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Recognition of the Species of Origin of Cells in Culture by Mixed Agglutination

I. USE OF ANTISERA TO RED CELLS

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Summary. Preliminary experiment on the mixed agglutination reaction suggests that this reaction will afford a useful method for identifying the species of origin of cells maintained in culture.

The reaction depends on the presence of antigens characteristic of the species, common to both tissue cells and red cells. Culture cells derived from man, ox, pig and rat could be distinguished one from the other. Fibroblasts of the mouse may be differentiated from those of the rat by means of a rat anti-mouse red-cell serum or a mouse anti-rat red-cell serum.

Experiments are reported on trial absorption procedures to render the sera completely species-specific in their reactions.

INTRODUCTION

There are many applications of *in vitro* cell culture today. The method, on the one hand, allows a full study of the biological behaviour of individual cells growing *in vitro* and, on the other hand, serves as an excellent tool for, for instance, the propagation and study of viruses

Characterization of cells in terms of their antigens is important for both these aspects of cell culture. In the first place if changes, transformation and possible mutation of cells in culture could be shown to be accompanied by definite antigenic changes, one would have added information as to the nature of the change. Secondly when cell lines are being used simply as tools for the study of virus propagation, antisera specific for certain lines of cells could serve as necessary checks against mistaken labelling or accidental contamination with other cells.

The object of the present paper, and further work to follow, is to report on the use of the mixed agglutination method, which, using selected sera, allows the easy demonstration of some of the antigens on individual cultured cells. The present study has been directed towards antigens characterizing the species of origin of cultured cells.

Mixed agglutination (Coombs, Bedford and Rouillard, 1956) is the specific clumping together, by antibody, of two types of cell — the cell under study (a cultured cell in the present work) and an indicator cell (usually a red cell) which possess an antigen in common.

Up till now the method has only been applied to the demonstration of blood group iso-antigens on cultured tissue cells (Kelus, Gurner and Coombs, 1959). If, however,

there were so-called species-specific antigens on cultured tissue cells, which were common also to red cells of the same species, then the mixed agglutination method (see Table 1) should also be applicable to the problem of recognizing the species of origin of cells growing in culture. Preliminary experiments to be reported here suggest that this is so.

TABLE 1

PRINCIPLE OF MIXED AGGLUTINATION AS A METHOD OF RECOGNIZING THE SPECIES OF ORIGIN OF TISSUE CELLS IN CULTURE

Red-cell antiserum	*Cultured tissue cells under study*	*Red-cell indicator system*	*Mixed agglutination*
Anti-human	Human	Human	+
Anti-human	Rat	Human	–
Anti-human	Pig	Human	–
Anti-rat	Human	Rat	–
Anti-rat	Rat	Rat	+
Anti-rat	Pig	Rat	–
Anti-pig	Human	Pig	–
Anti-pig	Rat	Pig	–
Anti-pig	Pig	Pig	+

MATERIALS AND METHODS

TISSUE CELLS USED IN THE TESTS

1. *Cultures*

Cells were cultured in 200 ml. Pyrex feeding bottles. The following cultures were used:

(*a*) *Cell lines.* Two lines of human cells — HeLa and KB — were used.

HeLa cells. The cultures used were derived from a sample of HeLa cells obtained from the Medical Research Institute, Mill Hill. The cells were grown in Eagle's medium supplemented with 10 per cent human serum. They were harvested, 6–7 days after subculture, by removing the growth medium and adding 5 ml. of sodium versenate (a 1 in 5000 solution in phosphate buffered saline) to the cell sheet. After 10 minutes' incubation at 37°, the cells were pipetted off the glass, transferred to a tube, centrifuged at 200 *g* for 5 minutes and washed once in growth medium. Finally, the cells were resuspended in 20 per cent glycerol in growth medium, frozen rapidly in an ethanol-CO_2 mixture, and stored at −70° until required.

KB cells. These were derived from a sample of KB cells obtained from the Research Institute (Animal Virus Diseases), Pirbright, and were grown in Eagle's or Puck's synthetic medium supplemented with 20 per cent bovine serum. They were harvested in the same way as the HeLa cells, and suspended for freezing and storage in 50 per cent glycerol in their own growth medium.

