[2] O'Conor, G. T., A. S. Rabson, A. Malmgren, I. K. Berezesky, and F. J. Paul, *J. Natl. Cancer Inst.*, **34**, 679 (1965).

[3] Rowe, W. P., and S. G. Baum, *J. Exptl. Med.*, **122**, 955 (1965); Schell, K., W. T. Lane, M. J. Casey, and R. J. Huebner, these PROCEEDINGS, **55**, 81 (1966).

[4] Huebner, R. J., R. M. Chanock, B. A. Rubin, and M. J. Casey, these PROCEEDINGS, **52**, 1333 (1964).

[5] Rowe, W. P., and S. G. Baum, these PROCEEDINGS, **52**, 1340 (1964).

[6] Rapp, F., J. L. Melnick, J. S. Butel, and T. Kitahara, these PROCEEDINGS, **52**, 1348 (1964).

[7] Rowe, W. P., these PROCEEDINGS, **54**, 711 (1965).

[8] Rapp, F., J. S. Butel, and J. L. Melnick, these PROCEEDINGS, **54**, 717 (1965).

[9] Rowe, W. P., S. G. Baum, W. E. Pugh, and M. D. Hoggan, *J. Exptl. Med.*, **122**, 943 (1965).

[10] Feldman, L. A., J. L. Melnick, and F. Rapp, *J. Bacteriol.*, **90**, 778 (1965).

[11] Black, P. H., and W. P. Rowe, these PROCEEDINGS, **50**, 606 (1963).

[12] Girardi, A. J., M. R. Hilleman, and R. E. Zwickey, *Proc. Soc. Exptl. Biol. Med.*, **115**, 1141 (1964).

[13] Atchison, R. W., B. C. Casto, and W. McD. Hammond, *Science*, **149**, 754 (1965).

[14] Hoggan, M. D., *Federation Proc.*, **24**, 248 (1965).

[15] Melnick, J. L., H. D. Mayor, K. O. Smith, and F. Rapp, *J. Bacteriol.*, **90**, 271 (1965).

[16] Marmur, J., *J. Mol. Biol.*, **3**, 208 (1961).

[17] Rose, J. A., P. R. Reich, and S. M. Weissman, *Virology*, **27**, 571 (1965).

[18] These procedures have been found to release 85% of the acid-precipitable DNA (labeled with tritiated thymidine) from adenovirus type 2 particles mixed with 10^8 nonradioactive KB cells. Furthermore, 90% or more of the acid-precipitable, tritiated thymidine-labeled material from adenovirus type 2 infected KB cells (exposed to tritiated thymidine 12–24 hr after virus inoculation) and uninfected KB cells, similarly labeled, was recovered by these procedures.

[19] Green, M., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 219.

[20] As measured by the homology technique, there was no detectable adenovirus type 2 DNA synthesis (less than 1% of that in control cultures) in infected KB cells treated with 2×10^{-5} *M* FUDR.

[21] Black, P. H., E. M. Crawford, and L. V. Crawford, *Virology*, **24**, 381 (1964).

[22] Nygaard, A. P., and B. D. Hall, *Biochem. Biophys. Res. Commun.*, **12**, 98 (1963).

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*IMMUNOFLUORESCENT STUDIES OF GROUP-SPECIFIC ANTIGEN OF THE AVIAN SARCOMA-LEUKOSIS VIRUSES**

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The sera of hamsters bearing tumors induced by the Schmidt-Ruppin (S-R) strain of Rous sarcoma virus (RSV) contain antibody which fixes complement when allowed to react with antigen prepared from cells infected with any one of several representative viruses of the avian sarcoma-leukosis group. The reacting antigen appears to be common to all members of the avian sarcoma-leukosis group and specific for this group.[1, 2]

In the present report, the sera of hamsters bearing tumors induced by the S-R virus were used in an immunofluorescent staining technique to examine cells infected *in vitro* with various viruses of the avian sarcoma-leukosis group and with other viruses. The results indicate that the antigen stained is probably the same

as that detected in the complement-fixation test. The distribution of the antigen in and on cells at various times after infection and the reactivity of the antigen with antibody before and after fixation of cell-virus preparations suggest that it is synthesized in the nucleus, is transferred to the cytoplasm, and then appears extracellularly, probably as an internal component of the virion.

