CELLULAR LOCALIZATION OF ROUS SARCOMA VIRUS AS STUDIED WITH FLUORESCENT ANTIBODY

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In the study of virus-induced neoplasms the fluorescent-antibody method provides a singular opportunity for investigating, within the limits of optical resolution, the cellular sites of origin and development of the virus (or specifically its associated antigens) and the tumor. The purpose of this communication is to present some of the qualitative and quantitative observations that we have made with this procedure in the study of Rous sarcoma virus.

Methods and Materials

Rous Sarcoma Virus.—The starting material for virus preparation was the harvest of wing tumors comprising the seventh passage in chickens of Rous sarcoma virus obtained originally from Dr. W. R. Bryan. His procedure of selecting the most rapidly developing tumors as virus-source material was used (1). The solid tumors were pooled and homogenized at a 1:5 dilution in isotonic saline containing 2 per cent rabbit serum, penicillin (100 units/ml.), and streptomycin (100 μg./ml.). The homogenate was shell-frozen and rapidly thawed three times and centrifuged at 2000 R.P.M. at room temperature for 20 minutes. The supernatant fluid constituted the standard preparation of Rous sarcoma virus which, after suitable dilution, was used in this work, Table I. When assayed by the chicken wing-web technique, the tumor-producing titer of the standard preparation was $10^{7.3}$ TD_{50} per gm. of original tumor, as calculated by the Reed-Muench formula. On the chorioallantoic membrane of the chick embryo the assay was $10^{6.5}$ pock-forming units per gm. of tumor.

Chickens and Chick Embryos.—White Leghorn chickens, 3 to 5 days of age, and chick embryos, 12 days of age, were obtained from Shamrock Farms, North Brunswick, New Jersey.

In the inoculation of wing web, the needle was passed through muscle so as to limit the loss of the inoculum by back flow along the needle track. For initiating the growth of wing tumors with attributes tabulated in Table I and Text-figs. 1 and 2, the standard preparation of Rous virus was diluted, as noted, and inoculated in 1 ml. quantity. Groups of 20 chicks were employed for each virus dilution, and 6 wing tumors of most rapid growth, or if in lesser number the entire yield, were chosen from each group for study. The wing tumors were harvested as soon as they attained an estimated diameter of about 10 mm.

Virus Assay.—Approximately one-half of each wing tumor, stored at −70°C., was homogenized, three times frozen and thawed, and centrifuged to yield a supernatant fluid (tumor extract) which was assayed by the chicken wing-web technique for tumor-producing activity, expressed as $\log_{10}$ TD_{50} per gm. of tumor. Five chickens were employed for the assay of each

decimal dilution of tumor extract. The inoculum was 0.2 ml. The data in Table I were the arithmetic means for virus extracted from tumors in each category.

The standard preparation of Rous virus was assayed for tumor-producing activity in the chicken wing web and the chorioallantoic membrane. The inoculum in either prodecure was 0.2 ml. Five chickens and 20 chick embryos were employed for each decimal dilution of virus. The chorioallantoic membranes were dropped conventionally and inoculated with a 20 gauge needle. Embryos were incubated at 100°F. for 6 days, and the membranes were harvested, placed on a glass plate, and with magnification and oblique illumination against a black background were examined for pocks. The average pock count was determined at two or more dilutions at which there was no confluence of tumor growth.

TABLE I

Assays of Virus and Attributes of Wing Tumors Produced by Rous Sarcoma Virus

No. of chickens	Tumor-initiating dose of virus*	Latent period	Age of tumor		Tumor diameter	Virus extracted from tumor‖	Sarcoma cells with Rous¶viral antigens¶
			from virus inoculation	from earliest appearance			
		days‡	*days*	*days*	*mm.*§	*log* TD_{50}*/gm.*	*per cent*
6	10^{-2}	6	9	3	8	8.2	12
6	10^{-4}	6	9	3	9	6.9	10
6	10^{-6}	9	15	6	7	6.0	5
4	10^{-7}	9	16	7	8	2.9	0.05
2	10^{-8}	12	16	4	9	2.8	0

* Dilutions of a standard, cell-free (three times frozen and thawed) preparation of Rous sarcoma virus.

