennyson and G. D. Pappas, Z. Zellforsch. *56*, 595 (1962).
[13] K. Meller and W. Wechsler, Acta Neuropath. *61*, 609 (1964).
[14] E. H. Craigie, J. comp. Neurol. *39*, 301 (1925).
[15] This work is supported by grant Nos. 1 R01 NS 8891-01 from the US Public Health Service.

## The Pathogenic Role of the Inflammatory Reaction in Poliomyelitis. Immunofluorescence, Electronmicroscopic and Virological Studies with Type 3 Poliovirus

In most histological studies of poliomyelitis, the inflammatory reaction has been regarded as a defense reaction. New information about the relationship of the virus to the inflammatory elements was obtained with immunofluorescence techniques, which have demonstrated the presence of virus antigen within the mesenchymal cells of the central nervous tissues, including the inflammatory elements[1-4]. These findings raise the question whether or not some of the inflammatory cells play a pathogenic role in the course of the infectious process. Our study deals with this problem and a preliminary report is given here. 98 rhesus and cynomolgus monkeys were inoculated intraspinally (0.1 ml) or s.c. (0.5 ml) with undiluted virus suspension of virulent and attenuated type 3 poliovirus of titre from 5.6 to 6.8 $\log_{10}$ $TCD_{50}$/0.1 ml. 2 control animals were given 0.1 ml of bovine-globulin conjugated with fluorescein isothiocyanate (FITC). The animals were sacrified at time intervals between 12 h and 21 days after inoculation. Light, fluorescence and electron microscopic studies, and virus assays, were carried out on the central nervous system of the inoculated animals.

The virulent strain produced the expected histopathological picture of severe, rapidly progressive neuronal damage with extensive inflammatory reaction. After intraspinal inoculation this inflammatory response comprised the early appearance of polymorphonuclear leukocytes and macrophages (within 12 h) and represented the 'secondary' non-specific reaction to neuronal destruction. Lymphoid cells and macrophages predominated later (between 48 and 72 h); in our opinion, this is the 'primary' specific reaction and represents the local immune response to viral antigen. A similar sequence of changes was observed 4 days after s.c. injection.

With the attenuated virus, lesions in the central nervous system only occurred after intraspinal inoculation. They were of the 'primary' specific character and were seen between 48 and 72 h. The majority of nerve cells were intact, the remainder exhibited degenerative changes, mostly of a reversible nature[5-8]. No changes were seen in the brain or cord after introduction of attenuated poliovirus by the subcutaneous route. In the animals inoculated with the virulent strain, 30–60% of the motor neurons showed some degree of fluorescence. This could be seen already after 12 h. With the attenuated strain of poliovirus, an exceptional neuron could be observed to fluoresce after an interval ranging from 48 to 72 h. However, fluorescence was seen after inoculation with either strain of virus in the non-neuronal elements, i.e. in the inflammatory and glial cells as well as in the vascular walls. After intraspinal inoculation, this finding was observed soonest, i.e. after 12–24 h, in the inflammatory cells of

[1] E. Kovacs, R. K. Baratawidjaja, J. K. Hamvas, L. Morrissey and N. Labzoffsky, Life Sci. *2*, 902 (1963a).
[2] E. Kovacs, R. K. Baratawidjaja and N. A. Labzoffsky, Nature, Lond. *2*, 497 (1963b).
[3] O. Palacios and K. Mannweiler, VIII. Europ. Symp. of Poliomyelitis (1963), p. 462.
[4] M. Kanamitsu, A. Kasamaki and M. Ogawa, Jap. J. med. Sci. Biol. *20*, 175 (1967).
[5] J. Simon, Thomayer's Sammlung (Zdrav. Naklad, Prag 1962).
[6] J. Simon, J. Hyg. Epid. *8*, 506 (1955).
[7] J. Simon, Arch. ges. Virusforsch. *15*, 220 (1965).
[8] J. Simon, Symp. Ser. in Immunobiol. Standardisation (S. Karger Verlag, Basel, New York 1967), vol. *8*, p. 31.

the traumatic reaction adjacent to injection site. From this time on, this type of fluorescence became more intense and widespread. In the control animals, fluorescence was observed in the phagocytic cells adjacent to injection site.

Electron microscopic examination carried out on the fifth post-inoculation day revealed intracytoplasmic crystalloid structures made up of particles, the size and shape of which corresponded to those of poliovirus. These crystalloid particle aggregates were repeatedly observed in mononuclear elements of the inflammatory infiltrates as well as in the endothelial cells of the intraspinal blood vessels. Nothing was seen in the nerve cells, which could be taken to signify the presence of poliovirus[9,10].

Virological assays showed that both virulent and attenuated viruses multiplied rapidly in the lumbar region after intraspinal inoculation, the maximum titre being achieved within the first 24 h. From the second day, a decrease was observed which was more pronounced for the attenuated virus. In the other regions of the central nervous system, a rising titre of virulent virus became apparent from the 3rd day, whereas no virus was detected with the attenuated strain.

Our findings indicate that inflammatory cells including activated microglial cells and cells of the vascular walls show a close association with the poliovirus. The immunofluorescence of some of the inflammatory cells, the electronmicroscopic demonstration of poliovirus-like crystals and the virus assays, all indicate the potential capacity of some of the inflammatory cells to replicate the poliovirus.

The fluorescence in the control animals suggest that this finding in some phagocytic cells of poliomyelitic monkeys may signify another process, i.e. virus uptake ('viropexis'). Whenever pinocytosis of an infectious particle occurs, the inflammatory cell can serve as a 'vector', providing local spread of virus.

To understand the development of the virulent infectious process it is necessary to keep in mind not only the pathogenic activities of the individual inflammatory cells, but also the circumstances under which these activities take place. The whole process can be compared to an 'epidemic' within the greatly enlarged population of closely packed cells of the inflammed nervous tissue. The crowding and mobility of the majority of these cells thus facilitates the local spread of infection. The nerve cells, which make up a small fraction of this cell population, become infected in the course of this 'epidemic' together with other non-neural potential host cells. They probably do not participate significantly in the virus replication, because their intracellular metabolism is rapidly damaged by the viral genome, often before the virus replication can be initiated. Their pathogenic role appears rather to rest on their ability to trigger the 'secondary' inflammatory reaction. The inflammatory elements are then responsible for replication and spread of the virus.

The results allow the conclusion that the inflammatory reaction, apart from its defensive function[11], plays an important pathogenic role in the development of poliovirus infection. Both defensive and aggressive mechanisms run parallel within the heterogeneous cell population of the focus of infection but opposing one another. Their intensity and mutual relationship will determine the course of the infectious process.

*Zusammenfassung.* Das Studium der sich entwickelnden entzündlichen Reaktion auf mit attenuiertem und virulentem Poliovirus infizierten Affen zeigte, dass diese in der Pathogenese der Poliomyelitis eine defensive und eine aggressive Rolle spielt. Die erstere umfasst eine unspezifische, sekundäre Antwort auf die Nervenschädigung (Neuronophagie) und eine spezifische, primäre Reaktion auf das virale Antigen (lokale Immunreaktion). Die letztere ist vor allem dadurch gekennzeichnet, dass einige der mobilen entzündlichen Elemente das Poliovirus replizieren und weiter verbreiten können.

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*Hampstead Laboratories, Holly Hill,*
*London N.W. 3 (England), 15 April 1970.*

[9] K. BLINZINGER, J. SIMON, D. MAGRATH and L. BOULGER, Experientia *24*, 1095 (1969).
[10] K. BLINZINGER, J. SIMON, D. MAGRATH and L. BOULGER, Science *163*, 1336 (1969).
[11] J. SIMON, G. PETERS, K. BLINZINGER, D. MAGRATH and L. BOULGER, in press.

## The Amphibian Lens: a Three Month Organ Culture[1]

Several years ago this laboratory reported that it is possible to maintain the ocular lens of the bullfrog (*Rana catesbeiana*) in organ culture for at least seven days[2]. We implied then that the culture system described would probably also permit cultivation for far longer periods. Data to be presented in the current account will show that this is so – that in fact one can maintain bullfrog lenses in culture for periods up to 3 months.

*Experimental.* Lenses were isolated from enucleated eyes by techniques that have been fully discussed elsewhere[3]. They were then cultured in either of 2 media. The first, 'A-199', consists of 88.3% water; 5% sodium bicarbonate (55 g/l); and 6.7% 10X 199[4] (Grand Island Biological Co., Grand Island, New York, USA). The second, 'R20', has the following composition: 5.3% 10X 199, 3.0% sodium bicarbonate (55 g/l), 71.7% $H_2O$ and 20% rabbit serum (Pel-Freez Biologicals, Inc., Rogers, Arkansas, USA). Both fluids are approximately isosmotic with bullfrog aqueous humor (225–235 mOsm) and have a pH of 7.1–7.2. Penicillin, 50 units/ml, and streptomycin, 50 µg/ml, were added to the media prior to explantation. Each lens was placed in a silicone

[1] This work was supported by United States Public Health Service Grant No. EY 00281-06 from the National Eye Institute.
[2] H. ROTHSTEIN, J. M. LAUDER and A. WEINSIEDER, Nature *206*, 1267 (1965).
[3] H. ROTHSTEIN, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1968), vol. 3.
[4] J. F. MORGAN, H. J. MORTON and R. C. PARKER, Proc. Soc. exp. Biol. Med. *73*, 1 (1950).