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PRODUCTION OF RUBELLA ANTISERUM IN HAMSTERS

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BHK 21 CELLS chronically infected with rubella virus can be serially passed in culture (Professor K. McCarthy, personal communication). The observation that most BHK cells that have been continually passed are oncogenic in hamsters (Foley and Handler, 1957), and the demonstration of viral antibodies in sera of hamsters with tumours induced with adenovirus type 12 (Huebner, Rowe and Lane, 1962), prompted us to study the effects of inoculating rubella virus-infected cells into hamsters. As a comparison with the prolonged stimulus of these infected cells, control animals were inoculated with a single dose of cell-free antigen.

MATERIALS AND METHODS

BHK 21 clone 13 cells, chronically infected with rubella virus, were grown in suspension culture in Eagle's medium containing 5 per cent. (v/v) inactivated foetal calf serum, 0.3 per cent. (v/v) tryptose phosphate broth, 0.1M glucose, 0.01 μ g per 100 ml (w/v) ferric nitrate, 100 units per ml of penicillin and 100 μ g per ml streptomycin at 35°C. The presence of virus antigen was checked by indirect (sandwich) immunofluorescent staining of acetone-fixed coverslip preparations of the cells with an immune human serum (haemagglutination-inhibition titre 1 in 512), with the usual tests for specificity (Nairn, 1964).

Fourteen hamsters, each weighing approximately 80 g, were given an inoculation into the interscapular space of 10^7 infected cells, 69 per cent. of which contained rubella antigen. Tumours developed in 2-3 wk, and when these were beginning to break down, 3-4 wk later, the animals were bled and killed. In order to test for the continued presence of antigen, smears were made of 11 of the tumours, and the cells of 1 tumour were cultured.

Cell-free antigen was obtained by alternately freezing and thawing the rubella virus-infected cells 3 times, followed by centrifugation and filtration. The antigen from 10^7 cells, which contained 5×10^5 ID₅₀ of infectious virus, was inoculated into the interscapular space of each of 10 hamsters as a control. After 6 wk the animals were bled and killed.

Estimation of rubella antibody in sera. Two methods were employed to assay rubella antibody in the hamster sera.

(i) *Haemagglutination-inhibition test.* Antigen was produced from the rubella virus-infected cells suspended in serum-free medium. A modification of the technique of Stewart and his colleagues (1967) was used. Pigeon red cells were substituted for chick red cells (Holmes and Warburton, 1967); cells from a single pigeon were used throughout. The dextrose gelatin barbitone diluent was adjusted to pH 6.2 (Clarke and Casals, 1958; Holmes

and Warburton). Antibody titre was expressed as the highest dilution of serum producing complete inhibition of haemagglutination.

(ii) *Titration of serum antibody by immunofluorescence.* The indirect method was employed: each serum dilution was tested on acetone-fixed cultures of the infected cells followed by anti-hamster globulin conjugated with fluorescein isothiocyanate (FITC.) Antibody titre was expressed as the highest dilution of serum that gave definite positive staining of rubella antigen.

Conjugates of hamster sera. Two pools of hamster sera were conjugated with FITC; one made up of sera with haemagglutination-inhibition titres greater than 1 in 64 (titre of pool 1 in 128), and the other with haemagglutination-inhibition titres less than 1 in 64 (titre of pool 1 in 16). Their direct staining properties were compared.

TABLE I

Antibody levels measured by haemagglutination-inhibition in hamsters inoculated with virus-infected cells, and with antigen alone

Serum titre	Number of sera at each titre in animals given an injection of	
	infected cells	antigen
8	0	1
16	2	8
32	4	1
64	3	0
128	2	0
256	1	0
512	1	0
1024	1	0

Number tested in infected cell group: 14; in antigen group: 10.

No antibody was found at dilution 1 in 8 in 9 sera tested before immunisation.

Immunofluorescent staining of smears of tumours and of cultures of tumour cells. Smears prepared from hamster tumours and cultured preparations of tumour cells were stained directly with a hyperimmune rabbit serum (rubella haemagglutination-inhibition titre 1 in 512) conjugated with FITC.

RESULTS

The pre- and post-inoculation sera were tested by haemagglutination-inhibition (HI), and a significantly higher level of antibody was obtained in the group of hamsters inoculated with the infected cells than in the group given antigen alone (table I: exact probability χ^2 test, 2 d.f., $P = 0.003$). When the post-inoculation sera of the former group were tested by fluorescent antibody staining, the levels of antibody found were comparable with those found by HI (table II).

Conjugates of the "high titre" serum pool stained specifically up to a dilution of 1 in 20; of the "low titre" serum pool to a dilution of 1 in 10. Both conjugates gave the minimal degree of non-specific staining.

The tumour smears and cultured cells showed rubella antigen.

DISCUSSION

Several methods have been described for the production of antiserum to rubella virus (McCarthy, Taylor-Robinson and Pillinger, 1963; Hutchinson and Thompson, 1965;

Best *et al.*, 1967; Estela, 1967; Gispen and Brand-Saathof, 1967; Hassan and Cochran, 1967). These differ in some respects, but all require repeated inoculations of relatively high titre rubella virus.

The finding that a specific antiserum to rubella virus can be prepared in hamsters by a single inoculation of BHK 21 cells chronically infected with the virus suggests that this method of antiserum production may be applicable to other viruses, provided that they can cause chronic infection in these cells. The amount of antiserum obtained from each hamster is small (approximately 2.5 ml) compared with that obtainable from rabbits, but the preparation of the inoculum is simple. The minimal degree of non-specific staining obtained with

TABLE II

Comparison of antibody levels measured by two methods in sera of hamsters inoculated with rubella virus-infected cells

Serial number of animal	Titre of serum in	
	haemagglutination-inhibition test	immunofluorescence test
1	16	20
2	16	20
3	32	40
4	32	40
5	32	40
6	32	40
7	64	40
8	64	80
9	64	160
10	128	80
11	128	160
12	256	160
13	512	320
14	1024	320

Titres of sera are expressed as reciprocals of serum dilutions. Geometric mean titres in haemagglutination-inhibition test, 74.2; in immunofluorescence test, 72.4.

immunofluorescence demonstrates the value of using virus cultured on the tissue of the antibody-producing species. In order to obtain sera of higher titre further experiments are planned, aimed at prolonging the life of the tumour and hence the antigenic stimulus.

Comparing the haemagglutination-inhibition and immunofluorescence tests in the serodiagnosis of rubella infection and for the detection of antibody elicited by past infection in man, Lennette, Schmidt and Magoffin (1967) found the tests to be of equal value. In their series the levels of haemagglutination-inhibiting antibody were higher than those of antibody assayed by immunofluorescence. In our series in hamsters the mean levels of antibody were similar when tested by the two methods, but the range of the haemagglutination-inhibition titres is less than that of Lennette *et al.*

The continuing presence of virus antigen in the tumour cells confirms that antigenic stimulus is prolonged. It is interesting that virus persists even in the presence of virus-specific antibody.

SUMMARY

In hamsters a single inoculum of BHK 21 cells chronically infected with rubella virus formed a tumour and stimulated antibody response to the virus. Haemagglutination-inhibition and titration by fluorescent staining were equally sensitive as tests for antibody.

The sera with higher HI titre were, when conjugated with fluorescein isothiocyanate (FITC), suitable for immunofluorescent staining.

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GERMICIDE FOR STERILISING THE SURFACE OF EGGS IN GNOTO BIOLOGY

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THE production of germ-free chickens starts with the assumption that the embryo inside the shell is sterile. If the outside of the shell can be successfully sterilised and the incubation completed under sterile conditions the chickens will be germ-free. Many workers since Nuttall and Thierfelder (1897) have attempted to find a suitable germicide for this purpose. Such a germicide should be capable of killing spores, yet at the same time it should not damage the developing embryo. Early workers (Nuttall and Thierfelder; Schottelius, 1908; Cohendy, 1912; Balzam, 1937) used mercuric chloride in various concentrations and at various times during incubation. The method was standardised by Reyniers *et al.* (1949), and mercuric chloride still remains the most commonly used germicide (Coates, 1968). Its use, however, often leads to a lower hatch rate and it is known, furthermore, that mercuric