(*b*) *Freshly isolated cells*

Ox kidney epithelial cells. These were obtained from adult bovine kidney cortex. The tissue was chopped finely, washed three times in phosphate buffered saline (P.B.S.) and incubated in 0.5 per cent trypsin (Light & Co.) at room temperature with constant stirring

until a sufficient degree of digestion had been obtained. The cells were then centrifuged at 200 *g* for 5 minutes, washed twice in growth medium, and finally resuspended in growth medium for inoculation of the culture vessels. The growth medium used was a mixture of Earle's buffered salt solution supplemented with 20 per cent bovine serum and ½–2 per cent yeast extract (Nutritional Biochemicals Corporation).

After 6–7 days in culture the cells were harvested using versenate and stored at −70° in 20 per cent glycerol in growth medium.

Pig kidney epithelial cells. These were obtained, cultured, harvested and stored in the same way as the bovine kidney epithelial cells.

Rat skin fibroblasts. The skins of rat foetuses at term were pooled, cut into small pieces and trypsinized (using 1 per cent 'Difco' trypsin in calcium- and magnesium-free Tyrode's solution) at 37.5° for approximately 10 minutes to free the epidermis. This was removed, and the remaining dermis trypsinized for a further hour; the undigested residues were then discarded, and the cell suspension centrifuged at 200 *g* for 5 minutes. The cells were washed once in growth medium and then resuspended in growth medium for inoculation of the culture vessels.

The growth medium used for most of these cultures was a mixture of Tyrode's solution, horse serum (not heat-inactivated) and 50 per cent chick embryo extract in the proportion of 6 : 3 : 1. Parker's medium No. 199, supplemented with 20 per cent bovine or rat serum has also been used.

After 6–7 days' culture the cells were harvested by removal of the growth medium and treatment of the cells with 5 ml. of 0.1 per cent trypsin (Light & Co.) in 0.9 per cent saline at 37° for 12–15 minutes. The cells were then pipetted off the glass into a tube and centrifuged at 200 *g* for 5 minutes. After being washed in growth medium they were finally resuspended in 20 per cent glycerol in growth medium, frozen rapidly in ethanol-CO_2, and stored at −70°.

Mouse skin fibroblasts. These were obtained from mouse foetuses at term using the same technique as for the rat fibroblasts. They were cultured in Parker's medium No. 199 supplemented with 20 per cent horse or mouse serum, and were harvested, frozen and stored in the same way as the rat cells.

2. *Cells Obtained Direct from Epithelial Surfaces*

Samples of oesophagus and ureter were obtained from freshly killed animals. The tissues were pinned out, washed well with 0.9 per cent saline, then gently brushed with a No. 5 artist's brush. The cells were transferred by gently rotating the brush in a tube containing 0.9 per cent saline, then centrifuged at 200 *g* for 5 minutes. After washing once in 0.9 per cent saline, the cells were finally resuspended in 20 per cent glycerol in 0.9 per cent saline, frozen in ethanol-CO_2, and stored at −30°.

3. *Preparation of Cell Suspension for Testing*

(*a*) *Recovery.* All cell suspensions were recovered from −30° and −70° by immersing the tubes in the 37° water bath and gently agitating until the suspensions thawed. From this point the cells were kept in an ice-water bath, and all centrifugations were carried out in a refrigerated centrifuge. Samples of approximately 0.5 ml. amounts were centrifuged at 200 *g* for 8 minutes and the supernatant fluid discarded.

(*b*) *Washing.* The deposited cells after initial centrifugation were resuspended in 1 ml. of diluent (ice-cold 1/200 N.R.S. diluent — see 'Reagents used in the mixed agglutination

test'), and again centrifuged at 200 *g* for 5 minutes. After one further wash in 1 ml. of diluent the cells were resuspended in diluent to give approximately fifteen to twenty cells per microscope field when viewed with a ×40 objective and ×10 eyepiece. They were used in this concentration for the tests.

Production of antisera to red cells

1. *Rabbit Antisera to the Red Cells of Man, Ox, Pig, Rat and Mouse*

These sera had been prepared at various times and since preserved at −20°. The rabbits received two courses of injections of 10 per cent washed red cells. Each course consisted of four to six injections of 2–5 ml. given either intravenously or intraperitoneally. The animals were bled about 10 days after the last injection. The sera obtained after the second course of injections were used for the mixed agglutination tests.

