## PATHOLOGY

### Experimental Myopathic Syndrome associated with Pituitary Activation of Thymus

FORMATION of lymphoid follicles with light germinal centres is generally considered as being characteristic of myasthenia gravis[1] and is regarded as one of the manifestations of the auto-immune nature of this disease[2]. However, quite similar changes are induced in the thymus with no symptoms of myasthenia by subcutaneous injection of Freund's adjuvant[3] or through direct administration to the thymus of various antigens[4], proving only that the thymus is involved in every enhanced immunization process. On the other hand, the relationship of myasthenia gravis to thymus pathology is beyond any doubt; yet the nature of this relationship is obscure.

We have suggested that a mutual endocrine relationship does exist between the thymus and hypophysis. If, therefore, some known or unknown pituitary hormone carries a thymotropic function, implantation of the pituitary cells directly into the thymus of intact animals might lead to proliferation and drastic activation of the function of various thymic structures.

Experiments have been carried out on non-inbred albino rats (100–150 g). Minute pieces from 5–10 rat pituitaries have been surgically administered to the thymus of each animal.

More than 75 per cent of the experimental animals (22 out of 27) on the 14th–60th day after implantation have become ever more depressed, constrained and motionless and when set on the edge of a table usually drooped their heads for many hours. Aspiration was enforced. These symptoms as well as rapid onset of fatigue revealed a disease greatly similar to myasthenia. The tactile skin sensitivity was increased. The healing of wounds in the myopathic rats was longer than in normal ones. Electrophysiological examination shows neuromuscular disorders in this syndrome.

The following are additional symptoms observed in some individual rats: changes in the ratio of serum proteins; symptoms of hæmorrhagic diathesis; appearance in the blood of a great number of large immature cells with unsegmented nuclei; anæmia—the fall of erythrocytes number without changing the type of erythropoiesis.

Control animals injected intrathymically with pieces of brain, posterior pituitary or with the Freund adjuvant did not reveal any of the foregoing clinical changes.

Living grafts of the anterior pituitary were observed in the thymuses of some myopathic rats killed on the 25th day after implantation. These thymuses were almost normal in appearance with insignificant prevalence of lymphoid elements. Extreme hyperplasia of the lymphoid tissue with abundant germinal centres and with proliferation of plasma cells were noted in the spleen and lymph nodes.

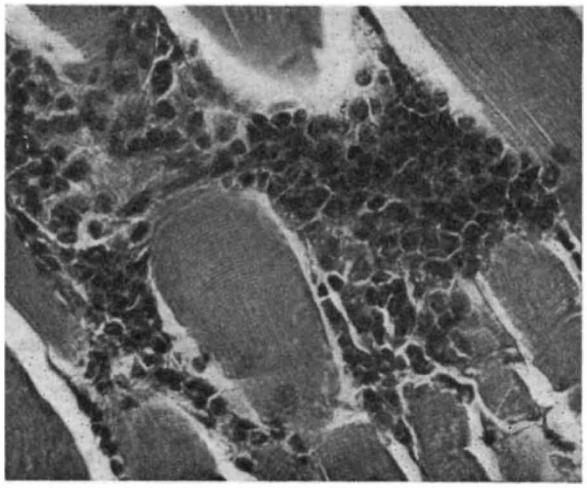

Fig. 1. Lymph cells in the striated muscle fibres of myophathic rat. (Hæmatoxylin and eosin, × *c.* 210)

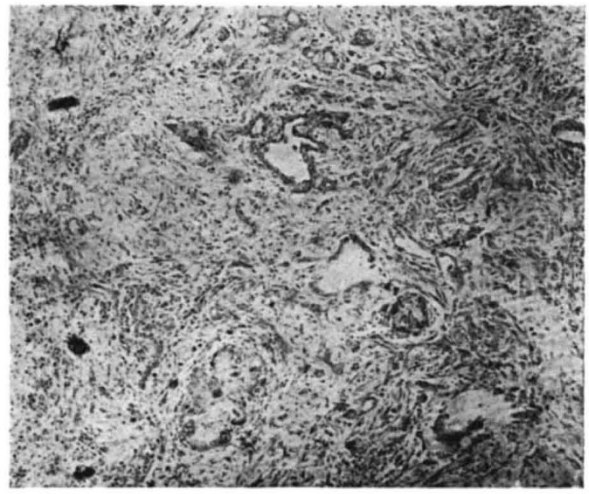

Fig. 2. Pituitary-activated thymus in cobalt-60 irradiated rat. Cavities lined with epithelium (× *c.* 52)

In some groups of striated muscle fibres of myopathic rats clumps of lymphoid cells and histiocytes with individual leucocytes between unchanged muscle fibres (lymph cells) were noted (Fig. 1).

In a rat with myopathic syndrome killed on the 80th day after pituitary implantation the thymus was reduced without any features of accidental involution. Ovoid lobules with nodular hyperplasia of the lymphoid tissue were noted in the upper part of the gland but without any follicles. In the lower part, lobules with thick fibrous capsule rich in mast cells, there occurred groups of epithelial and lymphoid cells surrounded by an abundance of fibrous cells. Lymph cells in the striated muscles were noted.

As to the morphological structures responsible for the myopathic syndrome, this question remains open. The lymphoepithelial thymoma-like structures previously described in this laboratory[5] have no relation to myopathic syndrome. The thymectomy of 2 rats with developed myopathia did not lead to recovery.

In some rats irradiated with cobalt-60 (200 r.) and then intrathymically implanted with the anterior pituitary unusual activation of the epithelial structure was observed which was different from that previously described[3]. The thymus was a little reduced in size, being composed only of reticular tissue in which many cavities of various size lined with monolayered epithelium were observed, some of them with pyroninophil homogeneous secretion. The whole thymus has an appearance of a secreting gland (Fig. 2). Lymphocytes were absent. Transient inhibition was observed in these rats. Such structures but less numerous were noted in control experiments with intrathymical implantation of hepatic tissue to irradiated rats. Such irradiated and pituitary activated thymuses can be used as a source for extraction of the thymus hormone. Preliminary experiments show that injection of such a thymus extract induces a marked but transient inhibition of the irradiated rats.

The foregoing experimental evidence proves the thymotropic action of the pituitary. Experiments show that this action is connected with the anterior lobe of the pituitary. Injection into the thymus of pure so far known pituitary hormones opens the possibility of deciding whether or not there actually does exist a specific pituitary thymotropin.

If the pituitary–thymus interrelation is mutual the thymus should act on the pituitary according to the feed-

back principle, and experimental implantation of the pituitary into the thymus of hypophysectomized animals should not either restore or change the syndromes developing after hypophysectomy. This theory, as well as the method of stimulating implantation of one endocrine gland into another, is of general significance and may be used for the examination of any pair of organs to reveal in one of them an unknown endocrine or hormone-inhibiting function with regard to the other organ. Results of autotransplantation of the pituitary into the thymus indicate the existence of the anti-hypophyseal action of the thymus.

George J. Svet-Moldavsky
Ninelle Spector
Lucie Ravkina
Laboratory of Virology,
Institute of Experimental and Clinical Oncology,
Academy of Medical Sciences,
Moscow, U.S.S.R.

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## Artificial Heterogenization of Tumours by means of Herpes Simplex and Polyoma Viruses

We have suggested elsewhere[1] the possibility of artificial heterogenization of malignant tumours by induction in tumour cells of new antigen determinants followed by exposure of these antigens to actively acquired or passively administered lymphoid cells or antibodies.

Experiments in this laboratory on artificial heterogenization of tumours with *Salmonella* and *Staphylococcus* antigens followed by treatment with corresponding antisera proved of low efficacy. Artificial induction of new antigens in tumour cells by means of 'infectious' and 'oncogenic' viruses is described here.

In these experiments sarcoma 237 induced in $C_{57}$ mice by means of 7,12-dibenz-(α)-anthracene and 2–5 times transplanted to these mice was used. The tumour nodes appeared on approximately the thirtieth day after inoculation. The tumour was minced without any trypsinization. The cells were filtered through 2 layers of gauze and infected *in vitro* with the herpes simplex virus (strain *El*-2 (kindly supplied by Dr. A. I. Shatkin) with low pathogenicity to mice has previously undergone about 80 passages in chick embryonic tissue cultures, titre $10^4$ $PFU_{50}$ (plaque forming units)) and then implanted in mice. The cells of grown infected tumours have once more been infected with the herpes virus and implanted in mice. This procedure was carried out three times.

Tumour cells of the third passage were once more infected *in vitro* with the herpes virus and different quantities of such virus-infected tumour cells ($10^4$, $10^5$ and $10^6$ cells) were administered at a rate of 0·2 ml. to the following groups of mice: first group was twice immunized at 10-day intervals with herpes virus alone 1 month before tumour cells inoculation; the second group was immunized with vaccinia virus according to a similar schedule; the third group consisted of non-immunized mice. The same amounts of non-infected tumour cells have been injected into immune and non-immune mice.

It will appear from Table 1 that the growth of tumours infected by herpes virus was specifically inhibited in the herpes virus immunized mice.

In the next experiment the cells of sarcoma 237 were mixed *in vitro* with *SE*-polyoma virus, containing 4,960 hæmagglutinating units per 1 ml. This strain has kindly been supplied by Dr. S. Stewart. After exposure for 2 h the cells were precipitated by gentle centrifugation and resuspended in Earle's solution and injected into mice.

Table 1. Artificial Heterogenization of Cells of Cancerogen-induced Sarcoma 237 by Means of the Herpes Simplex Virus

| Mice | Herpes virus-infected tumour cells | | | Non-infected tumour cells | | |
|---|---|---|---|---|---|---|
| | $10^4$ | $10^5$ | $10^6$ | $10^4$ | $10^5$ | $10^6$ |
| Immunized with herpes virus | 3/14* | 6/15 | 15/15 | 14/14 | 14/14 | 14/14 |
| Immunized with vaccinia virus | 14/14 | 13/14 | 14/14 | nt† | nt | nt |
| Non-immune | 14/14 | 12/14 | 14/14 | 12/12 | 14/14 | 13/14 |

* Denominator indicates number of inoculated mice; numerator, number of tumours which have developed.
† nt, not tested.

Ripe tumours were minced and repeatedly treated with the polyoma virus *in vitro*. The cells treated with polyoma virus as well as non-treated control sarcoma cells were injected in doses of $10^4$, $10^5$ and $10^6$ per mouse to the following groups of mice: (1) twice immunized with polyoma virus at 10-day intervals; (2) immunized with vaccinia virus; (3) non-immune control mice.

Absence of antibodies to polyoma virus was tested in the sera of the latter group of mice by the hæmagglutination inhibition test.

It is clear from Table 2 that growth of sarcoma 237 infected with polyoma virus is inhibited in polyoma-immunized mice.

The immunological nature of the growth inhibition phenomenon of heterogenized tumours is obvious from its passive transmission by means of lymphoid cells.

Fresh $C_{57}$ mice were injected with polyoma virus-treated cells of sarcoma 237 ($10^4$ cells per mouse) and 48 h thereafter half these mice received lymphoid cells from the $C_{57}$ mice actively immunized with the polyoma virus ($10^4$ lymphoid cells intravenously + $10^6$ intraperitoneally). A second group of mice received the same quantity of lymphoid cells from vaccinia-immunized mice. Tumour growth in these two groups of mice was 3/14 and 14/14 respectively. The experiment shows the possibility of subsequent immunological action on the artificially heterogenized tumour.

The foregoing experiments show that tumour cells are artificially heterogenized by herpes and polyoma viruses and that immunity acts specifically on these newly artificially induced antigens. Another alternative, namely, that tumour growth is stimulated by means of virus in non-immune mice, and that this effect is inhibited in immune animals, can probably be excluded by a comparison of growth of infected and non-infected tumours.

The result of artificial heterogenization of tumour cells by means of herpes virus complies with our previous suggestion that natural heterogenization of infected cells is a general property of viruses[2].

Inflammatory lymphoid cell infiltrates common in virus disease of birds and mammals are a host reaction on the cells heterogenized by means of virus[3].

The principal problem is artificial heterogenization of ripe tumours followed by immunological treatment. For these purposes it is necessary to examine artificial heterogenization by means of various cytotropic organisms and compounds.

In one of the pioneer papers on viral oncolysis, Pierce and Rivers[4] have pointed out that growth of Brown–Pierce carcinoma infected with virus III is to some degree inhibited in rabbits immune to this virus. They regarded this effect as a non-specific one. It might be suggested, however, that here we are dealing with a specific immunological effect on an artificially heterogenized by virus III carcinoma.

Table 2. Artificial Induction of 'Polyoma' Antigen in Cells of Sarcoma 237*

| Mice | Polyoma-infected tumour cells | | | Non-infected tumour cells | |
|---|---|---|---|---|---|
| | $10^4$ | $10^5$ | $10^6$ | $10^4$ | $10^5$ |
| Immunized with polyoma virus | 0/18 | 4/20 | 19/19 | 18/19 | 20/20 |
| Immunized with vaccinia virus | 20/20 | 18/19 | 20/20 | nt | nt |
| Non-immunized | 20/20 | 20/20 | 19/20 | 17/19 | 19/20 |

* Legends same as in Table 1.