T antigen appeared normal, but raising their temperature to 37° was followed by a rapid hundred-fold increase in V antigen and the appearance of cytopathic changes. These findings indicate that the synthesis of tumor antigen is less temperature dependent than is the synthesis of virus antigen. The thermal separation of the synthesis of tumor and virus antigen confirms the finding obtained previously with DNA inhibitors—that is, formation of tumor antigen is not dependent upon the synthesis of virus.

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Blood Group Differentiation Using Fluorescent Antibodies. (30633)

BYRON A. MYHRE (Introduced by A. J. Quick)
Department of Pathology, Marquette University Medical School and Milwaukee Blood Center, Milwaukee, Wis.

Considerable quantitative work has been performed with human blood groups using antisera labelled with radioisotopes(1-4), but the use of fluorescent labelled antibodies has been confined almost exclusively to the qualitative localization of antigens. Several recent reports have demonstrated the practicality of using fluorescent compounds for quantitating antigen-antibody reactions. Head(5) presented a study of diphtheria toxin in which diphtheria antitoxin was labelled with fluorescein and allowed to react with the toxin forming a precipitate. The precipitate was dissolved in formamide, and measured for resulting fluorescence. A similar approach was used by Fox(6) who agglutinated streptococci with fluorescein labelled anti-streptococcal serum, dissolved the agglutinated organisms with 1N NaOH, and measured the fluorescence of the solution. Tengerdy(7) employed systems of either human or bovine gamma globulin and their corresponding fluorescein labelled antibody. The fluorescence of the antibody solution was measured before and after addition of the antigen and the decrease in the amount of fluorescence of the supernatant solution was used as an indication of the amount of antibody combined with the antigen. This report deals with the application of the above procedure to human blood groups.

Materials and methods. Seventeen human antisera for various isologous blood groups of various titers have been labelled with fluorescein isothiocyanate (F.I.T.C.) on Celite (Calbiochem, Los Angeles, Calif.) using the meth-

TABLE I. Representative Studies of Fluorescent Labelled Human Blood Group Antisera and Cells. Fluorescence of the supernatant is given in units, one of which is equivalent to the fluorescence given off by 26 mμg of potassium fluorescein freshly prepared in 5 ml of distilled water, in all tables. Agglutinating titers are given as dilution of the serum which give a 1+ microscopic reaction.

a. Fluorescein labelled anti-A serum and A or O cells.

Anti-A agglutinating titer	1-5120	1-2560	1-1280	1-640	1-320	1-160	1-80	1-40
O Cell	33,500	13,000	9,000	5,800	3,000	1,500	800	250
A Cell	30,000	12,000	7,300	3,900	1,500	400	250	100

b. Rhodamine labelled anti-B serum and B or O cells.

Anti-B agglutinating titer	1-32	1-16	1-8	1-4	1-2
O Cells	48	27	11	7	3.5
B Cells	28	15	7.5	5	2.5

c. Fluorescein labelled anti-Rho (D) serum and Rho (D) positive and negative cells.

Anti-D agglutinating titer	1-512	1-256	1-128	1-64	1-32	1-16	1-8
D− Cells	325	280	140	64	45	35	15
D+ Cells	210	140	18	26	15	14	10

od of Rinderknecht(8). The antisera were absorbed with charcoal (0.01 g/1 ml) using Chadwick's technique(9) to remove most of the unattached fluorescein and were either centrifuged or filtered through a Millipore filter to remove the charcoal. After charcoal absorption, the antiserum titer usually was found to be unchanged, but there was a 10-fold decrease in the total fluorescence of the antiserum. The sera were stored at −20°C. It was found necessary to absorb them again with charcoal before use.

Fluorometric measurements were made with a Turner Model 111 photofluorometer using the standard sensitivity door. When measuring fluorescein the 2A and 47B filters were used as exciter with a 2A-12 as a barrier filter, while a 1-60 and 58 exciter and a 23A barrier were used when measuring rhodamine. Measurements were usually taken using the 1× or 3× light ports. Fluorescence beyond the scale of the instrument was compensated by dilution of the sample or using neutral density filters in the light path while too little fluorescence was corrected by using the 10× or 30× ports.

Red cells from previously grouped individuals were drawn in NIH formula A, ACD solution and stored at 4°C. Prior to use, they were washed 3 times with buffered physiological saline (0.9% W/V NaCl in water with 0.01 M PO_4 buffer pH 7.3) and reconstituted to a 2% suspension (100,000 ± 10% red blood cells/mm^3) with the buffered saline. One-tenth ml of the cell suspension was added to an equal volume of labelled antiserum and the resulting solution was allowed to agglutinate in the dark. Five ml of buffered saline were added to the mixture which was then centrifuged at 5000 R.P.M. (3400 R.C.F.) for 5 minutes. An aliquot of the supernatant solution was measured for residual fluorescence.

Results and discussion. A representative human anti-A antiserum was labelled with F.I.T.C. The initial agglutinating titer of the unlabelled serum was 1-4096, while after labelling and charcoal absorption the titer was still 1-4096. Human group O and A red cells were added to separate samples, allowed to incubate at room temperature for 5 minutes, and the fluorescence of the supernatant solution was determined. Table 1a shows that O and A cells could be differentiated fluorometrically through a large series of antiserum dilutions. Best results were obtained with a labelled antiserum having an agglutinating titer greater than 1-20. This table shows some inhibition of antibody binding at high levels of antibody concentration

TABLE II. Study Employing Fluorescein Labelled Anti-A Serum and Cells of Groups A_1, A_2, A_3, B, O, and a Saline Control.

Serum No.	Dilution	Cells					
		A_1	A_2	A_3	B	O	Saline
1	1-4	140	256	—	430	610	620
1	1-8	130	190	—	270	310	310
2	1-8	96	98	140	160	166	170

such as has been reported previously by Masouredis(10).

In a like manner several anti-B sera were labelled with Lissamine rhodamine to test another antiserum and dye. Blood group B or O cells were added to the antiserum and the supernatant measured. The results (Table 1b) are comparable to those shown in Table 1a, except that the antiserum had a much lower initial titer, and that rhodamine did not give as brilliant fluorescence as did the fluorescein.

Table 1c shows a study performed with a fluorescein labelled anti-Rho (D) antiserum and Rh positive and negative cells. The only variations in this procedure were incubation at 37°C for 1 hour and the use of a low ionic strength medium (0.15% W/V NaCl in 6% glucose) for washing and suspending the red cells, for diluting the antiserum at the time of reaction, and for diluting the supernatant prior to its final measurements. The results in this study are felt to be comparable to those of the other antisera.

A series of experiments was performed to determine if this method could differentiate one human red cell subgroup from another. Blood was drawn from individuals who were groups O, B, A_1, A_2, and A_3. These cells plus a saline control containing no cells were reacted with 2 different fluorescent anti-A sera. Representative results at several dilutions are shown in Table II. The A_1 cells removed usually 30% more antibody from the system than did the A_2 cells. The A_3 cells decreased the fluorescence of the antiserum by about 15% in one attempt using these cells. Group B cells apparently remove some cross reacting antibody, since there was consistently slightly less fluorescence in the supernatant solution of the B cell suspension than in that of the O cells. The fluorescence of the saline control was comparable to the supernatant of the O cells.

Summary. Human antisera against isologous blood groups have been combined with fluorescent chemicals and used in the study of red cell-antiserum reactions. The decrease in fluorescence of the antiserum measured after addition of red cells is used as an indication of the amount of antibody combining with the cells. By this technic, cells have been differentiated by ABO group, subgroup and Rh type.

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