2. *Rat Anti-Mouse Red-Cell Serum*

Ten white rats each received ten subcutaneous injections of mouse red cells (strain BALB/c) over the period of a month. Each injection consisted of 0.5 or 1 ml. of a 10 per cent suspension of washed red cells. The rats were killed and bled 10 days after the last injection. The pooled sera had an agglutinating titre against mouse red cells of 128.

3. *Mouse Anti-Rat Red-Cell Serum*

Forty BALB/c mice were injected with rat red cells with a schedule as just described. Each injection consisted of 0.2 ml. of a 10 per cent cell suspension. The sera finally obtained were pooled and the agglutinating titre for rat red cells found to be 128 also.

Absorption of antisera

Preparation of Lymphoid Cells for Absorption Purposes

Lymph glands, thymuses and, in the case of the mouse, spleens were obtained from recently killed animals. The glands were cut into fine slices with a razor-blade and the lymphoid cells shaken out into ice cold Tyrode's fluid. The suspensions were filtered through gauze, washed twice in cold Tyrode's fluid and then, as approximate 10 per cent suspensions, treated with an equal part of neutralized 1 per cent formalin solution in Tyrode's fluid. After 24 hours at +4° with occasional shaking the cells were washed once to remove the formalin and stored at −30° as a 20 per cent suspension in 20 per cent glycerol in saline. For absorption, these suspensions were thawed and washed once in saline. Cells treated in this way did not form large clumps but remained intact and free of clumping.

Preparation of Autoclaved Red Cells

Red cells were washed free of plasma and as 25 per cent suspensions in saline were autoclaved at 20 lb. pressure for 15 minutes. The chocolate-coloured mass was centrifuged and the deposit washed twice in saline. Finally the insoluble material was resuspended in 0.5 per cent phenol in saline and stored at +4°.

Actual Absorptions of Sera

The heat-inactivated sera were diluted 1/2 or 1/5 before absorption and were usually treated two to four times with the absorbing material, which, as a packed deposit, would amount to 25–30 per cent by volume of the serum to be absorbed.

Reagents used in the mixed agglutination tests

Antisera

The red-cell antisera were heat-inactivated at 56° for ½ hour before being used in the tests.

N.R.S. Diluent

Normal rabbit serum diluent (N.R.S. diluent) was used to keep the cells from sticking to the glassware. This consisted of saline, buffered at pH 7.2 by adding 10 ml. Sörenson's buffer to each litre of 0.9 per cent NaCl, to which one part heat-inactivated normal rabbit serum (absorbed with whichever red cells were being used in the test) had been added to every 199 parts of the salt solution.

Red-Cell Indicator Suspension

This consisted of a suspension of the appropriate washed red cells in a 0.5 per cent suspension.

As ox red cells may be 'inagglutinable' (Coombs, Gleeson-White and Hall, 1951) these were previously treated with papain before use to render them more agglutinable (see also Hawes and Coombs, 1960).

Treatment of Ox Red Cells with Papain

The red cells were centrifuged, washed three times and finally resuspended to give a 20 per cent solution in 0.9 per cent saline. To this suspension was added an equal volume of papain solution; this consisted of a mixture of one volume of 0.25 per cent papain (B.D.H.) one volume of 3.6 per cent disodium hydrogen phosphate ($Na_2HPO_4.12H_2O$), and two volumes of 0.2 per cent L-cysteine hydrochloride. The resultant suspension was incubated in the 37° water bath for 15 minutes, after which the red cells were washed three times in 0.9 per cent saline.

Glassware

All the glassware used in the tests was silicone treated.

Mixed agglutination test

To two drops of serum dilutions in 50 × 10 mm. siliconed tubes were added two drops of the appropriate tissue cell suspension. The tubes were incubated on a slowly revolving machine at room temperature for 1 hour.

The tissue cells were then deposited by centrifuging at 200 *g* for 5 minutes in a refrigerated centrifuge, and washed twice in cold diluent, finally being resuspended in one drop. To each of these was added one drop of the appropriate red-cell suspension and the mixture was centrifuged again at 200 *g* for 2 minutes to bring the cells into contact.

The deposited cells were lifted gently with a siliconed capillary tube (100 × 1.2 mm.) and, together with a little of the supernatant fluid, carefully placed on a siliconed slide. Finally, a siliconed coverslip was very gently placed in position and sealed with paraffin wax.

