abnormal kidneys were still present. The blood-pressure fell progressively after the transplant, and subsequent removal of the two abnormal kidneys led to no further fall in blood-pressure. It is difficult to visualize that this transplanted kidney was destroying a circulating vasoconstrictor, and it certainly was capable of restoring the excretory situation in the recipient to normal. However, Hume (1958) has reported a case with a similar transplant in which the blood-pressure did not fall until the two abnormal kidneys were removed. This suggests most strongly that we have to expect a wide variety of situations, different from patient to patient, as an explanation for the prevailing level of blood-pressure, and in man no full analysis has been given of any one situation.

[*The second Goulstonian Lecture, together with a list of references, will appear in our next issue.*]

A "POLYOMA" VIRUS DERIVED FROM A MOUSE LEUKAEMIA

BY

G. NEGRONI, M.D.

R. DOURMASHKIN, M.D.

AND

F. C. CHESTERMAN, M.B., B.S., M.I.Biol.

Division of Experimental Biology and Virology, Imperial Cancer Research Fund, Mill Hill, London, N.W.7

[WITH SPECIAL PLATE]

Stewart and her colleagues (Stewart, Eddy, Gochenour, *et al.*, 1957; Stewart, Eddy, Haas, and Borgese, 1957; Eddy, Stewart, and Touchette, 1958; Eddy, Stewart, Young, and Mider, 1958; Stewart, Eddy, and Borgese, 1958; Stewart and Eddy, 1959) isolated a filterable agent and found it to produce a wide variety of tumours when inoculated parenterally into very young mice, hamsters, and rats. This "polyoma" virus was present in extracts of induced mouse parotid-gland tumours and in spontaneous leukaemias, and multiplied in cultures of monkey kidney or mouse-embryo cells. The virus was cytopathogenic for the cultures, resisted heating to 56° C., was insensitive to ether, and haemagglutinating for guinea-pig, rat, and human Group O cells at +4° C., but not at room temperature.

Tissue-culture Studies

A virus with very similar properties has now been isolated by us. It was derived from the spleen of a male AK mouse with spontaneous lymphocytic leukaemia, by inoculation of the cells into replicate, seven-day-old cultures of trypsinized embryos from C3H or C57 mice. The cells were grown in roller tubes in a medium comprising lactalbumin hydrolysate, calf serum, and Hanks's salt solution (with added antibiotics). After seven days cytopathic changes were observed in some cultures, and cell-free media from such cultures were used to infect fresh embryo cells. The cytopathic changes appeared within ten days of infection, and were characterized by rounding-up of the cells and a degeneration of their nuclei, which was particularly apparent in Feulgen-stained preparations.

The fluids from these cultures infected fresh mouse embryo cultures at a dilution of 10^6, and the infectivity was not reduced by centrifugation followed by filtration through bacteria-tight sintered glass filters. Like the Stewart polyoma agent, this virus agglutinates guinea-pig erythrocytes at +4° C, is heat- and ether-resistant, and its infectivity for cell cultures is neutralized by rabbit antivirus antiserum.

Infection of Mice and Hamsters

Cell-free media from infected cultures in which cell degeneration was pronounced were inoculated parenterally into newborn C3H mice and into golden hamsters up to five days of age. Within 28 days the hamsters died or were killed, and were found to have blood-stained peritoneal effusions and haemorrhagic lesions in the liver, uterus, and lungs, and multifocal sarcomas of the kidney and heart and sometimes of the liver. Emboli were present in the lungs and thrombi in the liver, and the vascular endothelium showed a general hyperplasia. Some of the animals died within 14 days in an apparent acute stage of the disease. These all showed typical haemorrhagic lesions and less frequently had tumours, though they had all been inoculated by the intraperitoneal route with cell-free medium from infected cultures.

There was a characteristic dwarfism in mice, which Stewart and her colleagues have also noted. Other animals had kidney sarcomas, and one female mouse killed 80 days after inoculation had multiple mammary carcinomas. These mammary tumours are being serially transplanted, and polyoma virus has been demonstrated by passage in tissue culture. Very young rats can also be infected with this virus, but, in general, lesions can be produced more consistently in hamsters than in mice or rats.

Control cultures of mouse embryo cells have never shown cytopathic changes, and neither the medium nor the cells produce tumours or the other lesions characteristic of polyoma virus after inoculation into mice and hamsters.

Cell-free extracts of the hamster tumours are not infective for newborn or adult hamsters. However, some polyoma virus must be present in these tumours, for the cell-free extracts produce cytopathic effects in mouse-embryo cell cultures and the cell-free medium from such cultures again produces haemorrhagic lesions and tumours in hamsters. The function of the culture in augmenting either the quantity or the virulence of the virus is being studied.

These hamster kidney sarcomas have been successfully transplanted subcutaneously into both male and female hamsters, and to date are in their tenth passage. The tumours develop at the site of inoculation, but grow progressively only in a proportion of the animals inoculated. In the first passage tumours grew in 11 out of 16 hamsters implanted, and in the second passage, from a rapidly growing tumour, in 7 out of 10. However, as the hamsters are bred in a closed colony and not by brother–sister mating, it is not surprising that some of the tumours tend to regress. The tumour-bearing animals do not develop metastases, and no haemorrhagic lesions have been observed. The virus is still present in the tumour, however, and may be demonstrated by the reculture of cell-free extracts in the way already described.

Cell cultures of these hamster kidney tumours have also been established, and these cells liberate virus into the culture medium. The cells, too, retain their

malignant properties and will grow progressively in a proportion of the hamsters into which they have been implanted.

Electron-microscopy of Infected Cells

Electron-microscopic studies have been made of tissue-culture cells infected with virus from a number of different sources: from the hamster kidney tumour, from one of the induced mouse mammary carcinomas, and from a (Gross) virus-induced leukaemia in a C3H mouse. Cells from cultures which showed typical cytopathic changes were collected after trypsinization, fixed with osmic acid, embedded in methacrylate, and sectioned in the usual way. The controls comprised uninfected cultures subsequently treated in the same way.

Independent of the source of the agent, the nuclei of the infected cells were found to contain particles ranging in size from 30 mμ to 40 mμ, round or slightly oval in shape and at times appearing to possess a less dense centre. Some cells had enormous nuclei filled with myriads of such particles which had completely replaced the normal structure. Occasional packed nuclei were seen to have an incomplete membrane as though the nucleus had burst. The cytoplasmic structure in such cells was not so abnormal as to suggest that the dissolution of the nuclear membrane was an artifact of fixation or inclusion.

The cytoplasm of infected cells also contained electron-dense particles, but there were far fewer particles in the cytoplasm than in the nucleus in the cells studied. Moreover, some of these particles were 50–60 mμ in diameter, and differed from the intranuclear particles in having an external membrane surrounding a central body; the latter closely resembled the intranuclear particle. Examples were found in which the cytoplasmic particles appeared to be embedded in the cell membrane or in the membrane of cytoplasmic vacuoles. However, extracellular particles were also seen which were identical with those in the nuclei. No particles corresponding to those described above have yet been seen in control cultures, and we infer that the particles are associated with infectivity.

The dimensions of the intranuclear particles correspond to those given by Kahler *et al.* (1959) for polyoma virus in purified preparations. Similar intranuclear particles have been seen by Banfield *et al.* (1959) in thin sections of cells from cultures infected with polyoma virus. Extracellular virus-like particles have also been described by McCulloch *et al.* (1959) in some parotid-gland tumours that arose in Swiss mice after the inoculation of an agent derived from a C3H mouse mammary carcinoma.

We are trying to correlate the high infectivity of the medium from infected cultures with the number and type of particle found by electron-microscopy. We are also studying the developmental cycle of the virus in the cell, and, despite the apparent low yield of virus in the hamster tumours, are searching for characteristic particles in the tumour cells.

Summary

A virus, which we have called "M.H. polyoma," with properties similar to Stewart and Eddy's "polyoma" virus has been isolated in tissue culture from the spleen of a leukaemic AK mouse. When inoculated into suckling mice, rats, and hamsters, the virus produced a variety of tumours and other lesions. Some of these tumours—renal sarcomata in hamsters and a mouse mammary carcinoma—were serially transplantable. In the hamster tumours the presence of the virus was only revealed by infecting tissue cultures of mouse-embryo fibroblasts. Electron-microscopy of infected tissue cultures showed many particles 30–40 mμ in diameter in the cell nuclei, and fewer, larger particles, with external membranes, in the cytoplasm.

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LYMPHADENOGRAPHY: ITS USES IN HAEMATOLOGY

BY

B. MALAMOS

S. D. MOULOPOULOS

AND

A. SARKAS

From the Department of Clinical Therapeutics, University of Athens

[WITH SPECIAL PLATE]

Lymphangiography by direct injection of contrast media into the lymphatics was performed as early as 1932 by Shdanow and by Menkes, but the idea remained unused clinically for 20 years, until Kinmonth (1952) described how a colloidal dye injected subcutaneously is picked up by the local lymph vessels, which thus become visible and can be surgically exposed for the introduction of an opaque medium and subsequent radiography. This technique was later improved upon by Kinmonth and co-workers so that the clinical application of lymphangiography became a relatively easy procedure (Kinmonth and Taylor, 1954, 1955). Kinmonth's team used the new method to investigate mainly disorders of the lymphatics (Kinmonth and Taylor, 1957).

Microlymphangiography and stereolymphangiography are but two examples of recent interest in this field (Bellman and Odén, 1957; Odén *et al.*, 1958), which, however, tends to refer chiefly to lymphatic vessels, as its name also implies. No work that we are aware of has utilized Kinmonth's technique in order to study the radiological findings from lymph glands (lymphadenography) in haematological diseases known to affect the lymphatic system.

The purpose of the present investigation was to visualize by radiography not simply the lymph vessels but mainly the lymph glands in several haematological diseases.

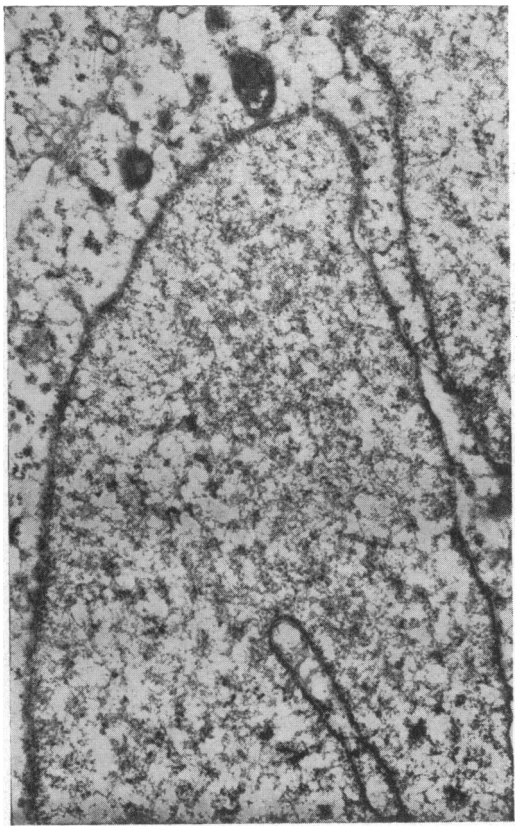

FIG. 1.—Cell from uninfected control culture. (×22,750.)

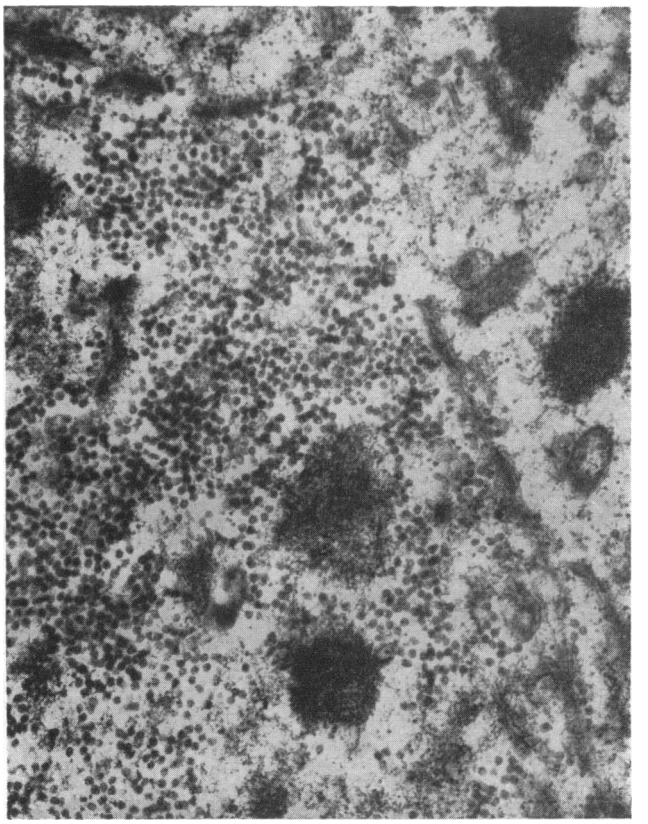

FIG. 2.—Cell with intranuclear particles. The nuclear membrane is incomplete. (×91,000.)

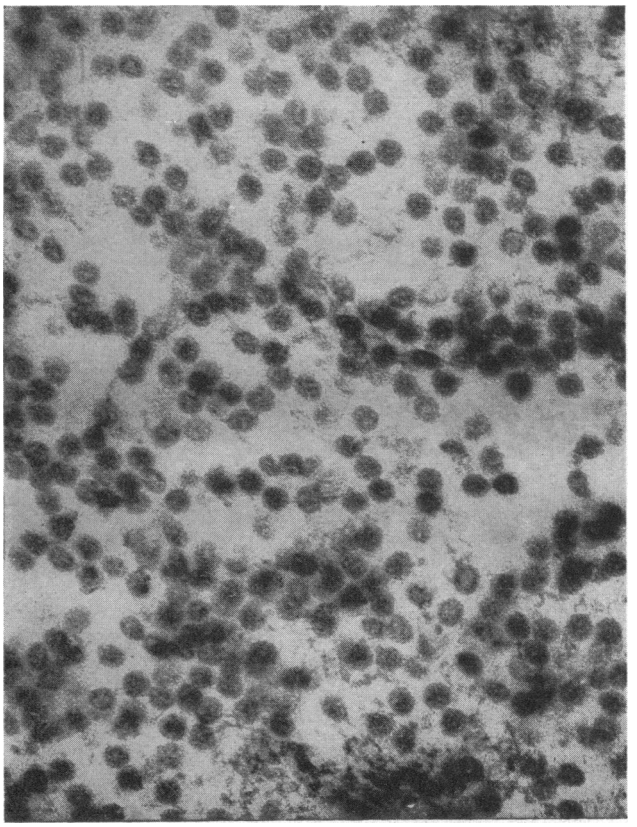

FIG. 3.—Intranuclear particles, some of which have a less dense centre. (×91,000.)

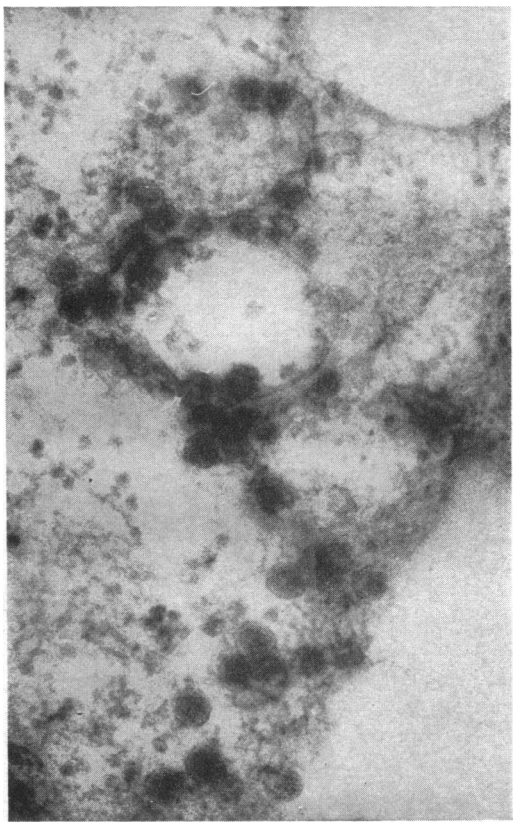

FIG. 4.—Cytoplasmic particles with external membrane. Some particles are situated on the cell membrane. (×91,000.)