foetuses from pregnant guinea pigs *not* injected with ϕX. It is unlikely that endotoxin contamination of phage preparations was responsible for the transplacental passage of virus because of virtual absence of biologically detectable endotoxin in the preparations of phage employed and the failure of an injection of endotoxin with phage to increase the amount of phage found in the foetal circulation. There was no apparent difference in phage transfer between foetuses of markedly different weights, 56-120 g.

The route of transplacental passage of phage is as yet uncertain. The failure to find a gradient in concentration from amniotic fluid to foetal blood (the latter sometimes had a higher phage concentration) suggests that phage is not transferred by this route. Previous studies of placental transfer of large proteins indicate that the splanchnopleure is the principal transfer organ in guinea pigs(7).

The question arises as to whether the placenta is an effective barrier in preventing intrauterine viral infections. The following findings are relevant to this question: a) in the guinea pig, the difference between maternal and foetal blood virus concentrations was approximately 10^5-10^6 and transfer usually was not demonstrated when maternal ϕX concentration was below 10^7/ml; b) the ϕX particle is an unusually small virus (approximately 250 angstroms in diameter). It is possible that its size or other particular properties(8) facilitates its passage into the foetal circulation.

These findings suggest that the placental barrier is usually an effective one in the presence of viremia of moderate intensity, provided, of course, that the placenta is free of infection and pathology. However, if there is a sufficiently high concentration of virus in the maternal circulation, passage of virus into the foetus may occur. Whether or not infection results will depend on a variety of factors, including the capacity of the placenta (9) and the foetus(10) to develop an immune response against the virus.

1. Blair, J. E., Reeves, D. L., *J. Inf. Dis.*, 1928, v42, 440.

2. Burckhardt, K., *Arch. Ges Virusforsch.*, 1940, v1, 140.

3. Kulangara, A. C., Sellers, M. I., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 207.

4. Medearis, D. N., Jr., *Am. J. Dis. Child.*, 1962, v104, 573.

5. Uhr, J. W., Finkelstein, M. S., Baumann, J. B., *J. Exp. Med.*, 1962, v115, 655.

6. Adams, M. H., in *Methods in Medical Research,* Year Book Publishers, Chicago, 1950, 2.

7. Brambell, F. W. R., Hemmings, W. A., Henderson, M., in *Antibodies and Embryos,* London, Althone Press, 1951, 5.

8. Brambell, F. W. R., Hemmings, W. A., Oakley, C. L., Porter, R. R., *Proc. Roy. Soc. Lond.* (sec. B) 1960, v151, 478.

9. Dancis, J., Samuels, B., Douglas, G., *Science,* 1962, v136, 382.

10. Silverstein, A. M., Uhr, J. W., Kraner, K. L., Lukes, R. J., *J. Exp. Med.*, 1963, v117, 799.

Received March 11, 1963. P.S.E.B.M., 1963, v113.

The Fluorescent Antibody Technique Applied to Titration and Identification of Antigens in Solutions or Antisera.* (28375)

FIORENZO PARONETTO (Introduced by Hans Popper)
Department of Pathology, The Mount Sinai Hospital, New York City

The fluorescent antibody technique has been used principally for localization of antigen or antibody in tissues or for identification of micro-organisms. The high sensitivity of the technique is caused by the fluorescent label and probably also by the ability to detect or use non-precipitating as well as precipitating antibody(1); the sensitivity is

* Supported by grants from Nat. Inst. of Arthritis and Metab. Dis., USPHS and by U. S. Army Medical Research and Development Command contract.

increased with indirect staining.

In the present work the fluorescent technique has been extended to titration and identification of antisera or antigens using cellulose acetate or agar as supporting media.

Material and methods. Antigen and antisera. The following antigens were used: bovine serum albumin (BSA),† human gamma globulin Fr. II,‡ and horse serum.† Antisera against BSA, HGG and HS (ABSA, AHGG, AHS) were prepared in rabbits and anti-rabbit gamma globulin antiserum (ARGG) in guinea pigs. Rabbit and human gamma globulin were isolated or purified by a DEAE cellulose column(2). Specificity of antisera was ascertained by immunoelectrophoresis according to Scheidegger(3).

Cellulose acetate fluorescent spot (CAFS) method. Nine 12 × 12 mm squares were drawn and numbered on cellulose acetate membrane filter discs§ 5 cm in diameter. The discs were placed on a 10 mg/ml antigen solution in a Petri dish, and the liquid was permitted to penetrate from underneath. When wet, they were submerged for 30 seconds. After light blotting the discs were placed in a vacuum desiccator for 10-15 minutes. The impregnated discs were fixed for 10 minutes at 5°C (using 95% ethanol for HGG and HS or 1% acetic acid in 95% ethanol for BSA) and washed 3 times for 3 minutes with pH 7.2 phosphate buffered saline (PBS). For direct staining, 4 lambda microdrops of serial dilutions of fluorescein labelled antiserum were placed with capillary melting point tubes into the center of each square. Then the discs were incubated in a moist chamber at 25°C for one hour and washed 3 times for 10 minutes with PBS. For indirect staining the discs were spotted in each square with serial dilutions of unconjugated antiserum prepared in rabbits, incubated for one hour at 25°C in a moist chamber, washed 3 times for 10 minutes with PBS, then stained for 60 minutes with fluorescein labelled ARGG. After a final washing with PBS, 3 times 10 minutes each, the discs were blotted and examined under illumination from an HBO 200 mercury vapor lamp with UG4 and UG5 exciter filters.