‡ Time elapsed from inoculation of virus to earliest macroscopic evidence of tumor.

§ Pathologist's measurement, a diameter of a cube of tumor.

‖ TD_{50}, the dose of virus which produces wing tumor in 50 per cent of chickens.

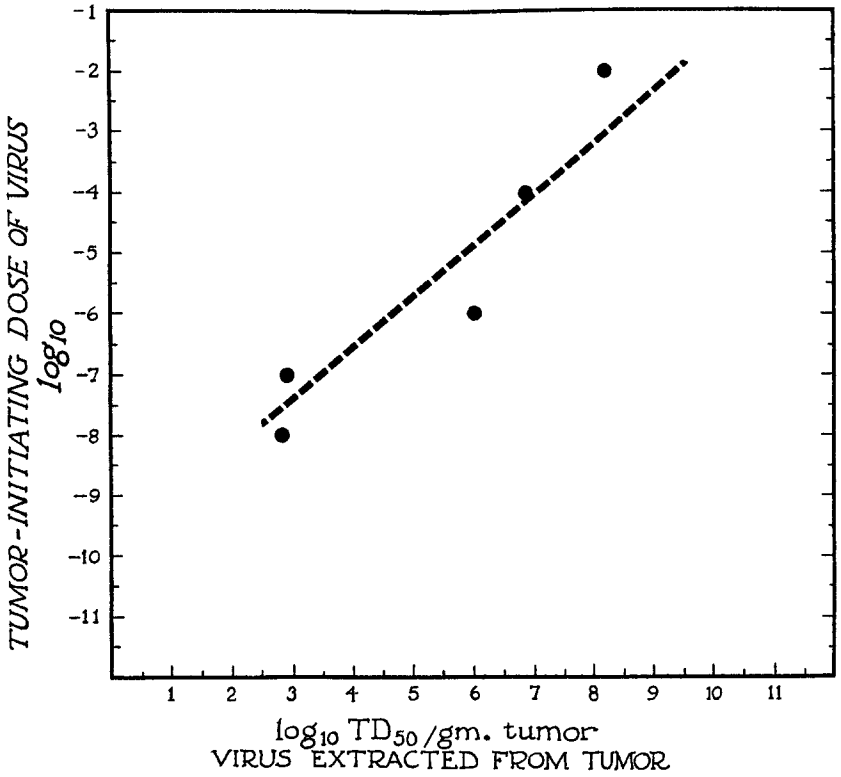
¶ Demonstrable with fluorescent antibody in frozen sections of the tumors.

Antiserum.—Chickens 8 weeks of age were inoculated in the wing web with a stock preparation of Rous virus that had been allowed to lose its infectivity by being maintained at 4°C. for 4 weeks (11). No formalin or other antiviral substance was added. A chicken received three semimonthly injections, each comprising 1 ml. of a 10^{-2} dilution. Two weeks after the third injection each chicken was inoculated with 0.2 ml. of a 10^{-2} dilution of fully infective Rous virus, a dose that would be expected to produce tumors in 100 per cent of susceptible chickens. Tumors did not occur in the immunized birds. Serum was obtained 3 to 5 weeks after the challenge with infective virus, stored at −70°C., and later assayed for virus neutralization titer. This undiluted antiserum in mixture with decimal dilutions of Rous virus produced a loss of 6 $\log_{10}$ in the end-point dilutions of virus when assayed by the wing web or the chorioallantoic membrane methods.

Fluorescent Antibody.—The crude gamma globulin fraction of chicken antiserum was obtained by fractionation with cold ethanol at 25 per cent concentration (2) and was conjugated with fluorescein isothiocyanate (12). The protein concentration in the reaction mixture was 20 mg. per ml., and fluorescein isothiocyanate was used in the ratio of 0.05 mg. per mg. of protein. Prior to use, fluorescent antibody in 1 ml. quantity was absorbed with 150 mg. of rabbit bone marrow powder. This powder was prepared from red marrow homogenate by acetone precipitation and thorough washing with isotonic saline (buffered at pH 7.6 with 0.01 M phosphate).

While the present study was carried out with the direct procedure, using fluorescein-labeled chicken antibody against Rous sarcoma virus, the indirect procedure has also been successfully employed. Unlabeled chicken antiserum against Rous sarcoma virus comprised the first layer of protein, and fluorescein-labeled rabbit antibody against chicken globulins was the final reactant.