Materials and Methods.—Cells: Cultures of chick embryonic fibroblasts (CEF) were prepared from individual embryos, which, except where noted, were from isolated dams of the White Leghorn line 15I.[3] Embryos from these dams were generally negative by the COFAL test.[4] Secondary cultures were grown and maintained on 9 × 22-mm coverslips in Leighton tubes with 1 ml of medium 199 containing 10% tryptose phosphate broth and 4% heat-inactivated calf serum. These cultures were incubated at 35°C in an atmosphere of 5% CO_2 and 95% air.

Cells, other than chick embryonic cells, were grown at 35°C on coverslips in sealed screw-capped Leighton tubes with 1 ml of medium. HeLa cells and primary cultures of calf kidney cells were grown in Eagle's basal medium with 10% heat-inactivated calf serum and changed to Eagle's basal medium with 1% fetal calf serum for maintenance. The LLC-MK_2 cells[5] were grown and maintained in medium 199 and 1% heat-inactivated horse serum. The RA (rubella-associated) cells, derived from LLC-MK_2 cells that were infected with rubella virus, were grown and maintained as described by Maassab and Veronelli.[6] BS-C-1 cells were grown as described by Hopps *et al.*[7]

Viruses: All viruses of the avian sarcoma-leukosis group that were studied were propagated, unless otherwise noted, in birds or eggs of line 15I. The S-R strain[8] of RSV was obtained through the courtesy of Dr. P. S. Sarma; the working pool of this virus was clarified homogenate of infected chorioallantoic membranes. Two different preparations of RSV designated CT 750 and CT 916 were originally obtained from Dr. Ray Bryan. Pools of these viruses were clarified extracts of 20% homogenate of wing web tumors. A preparation of Bryan's high titer strain of RSV, designated 82E, was kindly furnished by Dr. P. K. Vogt and was used without passage. RPL-12 virus produces visceral lymphomatosis and erythroblastosis[9]; a pool of this virus designated L 37 was prepared as described by Burmester and Gentry[10] from livers of birds with erythroblastosis. A similar pool of Rous-associated virus (RAV)[11] was prepared from livers of birds which developed leukosis following inoculation with virus which was kindly furnished by Dr. H. Rubin. Plasmas from viremic birds were used for pools of myeloblastosis virus, BAI strain A, and erythroblastosis virus, Engelbreth-Holm's strain R.[12]

Viruses, other than those of the avian sarcoma-leukosis group, that were used in the present study were as follows: Gallus adeno-like virus,[13] GAL, received from Dr. G. R. Sharpless and propagated in CEF; influenza viruses: type A, Asian strain A_2/AA/60, 12th passage in calf kidney cell cultures after isolation and 70 passages in chick kidney cell cultures; type B, strain B-Colorado-65, 15th passage in calf kidney cell cultures after isolation and 3 passages in rhesus monkey kidney cell cultures; parainfluenza viruses: type 1, strain Sendai, 8th passage in embryonated eggs after 15 passages in mice; type 2, Greer's strain, 1st passage in LLC-MK_2 cells after 17 passages in HeLa cells; type 3 (hemadsorption virus, type 1), received from Dr. R. M. Chanock and passaged 58 times in HeLa cells; mumps virus, received from Dr. J. F. Enders and passaged 4 times in eggs; Newcastle disease virus, strain 46-9679, passaged 27 times in eggs; rubeola virus, Edmonston strain, 38th passage in LLC-MK_2 cells; respiratory syncytial virus, Long's strain, passaged in a continuous line of human cells, the HL cells, by Dr. H. M. Maassab; simian virus 40, strain 776, passaged in BS-C-1 cells; adenovirus, type 2, originally obtained from Dr. R. J. Huebner, passaged 17 times in HeLa cells.

Infection of cultures: The various viruses were added to the medium of the indicated cell cultures to give a final dilution of 10^{-2} of the stock preparation. The cultures were then incubated at 35°C. At 24-hr intervals, for the next 5 days, coverslips were removed from the cultures and fixed for immunofluorescent staining.

Hamster sera: Newborn Syrian hamsters were inoculated subcutaneously with 0.05 ml of undiluted S-R virus, and some of the resulting tumors were transplanted to weanling hamsters. The latter animals were bled before receiving transplants and all animals were bled at weekly intervals after their tumors were about 1 cm in diameter. Sera from individual bleedings were stored at −20°C until needed for testing.