Reversing the procedure, discs were soaked in antisera either undiluted or diluted 1:5 with PBS, desiccated, fixed in cold 95% ethanol, spotted in the direct staining with dilutions of fluorescein-labelled antigen or in the indirect straining with dilutions of a 10 mg/ml solution of antigen followed by specific fluorescein-labelled antiserum. Incubation time and washings were similar to the previous procedure. Unrelated antigens and antisera and the blocking procedure were employed in control discs.

Fluorescent impregnated agar (FIA) method. An amount of 0.25 ml of a 5% solution of agar‖ in distilled water was thoroughly mixed at 56°C with 0.05 ml of a 10 mg/ml antigen solution previously warmed to 56°C. The mixture was poured into a 10 × 8 × 5 mm mold of aluminum foil which was chilled at −20°C for 30 minutes and then quick-frozen in a dry-ice isopentane mixture. Such impregnated blocks were now handled as "tissue" in the fluorescent antibody technique: they were sectioned at 5 mμ in a cryostat, fixed, washed, stained, mounted in Elvanol(4) and photographed as described previously(5,6). Direct and indirect staining was used. Agar blocks were also impregnated with antiserum and treated with fluorescein-labelled antigen or with unconjugated antigen followed by fluorescein labelled specific antiserum. Agar blocks impregnated with saline or unrelated antigens and antisera were used for controls. Specificity of staining was also demonstrated by suppression of specific fluorescence by unconjugated antiserum.

The microring precipitin test was performed(7) using 2 mm diameter glass tubes. In all methods titers were expressed as the reciprocal of the maximum dilution showing positive reaction. Fuorescein-labelled antisera were adsorbed twice before use with pork liver powder.

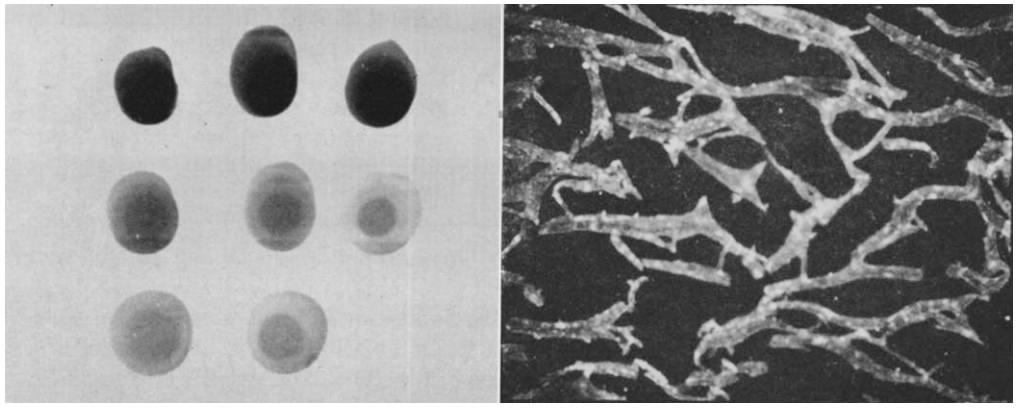
Results. Positive reactions in the CAFS method appeared as apple green spots, the brightness of which decreased with the dilu-

† Pentex Laboratory, Kankakee, Ill.

‡ Donated by American National Red Cross, Washington, D.C.

§ Consolidated Laboratories, Chicago, Ill.

‖ Bacto Agar Difco, Detroit, Mich.



A B

FIG. 1. A. Cellulose acetate disc impregnated with BSA and spotted with serial dilutions of rabbit anti-BSA antiserum followed by fluorescein labelled anti-rabbit gamma globulin. The first positive fluorescent spot corresponds to a serum dilution 1:64, the last to 1:4096; the right lower spot, a dilution of 1:8192, does not fluoresce. B. Section of agar block impregnated with HGG, treated with fluorescein labelled anti-human gamma globulin antiserum. Note the specific fluorescence of a network with brighter spots (×150).

tion of the antigen or antiserum (Fig. 1A). The control spots exhibited blue-gray or no fluorescence.

Positive reactions in the FIA method appeared as an irregular apple green network with occasional bright green dots (Fig. 1B). The use of direct or indirect staining and the dilution of antigen or antiserum affected the sensitivity of the method (Table I). In a system in which serial dilutions of antisera (ABSA, AHGG, AHS) or antigens (BSA, HGG, AHS) were tested against a fixed amount of corresponding antigens or antisera and the direct staining was used, the sensitivity of the ring test was higher or similar to the CAFS or FIA methods. When serial dilutions of antisera were tested against a fixed amount of antigen, and the indirect

TABLE I. Comparison of Titers of Precipitin Ring Test, Cellulose Acetate Fluorescent Spot (CAFS) and Fluorescent Impregnated Agar (FIA) Methods.

AG or AB*	Dilutions of FL†	Titers: Ring method	Titers: CAFS method	Titers: FIA method
			Direct straining	
BSA	ABSA	64	8	64
HGG	AHGG	64	8	16
HS	AHS	16	2	16
ABSA	BSA	2048	16	4
AHGG	HGG	512	32	512
AHS	HS	2048	4	2

AG or AB	Dilutions of	FL‡	Titers: Ring method	Titers: CAFS method	Titers: FIA method
				Indirect straining	
BSA	ABSA	ARGG	64	4096	256
HGG	AHGG	ARGG	64	512	512
HS	AHS	ARGG	32	2048	128
ABSA	BSA	ABSA	2048	2048	64
AHGG	HGG	AHGG	1024	512	1024
AHS	HS	AHS	8192	4096	512

* AG: antigen solution 10 mg/ml—AB antiserum diluted 1:5 with PBS.
† FL: fluorescein labelled.
‡ Used only for CAFS and FIA methods.

staining was employed both the CAFS and FIA method were more sensitive than the ring test. When serial dilutions of antigens were tested against a fixed amount of antisera, and the indirect staining was used the sensitivity of the ring test was similar or slightly higher than the CAFS and FIA method.

Discussion. Cellulose acetate or agar have been previously employed as supporting media for spot precipitin reactions(8,9). Fluorescent spot tests on microscopic slides have been described for detection of antibodies to DNA(10), and thyroglobulin(11) but are not feasible with less viscous antigens which do not adhere to the slides. Agar as supporting medium for antigens in solutions has been recently recommended. Thirty-five micron-thick sections have been employed in this procedure(12).

The CAFS and FIA methods are particularly sensitive with indirect staining and with serial dilution of the antisera tested. The CAFS method has the advantage of simplicity of performance and reading; it requires only a few lambda of antiserum and a simple source of U.V. light. The FIA method is essentially the same as the fluorescent antibody technique applied to tissues. Since agar serves as the substrate to which antigen is bound, a greater variety of fixatives can be used; the substrate in the CAFS method, however, limits the number and types of fixatives to those which do not dissolve cellulose acetate. The possibility of non-specific binding of protein to either the cellulose acetate or agar requires the performance of adequate controls.

The two fluorescent methods have been used a) to titrate antisera, especially when only small amounts are available, b) to determine the proper fixative for a given antigen,¶ c) to establish the proper concentration of fluorescein-labelled antisera in tissue investigations, and d) to detect antigen in solutions to which specific antisera are available. The CAFS method can also be applied to localization of antigens after electrophoresis. These methods may also be useful in detection of serum auto-antibodies using tissue or organ extracts and in purification of the extracts.

Summary. Cellulose acetate discs or agar are useful supporting media for titration or identification of antigen or antibody. The cellulose acetate fluorescent spot (CAFS) method is simple to perform and read and requires only a few lambda of antiserum or antigen; the fluorescent impregnated agar (FIA) method is essentially the same as the fluorescent antibody technique applied to tissue. In the titration of ABSA, AHGG and AHS both fluorescent methods were more sensitive than the ring precipitin test, when the indirect staining was used. In addition the methods permitted selection of proper fixatives for a given antigen and determination of the proper concentration of labelled antisera for demonstration of tissue antigen.

The author wishes to express the most grateful appreciation to Miss Sara Echeverria Cruz for her valuable and highly competent technical assistance and to Dr. Richard Horowitz for helpful discussion of the manuscript.

¶ For example, it was found that propanol or 1% acetic acid in 95% ethanol are better fixatives for BSA than acetone or ethyl alcohol.

1. Nairn, R. C., *Fluorescent Protein Tracing,* Livingstone Ltd., Edinburgh, 1962, 53.

2. Levy, H. B., Sober, H. A., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 250.

3. Scheidegger, J. J., *Internat. Arch. Allergy,* 1955, v7, 103.

4. Rodriguez, J., Deinhardt, F., *Virology,* 1960, v12, 316.

5. Paronetto, F., Rubin, E., Popper, H., *Lab. Invest.,* 1962, v11, 150.

6. Cohen, S., Ohta, G., Singer, E. J., Popper, H., *J. Exp. Med.,* 1960, v11, 285.

7. Boyd, W. C., *Fundamentals of Immunology,* 3rd Ed., Interscience Publ., New York, 1956, 660.

8. Feinberg, J. G., *Nature,* 1962, v194, 307.

9. ———, *ibid.,* 1961, v192, 985.

10. Friou, G. J., *Arthr. Rheum.,* 1962, v2, 407.

11. Crawford, H. J., Wood, R. M., Lessof, M. H., *Lancet,* 1959, v2, 1173.

12. Allen, J. C., *Fed. Proc.,* 1962, v21, 15.

Received March 12, 1963. P.S.E.B.M., 1963, v113.