RESULTS

I. REACTIONS OF FIVE UNABSORBED RED-CELL ANTISERA WITH CULTURED CELLS FROM FIVE ANIMAL SPECIES

Red-cell antisera were made against the red cells of man, ox, pig, rat and the mouse. The antisera used were second-course sera prepared as described under Materials and Methods. The sera were titrated in mixed agglutination reactions and tested against human, ox, pig and rat cultured cells. Cultured mouse cells were also used in some tests. The type and source of the cultured cells is given under Materials and Methods. The results of these mixed agglutination tests are recorded in Table 2. The appearance of a negative and positive reaction is shown in Fig. 1*a* and 1*b*.

TABLE 2

MIXED AGGLUTINATION OF ANTISERUM-TREATED TISSUE CELLS AND INDICATOR RED CELLS

Rabbit anti-red-cell sera	*Indicator red cell*	*Cultured cells*					
		Human		*Ox kidney epithelium*	*Pig kidney epithelium*	*Rat fibroblast*	*Mouse fibroblast*
		HeLa	*KB*				
Anti-human, Gp. O	Human, Gp. O	*1280*	*640*	0	80	0	
Anti-ox	Ox (papain)	40	40	>*5120*	80	40	
Anti-pig	Pig Gp. O	0	0	20	*160*	0	
Anti-rat	Rat	0	5	0	0	*160*	160
Anti-mouse	Mouse	0		40	0	160	*1280*

Figures indicate the titre giving positive mixed agglutination.
(papain) = papain treated.
0 = <5.

The first point to be commented on is that the strong mixed agglutination which occurred, indicated that tissue cells have antigens in common with red cells. This was an essential pre-requisite to any further work. The next encouraging finding was that the reactions with the homologous tissue cells were in all instances, except in the case of rat and mouse, by far the strongest and if the red-cell antisera were used at a certain dilution a specific testing reagent was already available.

However, as may be seen from Table 2 a considerable degree of cross-reaction was also observed. This, however, was really only to be expected as the red-cell antisera were not previously absorbed in any way.

II. ATTEMPTED ABSORPTION OF THE RED-CELL ANTISERA TO MAKE THEM SPECIFIC FOR RECOGNIZING THE SPECIES OF ORIGIN OF CULTURED CELLS

To achieve this end is not quite as easy as might appear at first sight. Considering the matter rationally we should expect little from absorbing the red-cell antisera with heterologous red cells only, as it is the antibodies to antigens on heterologous tissue cells which happen to be common to the homologous red cells that absorption should be aimed at removing. The ineffectiveness of absorptions with heterologous red cells only was confirmed by experiment.

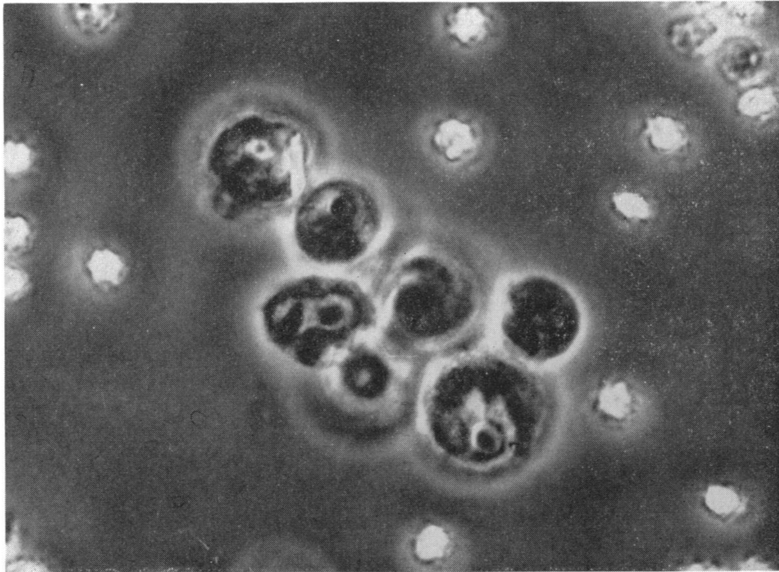

FIG. 1*a*.

