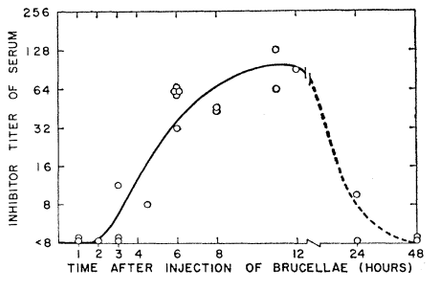

Fig. 1. Inhibitor titers in individual chicken sera collected at different times after intravenous inoculation of 10^{10} brucellae. Titers are expressed as inhibitor units per 3.0 ml of serum.

injection of *Serratia marcescens* (1.5×10^9 viable organisms), *Shigella sonnei* (4.4×10^9), *Bacillus subtilis* (2.8×10^8), and *Staphylococcus albus* (2.2×10^9) did not produce detectable inhibitor. The viable counts varied, although approximately the same mass of the different organisms (adjusted to the turbidity of approximately 5×10^9 brucellae per milliliter) was inoculated. Negative results also were obtained after the inoculation of 5.0 mg of bacterial endotoxin. On the other hand, intravenous injection of 10^9 plaque-forming units of Newcastle disease virus produced a high titer of inhibitor in chicken serum at 6 hours. This result in chickens confirms the observations made by Baron and Buckler in mice (*2*).

An attempt was made to rule out the possibility that breakdown products of brucellae in vivo present in the serums had some direct effect on the cells, thus inhibiting plaque formation. Lysates of 10^{10} live brucellae were prepared by the method Carver and Naficy (*5*) used to make bacterial inhibiting factor, a constituent of certain bacteria which interferes with the replication of group A arboviruses in cell culture. The capacity of vesicular stomatitis virus to produce plaque formation in cell cultures exposed to these lysates for 20 hours did not differ from that in untreated cell cultures. In addition, chick embryo cell cultures exposed to 100 μg of *Escherichia coli* endotoxin (0111:B4) for 20 hours did not show any difference in plaque count from control cultures. These results tend to rule out any carry-over in serums of bacterial products which might have an inhibiting effect on plaque formation. Another inhibitor, termed "serum sparing factor" by Gledhill (*6*), which is produced in mice by endotoxin, also is unlikely to be the inhibitor produced by injection of brucellae into chickens because of the failure of endotoxin to elicit inhibitor production in this species. Consideration must also be given to the possibility (*7*) that foreign RNA or DNA of diverse origins can stimulate interferon production in cell-culture systems. It should be noted that *Escherichia coli* RNA, the only bacterial nucleic acid tested, did not induce interferon production (*7*). In our work, intravenous injection of chickens with 3.0 mg of rabbit liver ribosomes yielded no interferon. The negative results obtained with several bacterial species, other than *Brucella*, also do not support the idea that foreign nucleic acids were responsible for our results.

The data show that a virus inhibitor with properties identical to those described for interferon is found in the blood of chickens after intravenous injection of large numbers of live, virulent brucellae. These organisms were selected because they are intracellular parasites in the hosts they commonly infect and are therefore suited to test the hypothesis that intracellular parasites other than viruses may induce interferon production by cells.

JULIUS S. YOUNGNER
WARREN R. STINEBRING
Department of Microbiology, University of Pittsburgh School of Medicine
Pittsburgh 13, Pennsylvania

References and Notes

1. A. Isaacs and J. Lindenmann, *Proc. Roy. Soc. London, Ser. B.* **147**, 258 (1957); M. Ho, *New Engl. J. Med.* **266**, 1258 (1962); R. R. Wagner, *Bacteriol. Rev.* **27**, 72 (1963); M. R. Hilleman, *J. Cellular Comp. Physiol.* **62**, 337 (1963).
2. S. Baron and C. F. Buckler, *Science* **141**, 1061 (1963).
3. From Dr. Lois Jones, Univ. of Wisconsin.
4. R. R. Wagner, *Virology* **13**, 323 (1961).
5. D. H. Carver and K. Naficy, *Proc. Soc. Exptl. Biol. Med.* **111**, 356 (1962).
6. A. W. Gledhill, *Nature* **183**, 185 (1959); *Symposium on bacterial endotoxins* (Rutgers University, 1963).
7. A. Isaacs, R. A. Cox, Z. Rotem, *Lancet* **1963-II**, 113 (1963); Z. Rotem, R. A. Cox, A. Isaacs, *Nature* **197**, 564 (1963); K. E. Jensen, A. L. Neal, R. E. Owens, J. Warren, *ibid.* **200**, 433 (1963).
8. We are indebted to Marion Kelly and Mrs. G. McIntyre for assistance. Supported by NIH grants AI-03414 and AI-03561.