Immunofluorescent staining: Cells on coverslips were fixed by immersion in acetone at 20°C for 3 min. They were then air-dried at 20°C for 30 min and stained either immediately or after being stored for several days at 4°C in sealed ampoules.

The fixed cells were exposed for 30 min at 35°C to hamster serum that was diluted 1:4 in phosphate-buffered saline (PBS), pH 7.6. After they were twice washed for 5 min with PBS, they were exposed to the fluorescein-conjugated antihamster globulin diluted 1:40, for 30 min at 35°C, and again washed twice with PBS. The antihamster globulin, prepared in goats and conjugated with fluorescein, was obtained from Baltimore Biological Laboratories and absorbed with hamster brain as described by Pope and Rowe.[14] Coverslips were then mounted in Elvanol[15] for examination with a Zeiss Ultraphot II microscope equipped with an Osram HB 200 mercury lamp, a BG 12 excitation filter, and OG 4 and GG 4 barrier filters.

Unfixed cells on coverslips, after rinsing with PBS, were similarly stained, but these were mounted in PBS.

When cultures were stained for complement, the acetone-fixed cells were exposed overnight at 4°C to a mixture of equal parts of heat-inactivated hamster serum diluted 1:2 and freshly reconstituted lyophilized guinea pig serum (Colorado Serum Co., Denver) diluted 1:5. After being washed, these cells were then exposed for 30 min at 35°C to fluorescein-conjugated anti-guinea pig serum diluted 1:40. This reagent was prepared in horses by Progressive Laboratories, Inc., and purchased from the Roboz Surgical Instrument Co., Washington, D.C.

Results.—Sera taken from hamsters before they received transplants of S-R virus-induced tumors and those from non-tumor-bearing hamsters or from hamsters bearing tumors induced with simian virus 40 did not stain noninoculated CEF cultures or those that had been fixed 48–72 hr after exposure to RAV. However, cells in the latter cultures were stained by sera from hamsters bearing primary or transplanted S-R virus-induced tumors. Generally, sera from those animals bearing primary tumors produced more intense staining than sera from those bearing transplanted tumors. Sera from hamsters with S-R virus-induced tumors did not stain cells of noninoculated CEF cultures.

Figure 1 illustrates the morphology of staining seen in RAV-infected and non-infected CEF cultures. No cytopathology developed in these cultures. Cells fixed at 48 hr after exposure to virus showed staining of intranuclear granules and diffuse staining of the cytoplasm (Fig. 1*a* and *d*). In the example illustrated, the intranuclear granules resemble an irregularly shaped inclusion body; however, in many experiments the granules were dispersed within the nucleus. There was always a nonstained peripheral area within those nuclei that contained inclusions. The intensity of staining varied among cells but in a given cell the nuclear inclusions and the cytoplasm stained with about equal intensity. In cultures fixed at 72 hr after exposure to RAV, most of the cells that showed diffuse staining of their cytoplasm showed less intense nuclear staining, but these cells had coarse stained granules over their surface (Fig. 1*e*). The extracellular granules were also distributed within a circumscribed area surrounding the affected cell, being present on the glass and on neighboring cells that showed little or no diffuse staining of their cytoplasm. Between 72 hr and 5 days after exposure to virus, the intensity of cytoplasmic staining tended to be less and the amount of extracellular granular material that stained had increased.

Cells or extracellular granules on coverslips from RAV-infected cultures, if not fixed before exposure to sera from hamsters with S-R virus-induced tumors, did not stain.

