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.

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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.

The resistance of polyoma immunized mice to implantation of some non-polyoma tumours as reported by Sachs[5] was probably due to casual infection with the polyoma virus and subsequent artificial heterogenization of these tumours.

Note added in proof. Recently, Sjogren[6] has shown that polyoma antigen can be induced in non-polyoma tumours by a very simple method. It was shown recently in our laboratory that vacuolating virus SV_{40} can induce strong artificial heterogenization of tumours.

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Protein Components of Amyloid

AMYLOID may be deposited in animal or human connective tissues in a variety of pathological circumstances. It consists mainly of protein, with up to 5 per cent of carbohydrates largely in the form of mucopolysaccharides. Previous attempts to characterize the protein moiety of amyloid have included : (*a*) Extraction with strong alkali followed by electrophoresis, revealing either α-1-, α-2-, and β-globulins with traces of albumin[1] : or α-globulin alone[2] ; (*b*) Extraction with strong alkali followed by analytical ultra-centrifugation, demonstrating a single component with a sedimentation coefficient of 6[2] ; (*c*) Extraction with concentrated aqueous urea followed by analytical ultracentrifugation, showing five fractions with sedimentation coefficients of 1, 6, 9, 16 and 23 (ref. 3); (*d*) Extraction with strong alkali followed by amino-acid analysis, which gave no precise protein identification but excluded collagen as a significant constituent on the basis of low hydroxyproline values[2]; (*e*) Fluorescent protein tracing in histological preparations, which is so sensitive a technique that trace contaminants would be revealed, has detected the presence of γ-globulin[4-6], β-1 complement[6], rheumatoid factor[6], and fibrinogen[6]; (*f*) Absorption of specific anti γ-globulin antiserum with purified amyloid substance, which produced no significant reduction in titre[7]; (*g*) Extraction with concentrated aqueous urea followed by immuno-diffusion and electrophoresis against specific antisera to albumin, γ-globulin, cæruloplasmin, orosomucoid, β-2 macroglobulin, rheumatoid factor, C-reactive protein, a pool of myeloma globulins, and whole serum ; none of these antisera precipitated the amyloid proteins[3]; (*h*) Extraction with strong alkali followed by preparation of an antiserum which, on immuno-electrophoresis against whole serum, gave precipitin arcs in the α-2 regions[8]. None of these methods has entirely surmounted the two main obstacles to precise identification of the protein components of amyloid. These are, first, the relative insolubility of this material in all but chemically vigorous reagents which are liable to degrade proteins and, secondly, the difficulty in obtaining amyloid free from incidental contamination by plasma proteins. The new method reported here appears to circumvent both these difficulties in the isolation of pure natural amyloid.

The technique involves cycles of : (*a*) brief ultrasonic disintegration into particles of macromolecular dimensions; (*b*) differential cold precipitation of the amyloid, which sometimes involves freezing, combined with numerous washings with neutral isotonic saline in order to remove all traces of incidentally trapped serum proteins. The substance so isolated is still in its original insoluble state and retains the tinctorial reactions (Schiff positivity, methyl violet metachromasia, positive congo red staining) characteristic of the parent amyloid deposits in the original tissues. The preparations from two human amyloidaccous kidneys were used to immunize rabbits and the antisera produced were analysed for their ability to precipitate human plasma proteins in immuno-electrophoresis.

Antiserum obtained with amyloid prepared from a patient with chronic syphilis reacted solely with 7*S* γ-globulin (Fig. 1). The antiserum produced by amyloid derived from a patient with chronic rheumatoid arthritis reacted with five plasma proteins ; very weakly with 7*S* γ-globulin, but very strongly with one α-1 and three α-2 components (Fig. 2). If the plasma proteins had been present in the amyloid preparations as a result of contamination from tissue fluids or blood they would most likely have been similar in both cases. However, since the antisera to each amyloid sample contained antibodies to different plasma proteins, the components revealed were probably integral with and peculiar to their respective amyloid substances. Neither sample induced precipitating antibodies to β-2-macroglobulin, to which class both rheumatoid factor and the Wassermann antibody belong[9], and thus these auto-antibodies were not components of the respective amyloid deposits. Hence, in opposition to Letterer's[10] belief regarding the pathogenesis of amyloid substance, it appears that neither of the amyloid samples tested contained sequestered products of an auto-immune reaction.

The present method of isolating amyloid, using only gentle physical means, seems to provide the substance pure and with its protein components undegraded. The findings with antisera prepared from such material may thus be accepted with less reserve than evidence obtained

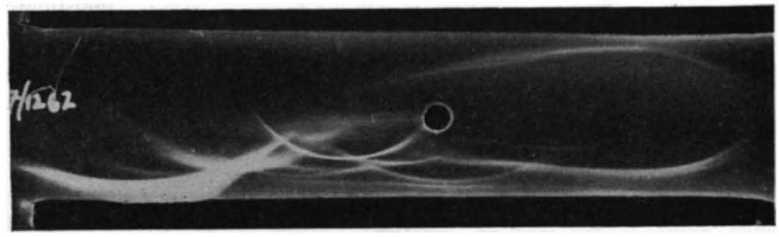

Fig. 1. *Upper trough*, antiserum to syphilitic amyloid; the only arc developed is to 7*S* γ-globulin, recognizable by its characteristic situation and curvature; *well*, whole human serum, anode to the left; *lower trough*, antiserum to whole human serum; *buffer*, borate-succinate; *p*H 8·6; M 0·05. N.B. The reduplication of the 7*S* γ-globulin lines on both sides is gel and/or Liesegang artefact

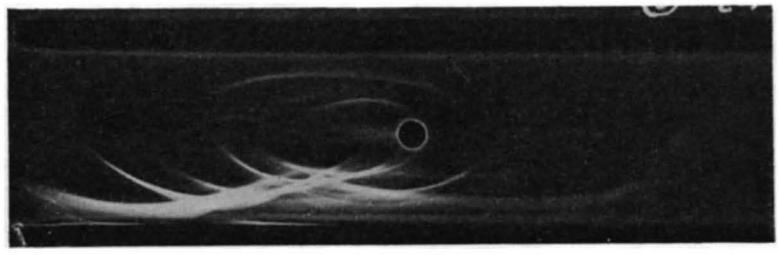

Fig. 2. *Upper trough*, antiserum to rheumatoid amyloid; the α-1 arc, two α-2 localized arcs, and an α-2 arc extending forward into the α-1 region are clearly visible. The 7*S* γ-globulin arc is too faint for photographic reproduction; *well*, whole human serum, anode to the left; *lower trough*, antiserum to whole human serum; *buffer*, barbitone; *p*H 8·2; M 0·05