31 March 1964

Immuno-osmophoresis, a Rapid and Sensitive Method for Evaluating Viruses

Abstract. *Characteristic antigen-antibody interactions in agar gel were obtained in 50 to 90 minutes in an electrical field with microgram quantities of three plant viruses, which were either in purified form or in crude plant juice. The method was faster and more senstive than agar-gel diffusion.*

Bon and Swanborn reported (*1*) that a precipitin reaction occurred within half an hour when an extract from the eye lens of cow and its homologous rabbit antiserum were applied simultaneously to separate wells in a thin layer of agar (*p*H 8.4 to 8.5) and exposed to an electrical field running parallel to the line connecting both wells. They suggested that this "very rapid method of immunoelectrophoresis" be named "immuno-osmophoresis," since the antibody movement was due to endosmotic flow. Plant viruses exposed to an electrical field at *p*H 7 are known to move in agar without loss of biological activity (*2*). We report here the immuno-osmophoresis of plant viruses at *p*H values near neutrality and its usefulness in serological studies aimed at quick identification and quantitative evaluation of these antigens of high molecular weight.

Electrophoresis was carried out in 1 percent agar in 0.01*M* tris-succinate buffer, *p*H 6.8 or 7.6, at 4 v/cm, in a cold room at 3°C, without additional cooling. The agar used was Difco Bacto agar, or Difco Noble agar, or agarose. Agarose, the neutral constituent of agar, was isolated from Noble agar according to Hjertén (*3*). The gel layer (16 by 13 by 0.9 cm) was prepared in a translucent plastic box (21 by 13 by 2 cm), and a strip 2.5 by 13 cm was left on both sides of the gel as electrode compartments. Two rows of six holes (0.6 cm in diameter) were arranged symmetrically in the middle of the gel. The distance between the centers of the holes in the direction of the electrical field was 2 cm. Fresh buffer solution was fed continuously into each electrode compartment at one end and the excess solution sucked off at the opposite end to maintain con-

Table 1. Sensitivity and range of immuno-osmophoresis.

Viruses	Free electrophoresis			Precipitin lines in		Minimum antigen detectable (μg)
	Mobility* (10^{-5}cm^{2}v^{-1}sec^{-1})	Ionic strength	References	Agar	Agarose	
TMV	−9.8	.05	(*7*)	+	+	0.06
AMV	−6.4	.10	(*8*)	+	+	4.0 (*12*)
SBM	−4.6	.02	(*9*)	−	+	2.0
TRSV	−1.38	.10	(*10*)	−	−	−
PVX	−0.40	.15	(*11*)	−	−	−

* At *p*H 7.0 except TRSV (= *p*H 6.5).

stant *p*H and temperature. The level of the liquid was controlled by the position of the tip of the suction tubes.

The viruses studied were tobacco mosaic virus (TMV), alfalfa mosaic virus (AMV), southern-bean mosaic virus (SBMV), tobacco-ringspot virus (TRSV) and potato virus X (PVX). These were chosen to cover a wide range of electrical mobilities (Table 1) and to include both rod-like and spherical viruses. The antigen solutions (0.1 ml) were placed in wells closer to the cathode. Homologous antiserums (*4*) (0.1 ml, usually 1/5 dilution in buffer) were placed in opposite wells closer to the anode. A water-saturated solution of *o*-nitroaniline (0.1 ml) was put in a well on the cathode side whenever the endosmotic flow was to be determined. This compound is neutral at *p*H 7 and above (*5*). To locate or confirm the position of the antigen at the end of an experiment, small cores of agar were sucked into capillaries at various distances on either side of the antigen well and prepared for electron microscopy (*2*) or used for infectivity tests. For infectivity tests two adjacent anode wells were filled with the same antigen, but one of the opposite cathode wells was filled with buffer instead of with antiserum.

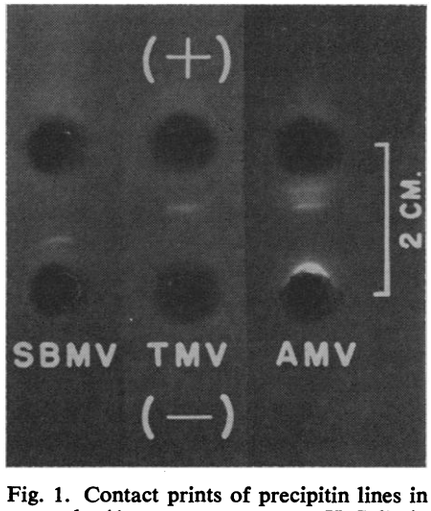

Fig. 1. Contact prints of precipitin lines in wet gels (1 percent agarose, *p*H 7.6) in ultraviolet light (at 360 mμ) on Agfa Copyrapid negative paper (low speed). Amount of antigens: SBMV 100 μg; TMV, 2.8 μg; AMV, 8 μg (*12*) in sap from *Nicotiana tabacum* (diluted 1/12). Antiserums were in 1/5 dilutions.

Precipitin lines resulting from the interactions of antigen and homologous antiserum were obtained with three of the five viruses studied (Fig. 1, Table 1). The location of these lines varied, depending on the antigen and the type of agar employed. The mobilities of tobacco mosaic and southern-bean mosaic viruses in agarose differed sufficiently to allow their detection in a mixture, when tested against a solution which contained antibodies against both viruses. The time of appearance of the precipitin lines depended mainly on the antigen. For tobacco mosaic virus it took about 50 minutes, for alfalfa mosaic virus about 65 minutes, and for southern-bean mosaic virus about 90 minutes before they became visible. The lines reached maximum intensity within several minutes after their appearance. They were clearly seen in transmitted white light, and lower antigen concentrations were visible than could be recorded photographically. In agar alfalfa mosaic virus produced a single precipitin line, whereas in agarose it produced two distinctly separated lines (Fig. 1) both of which contained virus-like spherical particles. Under standard conditions purified tobacco mosaic virus produced a single line. However, when the distance between the antigen and antiserum wells was increased from 2 to 3.5 cm, two closely spaced lines were obtained. The line closer to the antiserum well did not contain rod-shaped particles. Lack of sufficient negative net charge prevented tobacco-ringspot virus and potato virus X from moving unequivocally towards the anode even in agarose, which has a much lower endosmotic flow by visible dye movement (7×10^{-5} cm^2 v^{-1} sec^{-1}) than Bacto agar or Noble agar (14×10^{-5} cm^2 v^{-1} sec^{-1}). Despite reports to the contrary (*1*), Bacto agar and Noble agar showed similar endosmotic properties.

Tobacco mosaic, alfalfa mosaic, and southern-bean mosaic viruses were detected in microgram quantities (Table 1) in purified and crude preparations. By comparing precipitin lines produced by known amounts of pure antigen with those from unknown amounts in sap from diseased plants, accurate quantitative estimates of the virus can be made in these complex solutions. This was shown by adding purified tobacco mosaic virus to sap from healthy tobacco leaves. High concentrations of normal proteins in undiluted sap tend to mask the precipitin reaction. This can be overcome by diluting the sap one-third or more with buffer. Immunoosmophoresis is considerably more sensitive than Ouchterlony's (*6*) agar-gel diffusion method (about 100 times more sensitive for tobacco mosaic virus) and at least 20 times faster. Other viruses with sufficient negative net charge at about *p*H 7 to have a free negative electric mobility of at least 4.6×10^{-5} cm^2 v^{-1} sec^{-1} will undoubtedly lend themselves to this method.

H. W. J. RAGETLI
M. WEINTRAUB
Research Station, Canada Department of Agriculture, 6660 N.W. Marine Drive, Vancouver 8, British Columbia

References and Notes

1. W. F. Bon and P. L. Swanborn, *Chem. Weekblad* **59**, 72 (1963).
2. M. Weintraub, H. W. J. Ragetli, P. M. Townsley, *Virology* **17**, 196 (1962).
3. S. Hjertén, *Biochim. Biophys. Acta* **53**, 514 (1961).
4. We acknowledge the gifts of antiserums from Dr. N. S. Wright (TMV, TRSV and PVX), Dr. J. H. Tremaine (SBMV), and Dr. J. B. Bancroft (AMV).
5. M. A. Jermyn and R. Thomas, *Nature* **172**, 728 (1953).
6. O. Ouchterlony, *Arkiv. Kemi. Mineral Geol.* **26B**, 1 (1948).
7. E. Kramer, *Z. Naturforsch.* **12b**, 609 (1957).
8. M. A. Lauffer and A. F. Ross, *J. Am. Chem. Soc.* **62**, 3296 (1940).
9. G. L. Miller and W. C. Price, *Arch. Biochem.* **11**, 337 (1946).
10. P. R. Desjardins, C. A. Senseney, G. E. Hess, *Phytopathology* **43**, 687 (1953).
11. F. C. Bawden and A. Kleczkowski, *Virology* **7**, 375 (1959).
12. According to A. F. Ross [*Phytopathology* **31**, 394 (1941)] *N. tabacum* infected with AMV contains about 1 mg of virus per milliliter of sap.
13. This paper is Station contribution No. 65.

27 February 1964