These observations suggest that the antigen, detected by the sera of the tumor-bearing hamsters, was synthesized in the nucleus, moved to the cytoplasm, and

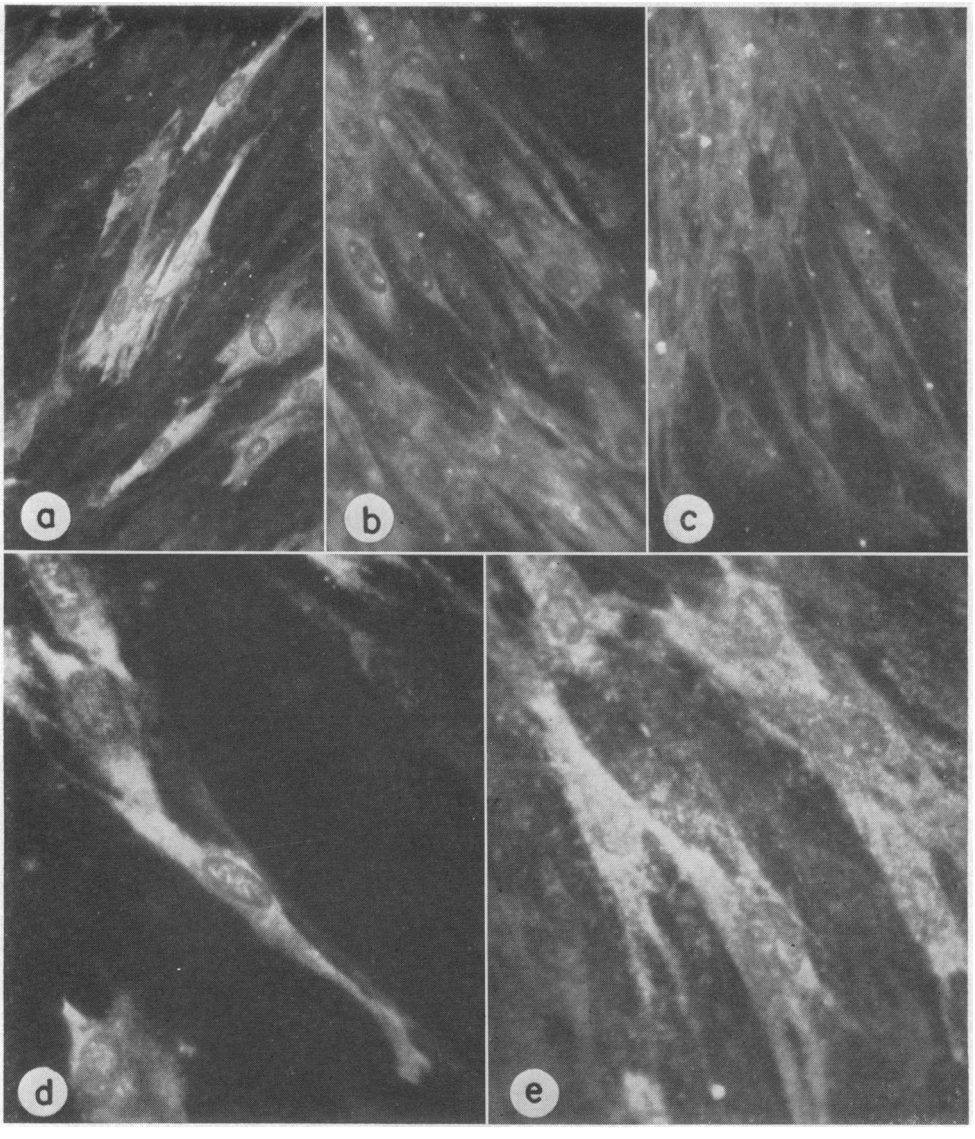

FIG. 1.—Immunofluorescent staining of RAV-infected chick embryonic fibroblasts with serum from hamster bearing S-R virus-induced tumor. (*a*) Cells 48 hr postinfection (p.i.) with serum of S-R tumor-bearing hamster. Magnification 340×. (*b*) Cells 48 hr p.i. with serum of a normal hamster. Magnification 340×. Photograph intentionally overexposed to reveal details of non-stained cells. (*c*) Noninfected cells with serum of S-R tumor-bearing hamster. Magnification 340×. Photograph overexposed. (*d*) Cells 48 hr p.i. with serum of S-R tumor-bearing hamster. Magnification 880×. Note nuclear staining. (*e*) Cells 72 hr p.i. with serum of S-R tumor-bearing hamster. Magnification 550×. Note staining of extracellular granules.

then appeared on the surface of the cell. Unlike the extracellular antigen stained with fluorescein-labeled antibody from chickens,[16] that detected in the present experiments did not stain unless the coverslip preparations were first fixed with acetone. These results would obtain if the antigen detected by the hamster sera is an internal antigen of the virus and that detected by the chicken sera is on the

viral surface. The viral capsule might be expected to prevent antibody from reaching the internal antigen and the capsule might be rendered permeable to antibody by treatment with acetone.

