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Observations on Induction of Resistance to Rous Sarcoma Cell Antigens in Hamsters

It has recently been shown that inoculation of mice and hamsters with several oncogenic viruses (polyoma, *SV* 40, myeloid and lymphoid mouse leukæmia) makes them resistant to subsequent transplantation of tumour cells induced by the same virus. Our observations on chickens naturally resistant to the Rous sarcoma virus, or chickens with regressed tumours, suggested that in some instances it was possible to induce resistance to Rous sarcoma cell antigens in chickens. If this were true then the Rous sarcoma is not basically different from the polyoma and other virus tumours. However, certain characteristics of the processes induced by Rous virus (the susceptibility of the adult chicken to this virus, the permanent presence of the virus in tumour cells, the short latent period of tumour induction) make it very difficult to investigate resistance to this tumour cell antigen in its natural host—the chicken. Therefore, it was interesting to investigate the possibility of inducing resistance to the Rous sarcoma cells in a mammal.

Sjögren and Jonsson[8] immunized mice with the Rous sarcoma virus (Schmidt–Ruppin strain) and inoculated the immunized and control animals 5–10 days later with a cell suspension of Rous sarcoma grown on isologous mice. This induced a very weak resistance in virus-immunized mice.

In our experiments adult golden hamsters were inoculated with Rous sarcoma virus (Carr strain). The tumour extract prepared on 0·1 M phosphate citrate buffer was centrifuged at 5,000 r.p.m. for 20 min and the supernatant was stored in sealed glass ampoules at −70° C. The oncogenic virus activity was tested by intracutaneous inoculation of chickens. The supernatant had a virus titre of 10^{-5}/ml. to 10^{-6}/ml. The hamsters were inoculated with the Rous virus 2–3 times. They received every day 1 ml. of the supernatant intraperitoneally and 2–3 ml. subcutaneously. After 7–24 days the immunized hamsters and controls (intact, or immunized with normal hen embryo tissues) were inoculated with sarcoma cell suspension. This tumour was first induced by Rous sarcoma virus (Carr strain) in new-born hamsters and then passaged with cells in adult animals of this species[9]. The cells were suspended in Earle's solution (pH ~ 8) and counted before inoculation. Each hamster was inoculated with $4{\cdot}5 \times 10^3$ to $4{\cdot}5 \times 10^6$ cells subcutaneously. The animals were observed 2 months after tumour grafting. The tumours were palpated every 2–3 days. The hamsters surviving at the end of the experiment were killed. All animals were autopsied. The results of three experiments are summarized in Table 1.

Table 1. Inoculation of Immunized and Control Hamsters with Tumour Cells

	No. cells in inoculum				Log
	$4{\cdot}5 \times 10^3$	$4{\cdot}5 \times 10^4$	$4{\cdot}5 \times 10^5$	$4{\cdot}5 \times 10^6$	TD_{50}
Immunized	0/27*	4/27 (14·8%)	8/27 (29·6%)	24/27 (88·8%)	5·3
Control	0/22	9/22 (40·9%)	18/22 (81·8%)	22/22 (100%)	4·3

* Figures in the body of the table denote number of hamsters with tumours over total number inoculated. Log TD_{50} denotes log of the cell dose giving 50 per cent positive takes in inoculated hamsters.

These results show that immunization of adult hamsters with Rous sarcoma virus induced some degree of resistance to the sarcoma cell antigens. However, the resistance in this case is not so strong as in the experiments with polyoma or murine leukæmia virus[1–5]. Further experiments are needed to test the specificity of transplantation resistance for Rous tumour and to elucidate the nature of the induced 'new cell antigen'.

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Diagnosis of Auto-immunity

In 1905 Osborne, Mendel and Harris[1] found that the extracts of certain beans had the capacity of agglutinating red blood cells. In 1949 Li and Osgood[2] used the extract of red beans in separating leucocytes from erythrocytes. Rigas and Osgood[3] described a method for the purification of phytohæmagglutinin. This method has been used by Difco Laboratories (Detroit) in preparing this material.

In 1959 Hungerford *et al.*[4] discovered that phytohæmagglutinin has a remarkable ability to initiate mitosis in cultures of leucocytes. Since then it has been extensively used in laboratories engaged in chromosome preparation. The nature of this mitogenic action was obscure until Hastings *et al.*[5] noted that phytohæmagglutinin had a leucoagglutinating activity. Rendon[6], using ^{14}C-labelled amino-acids, demonstrated that the leucocytes in tissue cultures produce a protein which migrates electrophoretically as γ-globulin. It produces fluorescent staining of all cells when incubated with fluorescent anti-γ-globulin. He concluded that the mitogenic action is probably an immune reaction.

This was followed by Pearmain *et al.*[7], who were able to produce mitosis in the lymphocytes from tuberculin-sensitive individuals by adding purified tuberculin to the cultures. Hirschhorn and his colleagues[8] produced the same effect in lymphocytes from individuals sensitized to diphtheria toxoid, pertussis vaccine and penicillin, using the appropriate antigen[3]. Not only microbial antigen but also tissue antigens have been used. Hashem *et al.*[9] found that lymphocytes from patients with infantile eczema were stimulated to undergo mitosis by extracts of human skin.

It seems that the technique of inducing mitosis appears to provide a useful and sensitive method for studying the mechanism of histo-compatibility. This communication describes a simple method which can be used to diagnose auto-immune diseases which have been attributed to release of previously sequestrated antigen (for example,