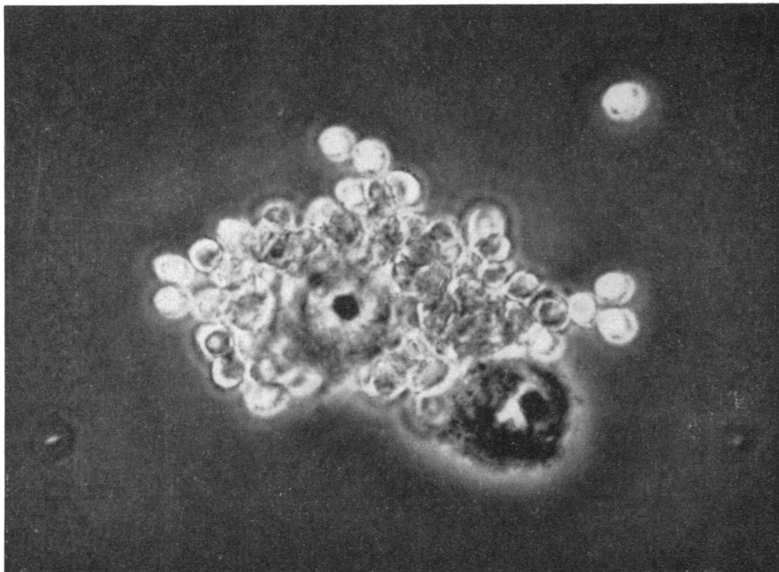

FIG. 1*b*.

FIG. 1. Mixed agglutination reactions on human KB cultured cells to demonstrate their species of origin.

a. negative reaction with anti-pig red-cell serum and pig red cells.

b. positive reaction with anti-human red-cell serum and human red cells.

Phase-contrast microscopy × 800.

Two other absorption procedures have been briefly investigated and it is possible that, after some degree of refinement, both may be useful in the pre-treatment of particular antisera. The first of these procedures which seemed sound on rational grounds involved absorbing the sera with lymphoid cells of the heterologous species. The second procedure involved treating the red-cell antisera with the corresponding homologous but autoclaved red cells. The rationale underlying this latter manœuvre was the following. It was thought possible that the 'species-specific antigens' on the red cell would be proteins and therefore thermolabile but that the cross-reacting or heterophile antigens would be polysaccharide and thus heat-stable. If this were the case absorption of say anti-pig red-cell sera with autoclaved pig red cells should remove the cross-reacting antibodies and render the serum specific for the 'species antigens' on pig red cells. So much for the theory. Each procedure was then tested empirically.

A. *Absorption with Lymphoid Cells of Heterologous Species*

As a satisfactory source of tissue cells to be obtained from different animal species, lymphoid cells were chosen as being most easily procured. These were obtained from lymph glands, thymuses or in some cases the spleen. The suspensions were treated mildly

TABLE 3

ATTEMPT TO MAKE RED-CELL ANTISERA SPECIFIC FOR DEMONSTRATING THE SPECIES OF ORIGIN OF CULTURED TISSUE CELLS BY ABSORPTION WITH HETEROLOGOUS LYMPHOID AND RED CELLS

Rabbit anti-red-cell	*Treatment of serum*	*Cultured cells*					
		Human		*Ox kidney epithelium*	*Pig kidney epithelium*	*Rat fibroblast*	*Mouse fibroblast*
		HeLa	*KB*				
Anti-human	Untreated	1280	640	0	80	10	
Anti-human	Abs. lymph cells and r.b.c. pig and ox	160	160	0	0	0	
Anti-ox	Untreated	40	40	>5120	80	40	
Anti-ox	Abs. lymph cells and r.b.c. pig and rat	0		2560	0	0	
Anti-pig	Untreated	0	0	20	160	0	
Anti-pig	Abs. lymph cells and r.b.c. ox		0	0	20		
Anti-pig	Abs. lymph cells and r.b.c. ox and human		0	0	(5)		
Anti-mouse	Untreated	0		40	0	160	1280
Anti-mouse	Abs. lymph cells and r.b.c. ox	0		(20)	0	160	1280
Anti-mouse	Abs. lymph cells and r.b.c. ox and gp kid.	0		0	0	80	1280

Abs. = absorbed; lymph = lymphoid; r.b.c. = red blood cells; gp kid. = autoclaved guinea-pig kidney.
Figures indicate the titre giving positive mixed agglutination — a figure in brackets indicates a weak reaction at this titre.
0 = <5.

with formalin as described under Materials and Methods to prevent subsequent clumping.

Sera previously diluted 1/5 were absorbed three or four times with approximately one-third the volume of packed lymphoid cells. Finally the sera received two absorptions with one-quarter the volume of packed red cells as indicated in Table 3. In this table, also,

are shown the results of testing these sera, both before and after absorption, for specificity in the mixed agglutination reaction on the various cultured tissue cells.

The method proved to be successful with the anti-ox and anti-mouse sera. With the latter serum further absorption with rat lymphoid cells, also reduced the homologous reaction as might be expected. With the anti-human red-cell serum absorption with pig and ox lymphoid cells considerably reduced the strength of the homologous reaction also. In the case of the anti-pig red-cell serum attempts to make the serum specific by this procedure failed — the homologous reaction being almost completely removed by heterologous lymphoid cells. The anti-pig red-cell serum, even unabsorbed, did not give a very high titred reaction on pig tissue cells. Two further sera also gave reactions of this strength.

Although this procedure of absorption was not without promise one could not rest satisfied with the results. Not only did the method completely fail with the anti-pig serum — but the reduction in the homologous reactions also indicated its failings. In many cases also, after absorption an annoying 'stickiness' remained in the reactions with the heterologous tissues. This stickiness could in no way be confused with a positive result but it was an indication that the perfect absorption had not been achieved.

B. *Absorption with Homologous Autoclaved Red Cells*

Autoclaved red cells were prepared as described under Materials and Methods. The sera, previously diluted 1/2 were absorbed two, three or four times with approximately one-third their volume of packed autoclaved homologous red cells and then, as before, tested for the specificity of their reaction with various cultured tissue cells (Table 4).

TABLE 4

ATTEMPT TO MAKE RED-CELL ANTISERA SPECIFIC FOR DEMONSTRATING THE SPECIES OF ORIGIN OF CULTURED TISSUE CELLS BY ABSORPTION WITH HOMOLOGOUS AUTOCLAVED RED CELLS

Rabbit anti-red-cell sera	*Treatment of serum*	*Cultured cells*			
		Human HeLa	*Ox kidney epithelium*	*Pig kidney epithelium*	*Rat fibroblasts*
Anti-human	Untreated	*1280*	0	80	10
Anti-human	Abs. auto. human r.b.c. × 2	*320*	0	80	2
Anti-human	Abs. auto. human r.b.c. × 4	*160*	0	20	0
Anti-ox	Untreated		*2560*	80	40
Anti-ox	Abs. auto. ox r.b.c. × 4		*320*	0	0
Anti-pig	Untreated		20	*160*	2
Anti-pig	Abs. auto. pig r.b.c. × 3		0	*40*	0

Abs. = absorbed.
Auto. r.b.c. = autoclaved red blood cells.
Figures indicate the titre giving positive mixed agglutination.
0 = <2.

This procedure for absorption failed with the anti-human serum. It was relatively successful with the anti-ox and anti-pig sera, although again, in both these cases, the homologous reaction was somewhat weakened. It is interesting that with the anti-pig serum better results were obtained using this method than were obtained with absorption procedure A — the converse was true with the anti-human serum.

It is obvious that until more is known about the nature of the antigens involved in this species recognition of tissue cells one will always be working very much in the dark. In the meantime every effort should be made to produce specific sera *de novo*.

With this end in view the next experiment was undertaken in the hope of producing such a specific serum *de novo* and also one capable of differentiating the closely related species of the rat and mouse.

III. PRODUCTION '*DE NOVO*' OF SPECIFIC ANTISERA AND ONES CAPABLE OF DIFFERENTIATING CULTURED TISSUE CELLS OF THE RAT FROM THOSE OF THE MOUSE

Rats were injected with mouse red cells and mice with the red cells of rats. After ten injections as described under Materials and Methods the animals were bled and the sera used in mixed agglutination tests on cultured fibroblasts from the rat and mouse and on tissue cells from other species. The highly specific nature of the reactions is indicated in Table 5.

TABLE 5

SPECIFIC MIXED AGGLUTINATION REACTIONS DIFFERENTIATING FIBROBLASTS OF THE RAT FROM THOSE OF THE MOUSE

Red-cell antiserum	*Indicator red cell*	*Cultured cells*				
		Human HeLa	*Ox kidney epithelium*	*Pig kidney epithelium*	*Rat fibroblasts*	*Mouse fibroblasts*
Rat anti-mouse	Mouse	0	0	0	0	16
Mouse anti-rat	Rat	0	0	0	32	0

Figures indicate the titres giving positive mixed agglutination.
0 = <2.

With these two sera, although the reactions with homologous tissue cells were quite definite, the strength of the mixed agglutination even with the higher concentrations of the serum was not as strong as that seen with the rabbit antisera and more care had to be taken setting up the slides for reading the tests. It is possible that this is to be explained by the fewer number of operative antigen sites. However, investigations are being directed into ways of strengthening these reactions. If this can be achieved this procedure of producing the red-cell antiserum in a closely related species might afford a very satisfactory way of obtaining specific antisera for this test.

IV. RECOGNITION OF THE SPECIES OF ORIGIN OF FRESHLY ISOLATED EPITHELIAL CELLS OF VARIOUS SPECIES

So far in this study it has not been verified that the 'species-specific' reactions shown hold for all lines of cells from the one species. The present experiment was undertaken partly with this in view and partly to see if the method was applicable to species differentiation of such different types of cells as epithelium of the oesophagus and ureter, not obtained in culture. These epithelial cells were obtained by gently brushing or scraping the lining of the oesophagus and ureter of a freshly killed animal or a recently dead person as described under Materials and Methods.

Anti-human, anti-ox and anti-pig red-cell sera treated as described under II A or II B to make them species-specific were used in the tests on the various epithelial cells. Table 6 shows that the method also holds for epithelial cells derived straight from the body and that the sera behaved fairly consistently.

TABLE 6

RECOGNITION OF THE SPECIES OF ORIGIN OF FRESHLY ISOLATED EPITHELIAL CELLS OF VARIOUS SPECIES

Rabbit anti-red-cell sera	*Cell type*	*From*			
		Man	*Ox*	*Pig*	*Rat*
Anti-human (Abs. method IIA)	Cultured	160	0	0	
	Epi. oesophagus	20	0	0	
	Epi. ureter	160	0	0	
Anti-ox (Abs. method IIB)	Cultured		320	0	0
	Epi. oesophagus	4	160	2	
	Epi. ureter	0	160	10	
Anti-pig (Abs. method IIB)	Cultured		0	40	0
	Epi. oesophagus	0	0	160	
	Epi. ureter	0	0	80	

Abs. = absorbed.
Epi. = epithelium.
Figures indicate the titre giving positive mixed agglutination.
0 = <2.

DISCUSSION

The results of the experiments reported in this paper suggest that the mixed agglutination reaction will afford a useful method for identifying the species of origin of tissue cells growing in culture. The need for such a method is emphasized by the growing number of aberrant cell lines which either because of transformation, contamination or other reason seem to be of uncertain parentage.

The reaction depends on the presence of antigens seemingly characteristic of the species which are common to both tissue cells and red cells. In this first paper we have summarily investigated red-cell antisera for their use in this test. Subsequent studies will be made with antisera to tissue cells.

As nothing is known concerning the nature of these antigens common to red cells and tissue cells, and which are characteristic of the species, absorption of the antisera to render them specific for the species is a matter for empirical experimentation. The two methods described under II A and II B have been tried but neither was wholly satisfactory. Further refinement of each, however, or, in other cases, a combination of the two may prove more satisfactory. Certainly the use of autoclaved ghosts instead of autoclaved red cells may cut down considerably the amount of non-specific absorption.

Perhaps what is most evident, is that, at the moment, the treatment necessary for each serum to render it absolutely specific is a matter of separate and special investigation. As mentioned in the text it would be very advantageous if a procedure of immunization could be established which lead to the production of antisera which were absolutely specific from the start and so obviate the necessity for subsequent absorptions. With this object in view further antisera should be produced in species of animals closely related to that of the red cells being injected.

In this paper only the broadest outlines of the problem have been investigated to see, as already mentioned, whether the method held sufficient promise. Now many lines of cells from the one species must be looked at and the complications likely to be encountered from adsorption of antigens on to the cells from the culture media investigated.

Finally, it is also possible that heterophile antigens may be of service in the serological differentiation of cultured cells. With this end in view the use of anti-Forssman sera are now being studied.

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