Sera from hamsters with S-R virus-induced tumors were tested for their ability to detect antigen in cells infected with those viruses listed in Table 1. After exposure to any of the avian sarcoma-leukosis viruses tested, CEF cultures contained cells that stained with the hamster serum. The distribution of antigen produced by these viruses was essentially the same as that produced by RAV. With each virus, antigen was seen first in the nucleus and the cytoplasm, and later on the surface of the cells. In cultures exposed to the Bryan strains of RSV or to the RPL-12 virus, as in those exposed to RAV, cells that stained were present at 2–3 days postinfection. Cultures exposed to preparations of S-R virus, myeloblastosis virus (strain A), or erythroblastosis virus (strain R) contained few, if any, cells that stained until 4–5 days postinfection. It is not known if the observed differences in the time after infection at which antigen-containing cells were first demonstrable reflect qualitative, rather than quantitative, differences among the viral inocula. Cells infected with those viruses that are not in the avian sarcoma-leukosis group developed cytopathology characteristic of the virus used; however, these cells did not stain with the sera from hamsters bearing S-R virus-induced tumors. Thus, such hamster sera when used in the immunofluorescent technique, as when used

TABLE 1

REACTIVITY OF VARIOUS VIRUS-INFECTED CELLS WITH SERA FROM HAMSTERS BEARING S-R VIRUS-INDUCED TUMORS, AS DETERMINED BY IMMUNOFLUORESCENT STAINING

Virus	Cells*	Staining reaction†
Rous sarcoma, Schmidt-Ruppin	CEF	+
" " Bryan's CT750	"	+
" " Bryan's CT916	"	+
" " Vogt's 82E	"	+
Rous associated, RAV	"	+
Lymphomatosis, RPL-12	"	+
Myeloblastosis, strain A	"	+
Erythroblastosis, strain R	"	+
Gallus adeno-like, GAL	"	0
None	"	0
Influenza, type A	CEF or CaK	0‡
" type B	" "	0
None	" "	0
Parainfluenza, type 1	HeLa	0
" type 2	"	0
" type 3	"	0
Mumps	"	0
Newcastle disease	"	0
Respiratory syncytial	"	0
Rubeola	"	0
Adenovirus, type 2	"	0
None	"	0
Simian virus 40	BS-C-1	0
None	"	0
Rubella	RA	0

* CEF = secondary cultures of chick embryonic fibroblasts; CaK = primary cultures of calf kidney cells; BS-C-1 = continuous line of green monkey kidney cells[7]; RA = rubella-associated line of monkey kidney cells.[8]

† Plus indicates that the culture, at some time between 1 and 5 days after exposure to virus, contained cells which stained.

‡ In CEF or CaK cultures exposed to influenza virus, strain A_2/AA/60, the nucleus of an occasional cell showed low-intensity staining with sera of hamsters bearing primary, but not transplanted, S-R virus-induced tumors. The morphology of this staining was unlike that seen in RAV-infected CEF cultures. The significance of this observation is under investigation.

in the complement-fixation test,[1, 2] detect antigen which appears to be common to viruses of the avian sarcoma-leukosis group and to be specific for the group.

In seeking additional evidence of the specificity of the staining reaction, it was reasoned that the production of antigen should relate to other viral functions. Since the S-R virus does not appear to be defective[17] and no associated leukosis virus has been found in preparations of the S-R virus,[4, 17] it was felt that with this virus, antigen production and transformation might relate. There had been little or no evidence of transformation in CEF cultures infected with S-R virus and incubated at 35°C. However, using the standard agar-overlay technique[18] and incubating the cultures at 38°C, S-R virus produced foci of transformed cells. These transformed cells stained with sera from hamsters bearing S-R virus-induced tumors. Only a rare cell that was not associated with a focus of transformation stained. Nuclear staining was much less intense in cells that were incubated at 38°C than those that had been incubated at 35°C.

The specificity of the staining reaction was also supported by the following observations. Progeny of line 7 chickens are resistant to tumor production by RSV.[19] Cultures of line 7 embryos after exposure to Bryan's RSV (CT750) or to RAV did not contain cells that stained with the sera of hamsters bearing S-R virus-induced tumors.

Concerning the relationship of the antigen which was stained in the present study and that which has been detected by complement fixation using sera of hamsters bearing S-R virus-induced tumors, the findings presented in Table 2 pertain. RAV-infected cells were not stained when heat-inactivated hamster serum was used as the sensitizing reagent in the indirect staining technique. These cells were stained when the heated serum from tumor-bearing hamsters was supplemented with fresh normal hamster or guinea pig serum, suggesting that a heat-labile serum factor was required for the binding of hamster globulin to the antigen. That fluorescein-conjugated antibody against guinea pig serum stained RAV-infected cells after they had been incubated with the heated hamster serum supplemented with guinea pig serum suggested that the reaction of hamster antibody and RAV-induced antigen had fixed guinea pig complement. The morphology and distribution of the antigen was the same, whether the fluorescein-conjugated antibody against guinea pig serum or against hamster globulin was used for staining RAV-infected cells.

TABLE 2

HEAT-LABILE FACTOR REQUIRED FOR IMMUNOFLUORESCENT STAINING OF RAV-INFECTED CELLS BY SERA OF HAMSTERS BEARING TUMORS INDUCED BY SCHMIDT-RUPPIN STRAIN OF ROUS SARCOMA VIRUS

Sensitizing Reagent*		Staining reagent†	
Treatment of serum of tumor-bearing hamster	Supplement	Fluorescein-conjugated antibody against:	Reaction
None	None	Hamster globulin	+
56°C, 10 min	None	Hamster globulin	−
56°C, 10 min	Normal hamster serum	Hamster globulin	+
56°C, 10 min	Guinea pig serum	Hamster globulin	+
56°C, 10 min	Guinea pig serum	Guinea pig serum	+

* Sera from hamsters bearing S-R virus-induced tumors or from normal hamsters were used at a final dilution of 1:4. Guinea pig serum was used at a final dilution of 1:10.

† Reagents containing conjugated antibody against hamster globulin or against guinea pig serum were used at a dilution of 1:40.

‡ RAV-infected cells sensitized with normal hamster serum or guinea pig serum did not stain with fluorescein-conjugated antibody against hamster globulin or against guinea pig serum. Noninfected cells did not stain with any of the combinations of sensitizing and staining reagents.

Discussion.—The evidence suggests that, using sera from hamsters bearing S-R virus-induced tumors, the immunofluorescent technique and the complement-fixation test[1, 2] detect the same antigen in cells infected with viruses of the avian sarcoma-leukosis group. This is supported by the conclusion of Berman and Sarma[20] that there was no unequivocal evidence of more than one sarcoma-leukosis antigen that reacted by immunodiffusion with sera from hamsters bearing S-R virus-induced tumors.

Sera from immune chickens neutralize avian sarcoma-leukosis viruses, but sera from hamsters bearing S-R virus-induced tumors do not,[1] suggesting that the two kinds of sera may react with different antigens. In previous studies[21–24] of the immunofluorescent staining of cells infected with these viruses, sera from immune chickens or turkeys have been employed. It was reported that the antigens stained were, in the vast majority of the infected cells, in the cytoplasm or on the surface of infected cells. However, each report noted that a very rare cell showed some nuclear staining. Malmgren, Fink, and Mills,[21] studying avian sarcoma tissue, stated that there were occasional poorly defined intranuclear masses which showed low-intensity fluorescence. Mellors and Munroe,[22] in a similar study, observed in a rare cell a discrete round intranuclear focus of fluorescence which seemed to be associated with the nucleolus. Noyes,[23] using CEF cultures infected with RSV, noted that very infrequently, in cells containing a large amount of cytoplasmic antigen, a central area in the nucleus stained faintly; he thought it unlikely that this was due to virus multiplication but that it might be due to diffusion of antigen. Vogt and Rubin[24] reported that "nuclear antigen" was found only in a very rare cell of RSV-infected CEF cultures and noted that this could have been due to occasional superposition of cytoplasmic antigen over the nucleus. The absence of prominent nuclear staining in the previous studies might have resulted from the use of avian sera that had little or no antibody against the nuclear antigen. However, since we found prominent nuclear staining in infected cells incubated at 35°C but not in those incubated at 38°C, it seems likely that temperatures of 38–40°C, which are usually used for CEF cultures infected with RSV, produced such rapid transfer of antigen from the nucleus that amounts sufficient to stain accumulated only rarely. The equal intensity of staining of nuclear inclusion and cytoplasmic antigen in any given cell of cultures incubated at 35°C suggested that, even at this temperature, antigen moved rapidly into the cytoplasm. In preliminary experiments, incubating infected cultures at temperatures below 35°C or treating them with *p*-fluorophenylalanine has not restricted further the transfer of antigen out of the nucleus.

Reports that deoxyribonucleic acid is involved in the replication of RSV[25] are of particular interest in relation to the possible intranuclear site of synthesis of the antigen detected in the present study.

In RSV-infected cells, those areas which stain with fluorescein-labeled antibody from immunized chickens have been found to coincide with those areas which contain virus particles.[24] Labeled antibody of avian origin also stains particulate antigen over the surface of nonfixed infected cells,[16] indicating that the antigen detected is readily available to the antibody and suggesting that this antigen may be present in the viral envelope or in the cell membrane. The similarities in appearance of the particles stained over the surfaces of infected cells in the present

study and those previously demonstrated using labeled antibody from chickens suggest that the antigen detected by sera from tumor-bearing hamsters is also a component of the virus. That the hamster sera stained this antigen only after the coverslip preparations were fixed in acetone is compatible with the suggestion that this antigen is not in the viral envelope but is an internal component of the virus. After the present investigation was completed, it was learned that Dr. P. K. Vogt (personal communication) has similarly interpreted his own unpublished observations on the ability of hamster or chicken antibody to stain fixed or nonfixed cells that were infected with viruses of the avian sarcoma-leukosis group. Vogt, Sarma, and Huebner[26] also have recently reported that the antigen, detected by complement fixation using hamster serum, is present in RSV-transformed chick cells that do not produce infectious virus, and they concluded that this antigen is not part of the viral coat.

Similarities between viruses of the avian sarcoma-leukosis group and influenza virus have been previously noted and similarities between the S-antigen of influenza virus and the antigen detected by sera from hamsters bearing S-R virus-induced tumors have been discussed.[1] The findings in the present investigation recall this comparison because the S-antigen or nucleoprotein of influenza virus is synthesized in the nucleus of the host cell, is transferred to the cytoplasm, and is present in the virion as the capsid which is antigenically distinct from the viral envelope.[27]

The principal advantage of the immunofluorescent staining technique described, as compared with the complement-fixation test using similar hamster sera,[1] is expected to be the former's ability to localize antigen in cells and tissues and to detect antigen in a few cells even when these are among many which contain no antigen.

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[1] Huebner, R. J., D. Armstrong, M. Okuyan, P. S. Sarma, and H. C. Turner, these PROCEEDINGS, **51**, 742 (1964); Armstrong, D., M. Okuyan, and R. J. Huebner, *Science*, **144**, 1584 (1964).

[2] Sarma, P. S., H. C. Turner, and R. J. Huebner, *Virology*, **23**, 313 (1964).

[3] Burmester, B. R., A. K. Fontes, N. F. Waters, W. R. Bryan, and V. Groupé, *Poultry Sci.*, **39**, 199 (1960).

[4] Sarma, P. S., H. C. Turner, and R. J. Huebner, *Natl. Cancer Inst. Monograph No. 17* (1964), p. 481.

[5] Hull, R. N., W. R. Cherry, and O. J. Tritch, *J. Exptl. Med.*, **115**, 903 (1962).

[6] Maassab, H. F., and J. A. Veronelli, *J. Bacteriol.*, **91**, 436 (1966).

[7] Hopps, H. E., B. C. Bernheim, A. Nisalak, J. H. Tjio, and J. E. Smadel, *J. Immunol.*, **91**, 416 (1963).

[8] Ahlstrom, C. G., and N. Forsby, *J. Exptl. Med.*, **115**, 839 (1962); Ahlstrom, C. G., N. Jonson, and N. Forsby, *Acta Pathol. Microbiol. Scand., Suppl.*, **154**, 127 (1962).

[9] Burmester, B. R., M. A. Gross, W. G. Walter, and A. K. Fontes, *J. Natl. Cancer Inst.*, **22**, 103 (1959).

[10] Burmester, B. R., and R. F. Gentry, *Poultry Sci.*, **35**, 17 (1956).

[11] Rubin, H., and P. K. Vogt, *Virology*, **17**, 184 (1962).

[12] Burmester, B. R., W. G. Walter, M. A. Gross, and A. K. Fontes, *J. Natl. Cancer Inst.*, **23**, 277 (1959).

[13] Sharpless, G. R., S. Levine, M. C. Davies, and M. E. Englert, *Virology*, **13**, 315 (1961).

[14] Pope, J. H., and W. P. Rowe, *J. Exptl. Med.*, **120**, 121 (1964).

[15] Rodriguez, J., and F. Deinhardt, *Virology*, **12**, 316 (1960).

[16] Vogt, P. K., and H. Rubin, *Virology*, **13**, 528 (1961).

[17] Hanafusa, H., *Natl. Cancer Inst. Monograph No. 17* (1964), p. 543.
[18] Temin, H. M., and H. Rubin, *Virology*, **6**, 669 (1958).
[19] Crittenden, L. B., W. Okazaki, and R. H. Reamer, *Natl. Cancer Inst. Monograph No. 17* (1964), p. 161.
[20] Berman, L. D., and P. S. Sarma, *Nature*, **207**, 263 (1965).
[21] Malmgren, R. A., M. A. Fink, and W. Mills, *J. Natl. Cancer Inst.*, **24**, 995 (1960).
[22] Mellors, R. C., and J. S. Munroe, *J. Exptl. Med.*, **112**, 963 (1960).
[23] Noyes, W. F., *Virology*, **12**, 488 (1960).
[24] Vogt, P. K., and H. Rubin, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 27 (1962), p. 395; Vogt, P. K., and N. Luykx, *Virology*, **20**, 75 (1963).
[25] Bader, J. P., *Virology*, **22**, 462 (1964); Temin, H. M., *Virology*, **23**, 486 (1964); Temin, H. M., these PROCEEDINGS, **52**, 323 (1964).
[26] Vogt, P. K., P. S. Sarma, and R. J. Huebner, *Virology*, **27**, 233 (1965).
[27] Francis, T., Jr., and H. F. Maassab, in *Viral and Rickettsial Infections of Man*, ed. F. L. Horsfall and I. Tamm (Philadelphia: J. B. Lippincott Co., 1965), 4th ed., chap. 30.

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*ON THE CULTURE OF A MICROORGANISM SIMILAR TO THE PRECAMBRIAN MICROFOSSIL KAKABEKIA UMBELLATA BARGHOORN IN NH_3-RICH ATMOSPHERES**

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Recent experiments in this laboratory with angiosperms, gymnosperms, invertebrates, fungi, and bacteria have shown that resistance to stress factors such as anoxia, extreme temperatures, desiccation, salinity, ionizing radiation, and combinations of these is more commonplace than is generally appreciated.[1, 2] This conclusion applies to O_2-free atmospheres containing appreciable amounts of nitrogen oxides[3] and to a variety of ammonia-rich gases as well.[4] In spite of the well-known toxicity of ammonia, even some angiosperms have been observed to tolerate 5–15 per cent NH_3 in methane or oxygen. *Penicillium*, yeasts, yeastlike forms, and other fungi, as well as *Pseudomonas*, *Clostridium*, and a wide variety of cocci and unidentified motile and nonmotile bacteria, show growth in these environments and have been repeatedly observed from soil samples incubated in such environments.

The concepts of stress tolerance extended by this work have been discussed in exobiological terms, but results of some of our experiments may also have significance with respect to paleobiology.

Methods.—Soil samples from England, Wales, Scotland, Belgium, Germany, British Guiana, Curaçao, and various locations in the United States have been inoculated into standard nutrient agar and incubated in air and NH_3-rich atmospheres. The medium consisted of 1.0 per cent Bacto-peptone plus 0.5 per cent beef extract in 2 per cent agar. Plates were routinely incubated at 25 ± 1°C in darkness. A few plates were kept under *ca.* 100 ft-c daylight fluorescent illumination to facilitate pigmentation. Routinely, a 1:1 ratio of methane and ammonia was used alone or combined with an equal volume of air. The experimental atmospheres had the following compositions: