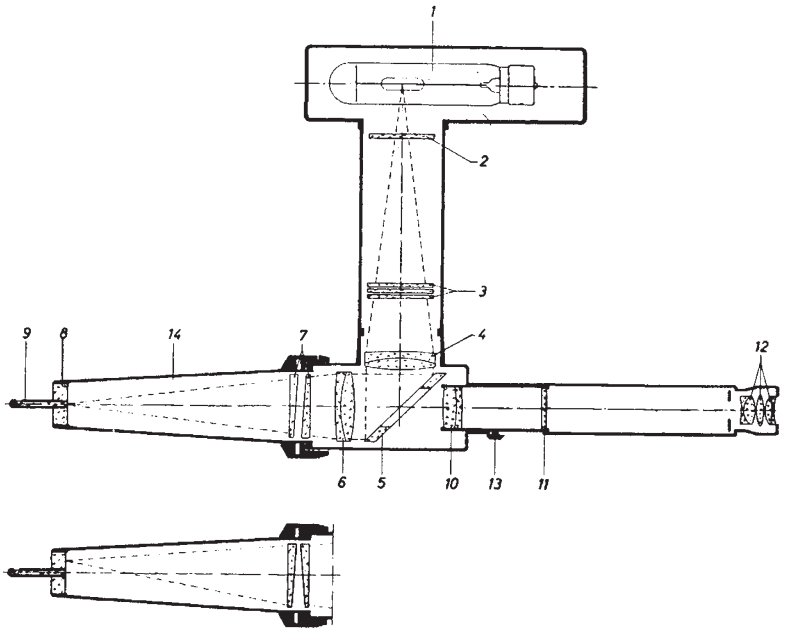

Fig. 1. The design of a fluoro-microscopic unit (the 'Cervicoscope') intended for the examination of the human cervix uteri

An instrument has been constructed (Fig. 1) for the examination of the human cervix†, but the principles are applicable to the design of instruments for the examination of other accessible organs. Incident illumination was used, the ultra-violet beam entering the main tube through a side-arm, to be deflected down to the objectives (Fig. 1, 8 and 9).

To avoid restriction of the objective aperture by the use of a prism or mirror to deflect the beam through 90°, as originally suggested by Ellinger and Hirt[1,2], a dichroic reflector with high ultra-violet reflectance and high transmission of ordinary light was used[5]. This increased the brightness of the image, which is known to vary with $(N.A.)_0^2/M$, where $(N.A.)_0$ is the numerical aperture of the objective, and M is the total magnification of objective and occular combined.

Further increase in image brightness was achieved by using an overall magnification of × 17, reducing to a minimum the distance between source and object, and illuminating a relatively small field with a condenser of higher numerical aperture. According to the fluorometric law of De Ment[14], the fluorescence intensity is proportional to the intensity of the optical excitant over wide ranges, and varies inversely as the square of the distance from the source of irradiation. In the living body, however, possible injury to cell membranes limits undue increase in the source intensity, hence this was kept within the safe range of 150–200 W, supplied by a high-pressure mercury vapour lamp with a specially constructed housing (Fig. 1, 1).

Since the area of the irradiated field at the object is equal to $A\ (N.A.)_s^2/(N.A.)_c^2$, where A is the effective source area, while $N.A._s$ is the numerical aperture of the lamp lens and $N.A._c$ is the numerical aperture of the condenser, it would have been possible to fill a large field by reducing the condenser aperture (Fig. 1, 4). This, however, would reduce the image brightness since the intensity of irradiation is proportional to $B\ (N.A.)_c^2$, where B is the image brightness. Therefore, it was preferable to illuminate a small field and obtain high image brightness by increasing the numerical aperture of the condenser. An alternative system is to fill a larger field by using a light source of large area and a lamp lens of high relative aperture.

To utilize a cross-filter system for excitation and inspection of the fluorescence of acridine orange, which is known to absorb in the region of 4000–4500 Å, a primary filter which cuts out all wave-lengths below 4000 Å was mounted between the lamp lens and the condenser (Fig. 1, 3), protected from the ultra-violet source by heat-resistant filter (2). The secondary filter (11), which cuts out all blue-violet rays (4000–4500 Å), was mounted between the objectives and ocular (12). This limits the fluorescence image to the green, yellow and red, which are the colours of monomeric, dimeric and polymeric acridine–nucleic acid complexes, respectively.

To avoid reflexion loss, the excitation rays are delivered to the object through a metal tube (14), and the fluorescence rays are afterwards transmitted back from the object to the ocular (12). An additional factor in image brightness was the use of a monocular unit, since the brightness is known to be reduced with a factor of more than 2 in all minocular fluorescence microscopes. The wedges (7) and button (13) are mechanical devices, respectively, for the illumination of successive fields and for focusing the fluorescence image.

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† The instrument, termed "The Fluorescence Cervicoscope", was designed by the author and constructed by J. D. Möller, Wedel, Hamburg, West Germany.

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IMMUNOLOGY

Type of Antibody Response in Mice immunized with Transplantable Tumours

Antibody in the serum of several animal species is confined to two main classes of proteins with sedimentation coefficients of approximately 7*S* and 19*S* respectively. The former class comprises molecules with molecular weights of at least 150,000 and includes both the 7*S*γ-globulins and the β_{2A}-globulins. The latter class is made up of β_2-macroglobulins with molecular weights of about 1,000,000. The type of antibody response appears to depend on a number of interrelated factors, probably the most important of which is the physicochemical nature of the stimulating antigen. Thus hæmocyanin[1], *A*2 influenza virus[2], and type III pneumococcal polysaccharide[1] were found to produce 7*S*γ-globulin, while sheep erythrocytes produced predominantly β_2-macroglobulins[1].

Various transplantable plasma cell tumours[3,4] and leukæmias[5] in mice have been described in the literature in recent years. Their availability has allowed a new approach to the study of malignant plasma cells and their effect on the serum protein systems in experimental animals.

An attempt has been made to follow the changes in the mouse γ-globulin system resulting from immunization with transplantable tumours by the use of analytical ultracentrifuge and gel-filtration techniques.

Mouse strains *A*, *C*, *C3H* and *C57BL* were used for these experiments. The transplantable mouse tumours comprised two mouse leukæmias, *AL3* and *EL4*, and two solid tumours, *AMT3* and *EP6*. The original tumours were kindly supplied by Dr. J. R. Batchelor, Guy's Hospital Medical School, London.

Batches of 20–100 mice of each strain were immunized with three injections of each of the heterologous tumours.

The first inoculum of approximately 5 million viable cells was given subcutaneously. The second and third injections of approximately 50–100 million cells were given intraperitoneally 4 weeks and 6 weeks respectively after the first inoculum.

Serum was obtained from the mice immediately prior to each successive immunization by bleeding the animals from the tail vein[6]. The mice were finally bled two weeks after receiving the last injection.

Hyperimmune sera from a batch of hybrid mice, designated *PH* mice, and from 129 mice, both immunized with *BP8* tumour, were also kindly provided for examination by Dr. J. R. Batchelor.

The degree of the serum antibody response, as measured by the hæmagglutinating activity against mouse red cells[7], varied considerably in the initial stages depending on the mouse strain and on the tumour type. After the final immunization, the hæmagglutination titres were all of the same order.

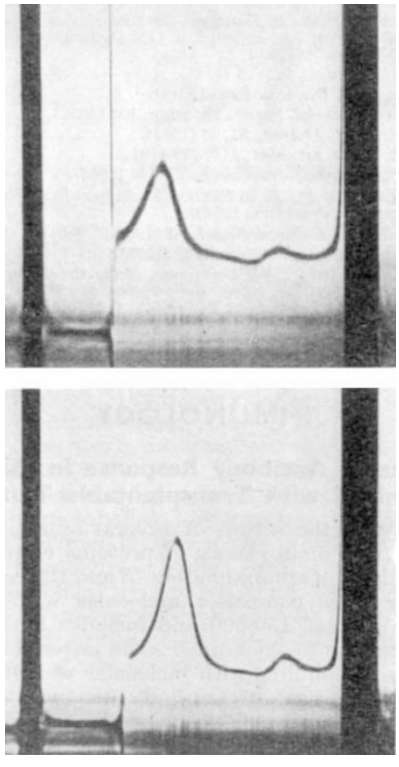

Fig. 1. Schlieren patterns obtained from sedimentation velocity experiments with mouse globulin preparations in the Spinco model *E* analytical ultracentrifuge. Speed, 42,040 r.p.m.; temperature, 20·0° C; photographs taken 52 min after reaching set speed. (*a*) Solution of normal globulins, phase plate angle, 40°; (*b*) solution of immune globulins, phase plate angle, 50°

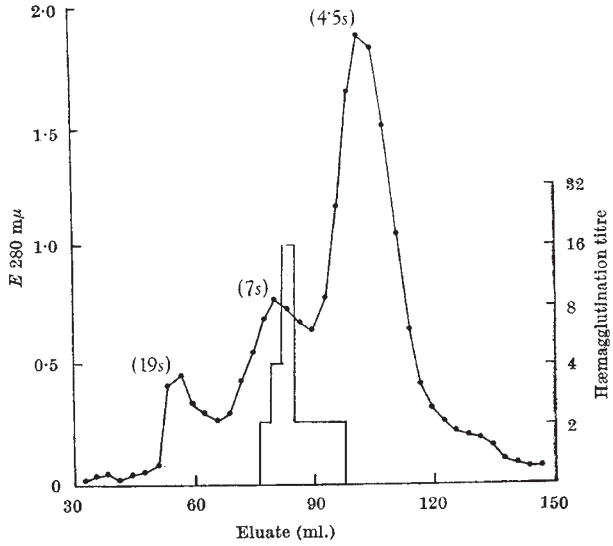

Fig. 2. Gel-filtration of mouse anti-tumour serum on 'Sephadex *G*–200' showing the distribution of antibody activity (open block) related to the eluted protein peaks

Equal volumes of these sera, from which most of the albumin had been removed by fractionation with sodium sulphate, were dialysed against 0·1 M *tris*-hydrochloric acid buffer, *p*H 7·4, in 0·2 M saline, and then restored to equal volumes. These protein solutions were centrifuged at 42,040 r.p.m. in a Spinco model *E* analytical ultracentrifuge operated at 20·0° C. No change in area of the faster sedimenting peak (19*S*) was observed in the Schlieren patterns of the immune sera as compared with the normal sera. However, the area of the slower sedimenting peak, identified as the 7*S* component and which had a sedimentation coefficient of 5*S* under these experimental conditions, was significantly increased in all the hyperimmune sera. This indicates an increase in the relative concentration of the 7*S* component following immunization (Fig. 1).

Samples of serum obtained from the mice 2 weeks after the final immunization were subjected to gel filtration on 50 × 1·8 cm. 'Sephadex *G*-200' (Pharmacia, G.B.) columns. In some cases most of the albumin was removed from the sera by fractionation with sodium sulphate in order to allow greater amounts of the mouse globulins to be loaded on to the columns. 0·1 M *tris*-hydrochloric acid buffer, *p*H 7·4 in 0·2 M sodium chloride, was used to elute the proteins from these columns.

The extinction at 280 mμ and the hæmagglutination activity of the eluted column fractions were measured (Fig. 2). The principal peaks eluted from the column were identified as 19*S*, 7*S* and 4·5*S* proteins in the analytical ultracentrifuge. The proteins contained in these broad zones were further identified by starch-gel electrophoresis. Hæmagglutinating activity was exclusively confined to the 7*S* proteins in all the immune sera examined.

The immune γ-globulin could be isolated by DEAE-cellulose chromatography of the mouse globulin fraction. This γ-globulin was identified by starch-gel electrophoresis and by its behaviour on sedimentation velocity analysis.

These results may be summarized as follows. As judged by hæmagglutinating activity and ultracentrifuge analysis, no evidence was found for the presence of 19*S* serum antibody during the course of immunization of several strains of mice with five heterologous transplantable mouse tumours.

This work has formed the basis for a detailed investigation in mice of the immune response to tumours[8,9]. It was supported by the Cowburn research fellowship,

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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,