Tissues and Frozen Sections.—Tissue specimens were obtained from 101 young chickens and 8 chick embryos after inoculation with Rous sarcoma virus: wing tumors, 50; wing muscle



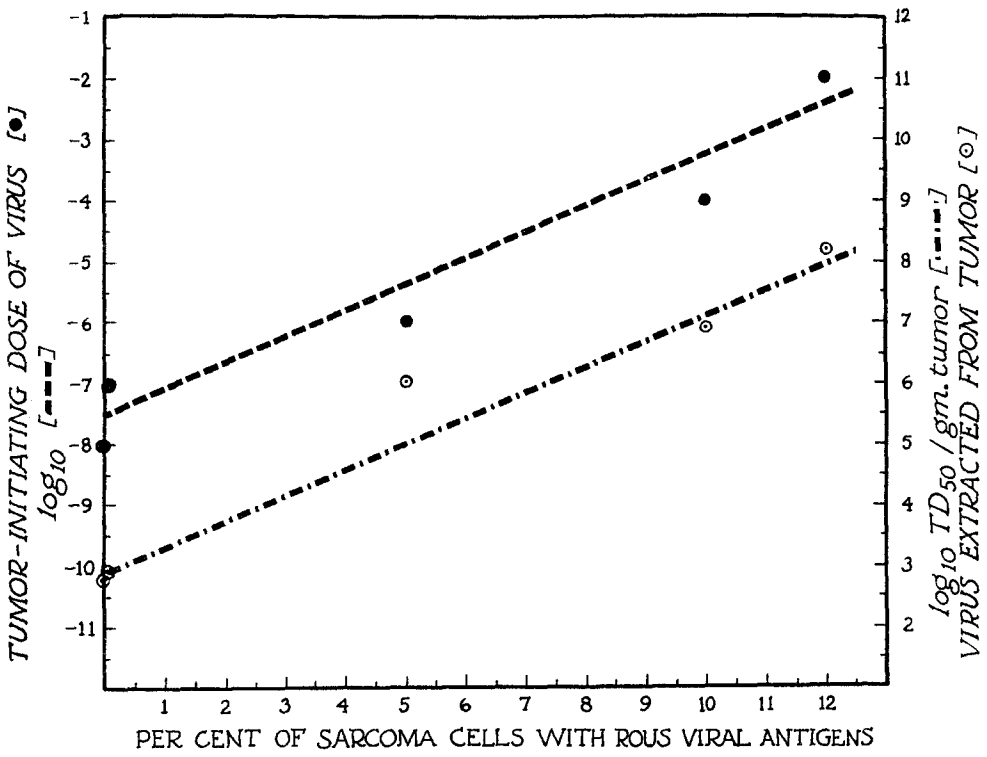
TEXT-FIG. 1. The concentration of extractable virus in wing tumors produced in chickens by various initiating doses (ordinate) of Rous virus.

and connective tissue, 33; lung tumors, 2; chorioallantoic membrane tumors, 8; and other tissues (heart, lung, liver, spleen, 4 each), 16. Control tissues were derived from 18 uninoculated normal chickens and 1 chick embryo: wing muscle and connective tissue, 12; heart, 6; liver, 6; spleen, 6; and chorioallantoic membrane, 1. In addition, wing muscle, spleen, and liver were received from 4 chickens which had been inoculated 27 to 41 days previously by Dr. Ben Burmester with his RPL 12 strain of virus that induces erythroblastosis and produces visceral lymphomatosis in about 80 per cent of chickens during the period of about 100 to 145 days after inoculation.

Fresh tissue blocks were placed in stoppered containers, frozen at −70°C. in a bath of dry ice and acetone, stored in a freezer at −25° to −30°C., sectioned in a microtome cryostat at an indicated thickness of 4 μ, dried for 10 minutes at room temperature, and stored in the

refrigerator in sealed containers. From the total of 158 tissue blocks which were accessioned during this study, 221 frozen sections were prepared and stained with fluorescent antibody.

Immunofluorescence Staining Procedures.—As needed, frozen sections were removed from the refrigerator, fixed with anhydrous acetone for 15 minutes at room temperature, air-dried, washed four times with buffered saline solution (pH 7.6), drained, blotted, and treated with



TEXT-FIG. 2. The per cent of sarcoma cells with Rous viral antigens demonstrated in sections of wing tumors with fluorescent antibody and related to the tumor-initiating dose of virus (upper curve with left ordinate) and the concentration of extractable virus (lower curve with right ordinate).

the appropriately absorbed fluorescent antibody or other reactant. Staining and blocking reactions were carried out for 60 minutes at room temperature.

The immunospecific staining patterns of Rous viral antigens discussed in this paper were obtained with fluorescein-labeled chicken antibody against Rous sarcoma virus. Absorption of this fluor with acetone-precipitated, saline-insoluble rabbit bone marrow powder reduced non-specific staining to imperceptible or negligible levels. Prior treatment of sections with unlabeled chicken antiserum against Rous sarcoma virus (but not chicken serum lacking such antibodies) prevented the staining of viral antigen with fluorescent antibody. Prior treatment of sections with unlabeled chicken antiserum, obtained from Dr. Ben Burmester and shown by him to neutralize avian leukosis virus (erythroblastosis and lymphotosis) and Rous sarcoma virus, substantially decreased the staining of Rous viral antigen with fluorescent antibody.

Conjugates prepared from heterologous antiserums did not stain the tissues of interest, nor did fluorescent antibody for Rous viral antigens stain the normal tissues obtained from uninoculated healthy young chickens (the serums of which lacked Rous virus neutralizing antibodies), chickens with latent leukosis virus infection (obtained from Dr. Burmester), and uninoculated chick embryos.

Fluorescence Microscopy and Photography.—These procedures have been described elsewhere (10). The exciter filters were UG5 (for visual work), UG2 (color photography), and BG12 (black and white photography). The barrier filters were BG23 and GG4 (for visual and color work) and OG4 and OG5 (for black and white photography).

The quantitative histological method of area sampling (5) with an eyepiece net micrometer was used to count the proportion of sarcoma cells containing Rous viral antigens. In randomly selected fields, additively covering the entire section without overlap, the number of intersection points falling on sarcoma cells containing apple-green foci of viral antigens was determined and divided by the total number of points falling on sarcoma cells of all kinds. This quotient multiplied by 100 gave the per cent of sarcoma cells containing Rous viral antigens. A total of 12,010 sarcoma cells in tissue sections from 17 wing tumors was evaluated in this manner. The tumors were produced by each of four dilutions (10^{-3}, 10^{-4}, 10^{-6}, and 10^{-7}) of Rous sarcoma virus (Table I). At each category of dilution, an average of 3,000 sarcoma cells in sections of 3 to 6 wing tumors were analyzed. Innumerable sarcoma cells lacking viral antigens were scanned in multiple sections of 2 wing tumors produced by a 10^{-8} dilution of virus.

Other Histological Procedures.—In almost all instances blocks of tissue were also fixed in neutral formalin, embedded in paraffin, cut at 4 μ, and stained with Harris' hematoxylin and picric acid–eosin, hematoxylin-eosin and alcian blue, and phosphotungstic acid–hematoxylin. Frozen sections, including those treated with fluorescent antibody as well as companions to these, were stained with hematoxylin and eosin.

RESULTS

The following descriptions pertain to the immunospecific, apple-green staining patterns imparted by fluorescent antibody to Rous sarcoma viral antigens in frozen sections of wing tumors (obtained under the experimental conditions summarized in Table I), wing muscle and connective tissue, and pulmonary tumors of young chickens and chorioallantoic membrane tumors of the chick embryo.

Wing Tumors.—If the tumor-initiating dose of virus is sufficiently high, an abundance of sarcoma cells are seen in low power fields to contain Rous viral antigens, Fig. 1. In such fields some of the virus-containing sarcoma cells tend to be elongated and to stream out in characteristic growth patterns, while others, more commonly plump than spindle-shaped, are distributed in random and loose array in tumor mucoid. At higher magnification, Figs. 2 to 11, variations in the cellular site (cytoplasm, cell membrane, and nucleus), distribution (particulate or homogeneous), and quantity (little or much) of viral antigens are seen. The smallest focus of viral antigens consists of one or more tiny round particulates which are visualized down to the limit of resolution of an oil-immersion objective and located in the cytoplasm, Fig. 5, or near the nuclear membrane, or on the cell membrane (and conceivably then extracellular).

The cytoplasmic foci of viral antigens may enlarge or coalesce to form coarse particulates, Fig. 3, aggregates, or packets, Fig. 6. Viral antigens below the limit of optical resolution had an indistinctly particulate, Fig. 7, or homogeneous distribution over a limited region, Fig. 8, or an extended area of the cytoplasm, Fig. 9. Particulate and homogeneous distributions of viral antigens sometimes coexist in the same cell. The maximum cellular content of viral antigens, as judged by the intensity and the area of cytoplasmic immunofluorescence, occurs in large sarcoma cells, notably those having plump, polyhedral, or bizarre shapes, Figs. 6 to 9. These cells tend to occur alone or in small groups and contrast with smaller neighboring sarcoma cells which may lack demonstrable antigen. While Rous viral antigens are localized almost exclusively in the cytoplasm, one, or rarely more than one, homogeneous round focus of immunospecific apple-green fluorescence of low intensity is occasionally present in the nucleus of sarcoma cells, Figs, 10 and 11. A nucleolar-like, spherical body with faint blue-violet intrinsic fluorescence is sometimes observed at the oil-immersion focal planes immediately below. Trace or larger amounts of viral antigens may coexist in the cytoplasm.

The quantitative relations between the tumor-initiating dose of Rous sarcoma virus, the infectivity titer of virus extracted from the wing tumor, and the per cent of sarcoma cells containing Rous viral antigens are shown in Table I and Text-fig. 2. If the tumor-initiating dose of virus is high (200,000 and 2,000 TD_{50} respectively provided by 10^{-2} and 10^{-4} dilutions of a standard preparation), approximately 10 to 12 per cent of sarcoma cells contain Rous viral antigens as demonstrated in frozen sections of wing tumors stained with fluorescent antibody. If the initiating dose of virus is low (2 and 0.2 TD_{50} respectively provided by 10^{-7} and 10^{-8} dilutions of the identical standard preparation), 0 to 0.05 per cent of sarcoma cells contain viral antigens. Although the precise nature of the curve relating log tumor-initiating dose and per cent of sarcoma cells containing viral antigens is not established for intermediate points in Text-fig. 2, the data obtained at high and low dilutions are statistically resolved, with a probability for chance occurrence of less than 0.01. Similar statements can be made for data pertaining to the titer of virus extracted from the wing tumors expressed as log TD_{50} per gm. of tumor and the per cent of antigen-containing sarcoma cells in sections of the wing tumors. In tumors yielding high concentrations of extractable virus (log TD_{50} equals 8.2 and 6.9), a substantial proportion of sarcoma cells (12 and 10 per cent, respectively) contain viral antigens; and in tumors with low concentrations of virus (log TD_{50} equals 2.9 and 2.8), only a minute proportion of sarcoma cells (0.05 and 0 per cent, respectively) contain viral antigens detectable with fluorescent antibody. The data obtained at high and low concentrations of extractable virus are statistically resolved, with a probability for chance occurrence of less than 0.01.

While the precise location of intermediate points is not established for the upper or the lower curves in Text-fig. 2 relating assays of virus in the tumor-initiating dose and in the tumor extract to the per cent of sarcoma cells containing viral antigens, it is to be noted that virus assay points do not deviate by more than 1 log unit from the hypothetical pair of parallel lines constructed in this figure.

Wing Muscle and Connective Tissue.—Microscopic foci of sarcoma cells containing Rous viral antigens were noted in sections of skeletal muscle and connective tissue in three of four wing specimens obtained from chickens at 4 days after inoculation of the wings with a 10^{-2} dilution of the standard, cell-free (three times frozen and thawed) preparation of Rous sarcoma virus. In one typical focus, comprising in all about 50 polyhedral-shaped sarcoma cells, approximately a dozen cells contained viral antigens, Figs. 12 and 13, while in another, Figs. 14 and 15, in which sarcoma cells had arisen along the needle track the majority (more than half) of the sarcoma cells in a microscopic field contained a finely particulate or homogeneous distribution of viral antigens. In three of four specimens, viral antigens were also localized in the skeletal muscle of the inoculated wing, Figs. 16 and 17, in a characteristic pattern of deposition near or on the sarcolemma. This immunospecific focal pattern of virus localization in muscle, which has also been observed on occasion in the muscle residues in fully established wing tumors, Fig. 19, is selective, affecting but a few of many muscle fibers, and is not obviously traceable by continuity to a neighboring focus of sarcoma cells. That is to say, foci of virus localization in muscle and in sarcoma cells occur independently in the same section and in one or two adjacent ones (the practical numerical limit of serial frozen sections). Moreover, viral antigens were focally localized as fine particulates on the sarcolemma of a few muscle fibers in two of four wings examined at 3 days after virus inoculation, Fig. 18, although sarcoma cells were not yet demonstrable in the tissue sections. Wing muscle and connective tissue obtained at 2 hours, 24 hours, and 2 days after virus inoculation did not contain viral antigens demonstrable with fluorescent antibody. Virus was not detectable in wing web by bioassay until 4 days after inoculation.

Others.—Rous viral antigens were observed in some sarcoma cells in a pulmonary tumor, Fig. 20, coexisting with wing tumor in a chicken which had been inoculated with virus 15 days previously. The intracellular distribution of viral antigens was qualitatively similar to that described for wing tumors. Viral antigens were not detected in the lungs of chickens at 24 hours and at 4 days after virus inoculation in the wing, although bioassay revealed detectable virus in the lungs as early as 24 hours after wing-web inoculation.

Viral antigens were present in sarcoma cells in the chorioallantoic membrane tumors of the chick embryo, inoculated at 11 days of age and harvested 6 days later, Fig. 21.

DISCUSSION

The dependence upon initiating dose of the amount of virus extractable from Rous sarcoma has been shown by the extensive work of Bryan, Calnan, and Moloney (1). Epstein (4) observed a statistically significant correlation between the incidence of Rous ascites tumor cells containing virus-like particles identified with the electron microscope and the tumor-producing activity of virus extractable from the tumor cells. Haguenau, Dalton, and Moloney (6) demonstrated a correlation between the particle count in ultrathin sections of wing tumors and the concentration of infective virus recoverable from these tumors. Particle counts and content of extractable virus were much greater in the category of tumors produced by a high, as contrasted with a low, initiating dose of Rous virus. These combined morphological and biological studies have shown therefore that the virus-like particles represent, or are quantitatively related to, the causative agent of Rous sarcoma.

With the exception of limited comments and illustrations presented elsewhere (8, 9, 11 *a*) and representing observations that for the most part were preliminary to the present investigation, there is but one other publication to date which deals with the fluorescent-antibody detection of Rous sarcoma virus. Malmgren, Fink, and Mills (7) illustrated the particulate and the diffuse distribution of viral antigens in the cytoplasm of sarcoma cells and also the presence of a poorly defined mass of viral antigens in the nucleus. They found that viral antigen-containing cells were very numerous in Rous sarcomas produced by large amounts of virus and were much less frequent in tumors induced with a low concentration of virus. Numerical counts for antigen foci were not given in their work, and the content of extractable virus in the tumors was not determined.

The present studies, in showing the quantitative proportional relations between the initiating dose, the recoverability, and the morphological detectability (as antigens) of Rous sarcoma virus in wing tumors, are in agreement with those previously cited. In addition, by reference to Table I and Text-fig. 2 it is seen that the least amount of Rous viral antigens detectable by fluorescent antibody in tissue sections is found in wing tumors with virus concentrations of about 10^3 TD_{50} per gm. Assuming that 1 gm. of tumor occupies about 1 cm^3. (10^{12} μ^3), a virus concentration of 10^3 TD_{50} per gm. becomes 1 TD_{50} per 10^9 μ^3. A tissue section with an area of 1 $cm.^2$ (10^8 μ^2) and a thickness of 10 μ has a volume of 10^9 μ^3, corresponding to a virus content of 1 TD_{50}. Therefore if localized in sufficient concentration, 1 TD_{50} of virus would apparently correspond to one immunofluorescent focus per tissue section, which was in fact the approximate number observed in sections of tumors of low virus concentration (log TD_{50} equals 2.9 per gm.). Assuming that one infectious (tumor-producing) unit of Rous virus comprises 50 spherical virus particles (4), with diameters of 70 mμ, the diameter of a sphere having a volume of 50 particles

equals 0.26 μ and the diameter of the sphere which encloses all of the particles is larger than that. Thus if a spherical aggregate of 50 virus particles corresponds to one tumor-producing unit of Rous virus, it would be optically resolved and, if sufficient in antigen content, would be detected as an immunofluorescent focus in a microscope. A solitary particulate focus of immunofluorescence with size near the limit of resolution of an oil-immersion objective is the smallect amount of Rous viral antigens detectable in an individual sarcoma cell. If representing infective virus, such an immunofluorescent focus apparently would correspond to one tumor-producing unit. However, one must acknowledge the possibility that the nucleic acid moiety of Rous virus may be by itself infective.

In several respects, the present work is in accord with the observations of Rubin (13). This investigator plated a known number of intact Rous sarcoma cells on the chorioallantoic membrane. Each tumor formed was found to arise from one virus-releasing cell, that is, each implanted cell could account for no more than one tumor. Moreover, an average of about one cell in eight (12.5 per cent) yielded a tumor on the chorioallantoic membrane. In the present study, when the initiating doses of virus corresponded to dilutions of 10^{-2} and 10^{-4}, 10 to 12 per cent of sarcoma cells contained Rous viral antigens demonstrable with fluorescent antibody. Rubin also found that enlarged cells practically always produced tumors and that some of these cells produced sufficient virus to infect the chorioallantoic membrane at 20 or 30 places. In the present investigation, large sarcoma cells, Fig. 9, contained the greatest quantity of Rous viral antigens per cell as estimated from the product or integral of immunofluorescence intensity and cytoplasmic area.

The most unexpected finding in the present study was the localization of Rous viral antigens on the sarcolemma of wing muscle, which at first thought was presumed to be a consequence of local diffusion of viral antigens from a neighboring focus of sarcoma cells in the interstitial fibrous tissue. That this explanation alone was not sufficient was suggested, however, by the observation that viral antigens were localized on the sarcolemma at 3 days after virus inoculation, at a time when sarcoma cells were not identifiable in the tissue sections. Nevertheless, a careful histological study of the muscle fibers in paraffin sections disclosed no cytopathic effects attributable to Rous virus. While this problem requires further investigation, it is of interest in respect to the recent studies of Ebert (3). In his experiments, chicken heart tissue and Rous sarcoma tissue were mixed and extracted together, and a cell-free preparation of Rous virus was made from the extract. When this conjoined extract was inoculated into the chorioallantoic membrane of the chick, it caused the formation of tumors containing striated muscle fibrils. Presumably some association between virus particles and muscle-cell constituents had occurred during extraction, and a transduction-like reaction had ensued.

SUMMARY AND CONCLUSIONS

A study has been made of the immunospecific, apple-green staining patterns imparted by fluorescent antibody to Rous virus in frozen sections of wing tumors produced in young chickens by various initiating doses of virus. Variations in the cellular site (cytoplasm, cell membrane, and nucleus), the distribution (particulate or homogeneous), and the quantity (little or much) of Rous viral antigens were seen. The per cent of sarcoma cells containing viral antigens was related to the tumor initiating dose of virus and to the infectivity titer of virus extracted from the wing tumor. With certain assumptions it was estimated that a quantity of virus approaching one tumor-producing dose (1 TD_{50}) per tissue section was detectable with fluorescent antibody.

Sarcoma cells containing viral antigens were identified as early as 4 days after inoculation of Rous virus. At this time viral antigens were also localized in the skeletal muscle of the inoculated wing in a characteristic focal pattern of deposition near or on the sarcolemma. This association between viral antigens and skeletal muscle fibers was observed also at 3 days after virus inoculation, when sarcoma cells were not yet demonstrable in the tissue sections.

Viral antigens were detected in sarcoma cells in lung tumors of young chickens and in tumors of the chorioallantoic membrane of chick embryos.

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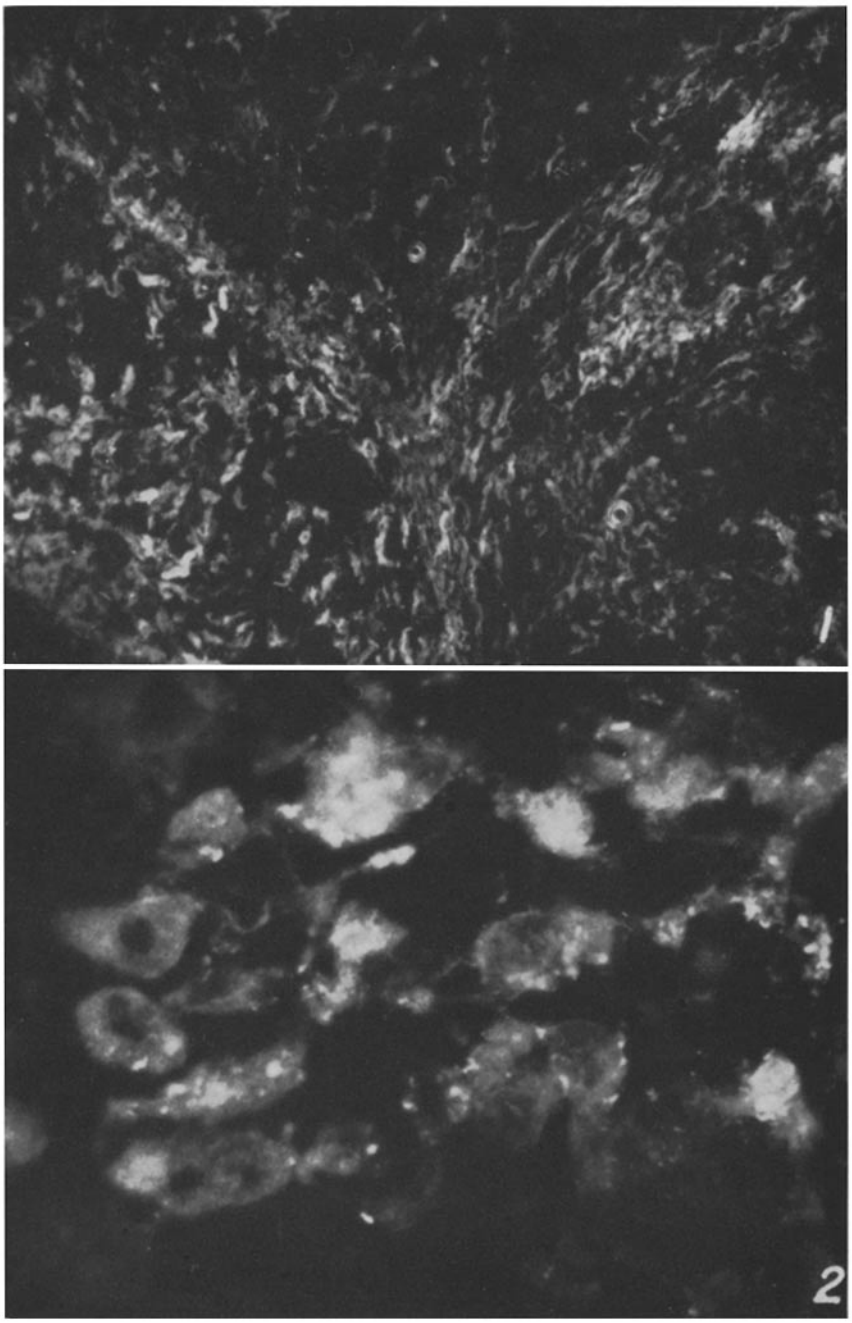
EXPLANATION OF PLATES

PLATE 81

The illustrations are fluorescence photomicrographs of 4 μ thick, frozen sections of chicken tissues stained with the direct fluorescent antibody procedure. The descriptions pertain to the immunospecific staining of Rous viral antigens.

FIG. 1. Rous sarcoma, wing tumor with log TD_{50}/gm. equals 7.8. Rous viral antigens (white areas) in the cytoplasm of many sarcoma cells. × 100.

FIG. 2. Wing tumor. