The cavities of the knee joints in animals with lesions of epiphyses showed necrosis of epiphyseal cartilage, oedema of the ligaments, thickening of the synovial membrane, and sometimes filling of the articular cavity with loose, richly vascularized connective tissue. The lymph nodes of the extremities showed hyperplasia, cellular dissociation and focal necrosis, giving rise to the appearance of plaques in tissue cultures. No differences were found between the lesions in mice inoculated into the bone marrow and into the joints.

On the basis of the results obtained in these conditions, it can be concluded that the Coxsackie B_1, B_3, B_4 and A_9 and ornithosis O.A.P. strain *Y.S.*224 viruses injected into the bone marrow or joints in mice induce osseous tumours which originate in the connective tissue. We anticipate that further experiments will allow their classification and evaluation of their malignity.

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[1] Markowa, J., *J. Bone and Joint Surg.*, **43** B, 95 (1961).
[2] Markowa, J., Marek, A., Popiela, T., and Zurek, W., *Chir. Narz. Ruchu i Ortop. Pol.*, **28**, 997 (1963).
[3] Markowa, J., and Marek, A., *Nature*, **195**, 351 (1962).

Use of Kieselguhr to increase Cell Production for Animal Virus Investigations

ONE of the difficulties involved in elucidating the structure of an animal virus is to produce sufficient virus for investigation. Wheatley[1] has shown that the yield of cells given by mice inoculated with Ehrlich ascites tumour could be increased by previous injection of a suspension of kieselguhr. Krebs ascites tumour cells, propagated in mice, can later be used for growing virus *in vitro*[2]. We show that kieselguhr can also be used to increase the yield of these cells, and furthermore, the latter resemble those produced in the conventional way in their response to virus infection. A simple method is thus available to provide larger quantities of cells for later virus investigations.

Krebs II ascites tumour cells, obtained originally from Dr. D. C. Roberts, were maintained by weekly intraperitoneal injection of 1×10^7 cells in 0·1 ml. of phosphate buffered saline (PBS) into genetically heterogeneous white mice. Either 0·2 per cent suspension of kieselguhr in PBS, or of PBS alone (0·1 ml.), was injected at the same time as the ascites cells. After 7 days the cells were withdrawn from both kieselguhr treated and control mice, washed to remove red blood cells by centrifugation and the cells counted; the percentage of cells permeable to stain was estimated with 0·2 per cent nigrosin in PBS.

Kieselguhr treated mice produced about 1·5 times as many cells as controls (Table 1). This figure, which supports the work of Wheatley[1], would be slightly higher if unstained cells only were considered. Furthermore, ascitic fluids from kieselguhr treated mice contained fewer red blood cells and the ascites cells thus required fewer washes to prepare for use.

Table 1. EFFECT OF KIESELGUHR ON PRODUCTION OF KREBS ASCITES TUMOUR CELLS

Treatment	Cells/mouse $\times 10^{-8}$	Percentage stained	Ratio of kieselguhr to control cells	No. of mice
Kieselguhr	6·1	9·9	1·6	7
Control	3·8	16·0		7
Kieselguhr	4·9	8·9	1·3	27
Control	3·8	6·4		27
Kieselguhr	7·3	9·6	1·4	37
Control	5·1	13·8		37
Kieselguhr	7·2	10·6	1·7	37
Control	4·3	10·9		37

Both kinds of cells were tested for their ability to support virus multiplication by infecting them with encephalomyocarditis (EMC) virus[2]. Virus yields, estimated both by haemagglutination and by plaque assay using Krebs ascites cells (from mice not given kieselguhr) in agar suspension[2], were the same, within the limits of experimental error, whether the ascites cells came from kieselguhr or from untreated mice (Table 2). Mice injected with kieselguhr gave on average 1·5 times as many cells (Table 1) and so there would be a 1·5-fold increase in virus output from the same number of mice.

Table 2. COMPARISON OF VIRUS YIELDS IN CELLS FROM KIESELGUHR TREATED AND CONTROL MICE

Treatment	Plaque forming units/ml. $\times 10^{-9}$	Haemagglutination units/ml. $\times 10^{-4}$
Kieselguhr	1·1	8
Control	0·9	8
Kieselguhr	2·3	16
Control	1·6	16
Kieselguhr	2·8	32
Control	3·4	32

The sensitivity of ascites cells from kieselguhr treated mice when used for plaque assay of EMC virus was compared with control mice using two virus preparations. The number of plaque forming units detectable was the same, within the limits of experimental error, when either type of cell was used (Table 3).

Table 3. COMPARISON OF ASCITES CELLS FROM KIESELGUHR TREATED MICE AND CONTROLS FOR PLAQUE TITRATION

Treatment	Plaque forming units/ml. $\times 10^{-8}$	Large plaque / Small plaque
Kieselguhr	4·6	1·00
Control	4·1	1·04
Kieselguhr	5·7	1·15
Control	6·0	1·10

The virus preparations used contained mutants differing in plaque size. The sensitivity of the two types of cells was the same for both large and small plaques (Table 3).

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[1] Wheatley, D. N., *Nature*, **202**, 1348 (1964).
[2] Sanders, F. K., Huppert, J., and Hoskins, J. M., *Symp. Soc. Exp. Biol.*, **12**, 123 (1958).
[3] Wheatley, D. N., *Nature*, **209**, 1255 (1966).

PHYSIOLOGY

Ultrastructure of the Membrane of Synaptic Vesicles

INVESTIGATIONS by Whittaker and Sheridan[1] on permanganate fixed preparations of isolated synaptic vesicles have recently confirmed that the membrane of such organelles has the structure of a "unit membrane"[2], but have also indicated that in this case the classical model should be corrected in order to take into account the results obtained on negative stained specimens which suggest a membrane structure consisting of two 40 Å layers with an intermittent hydrophilic space between them.

In the course of experiments initiated with the aim of investigating possible morphological differences between synaptic vesicles and the axonic vesicles which are found in non-synaptic regions of peripheral nerves, we observed that in tissue specimens fixed in glutaraldehyde followed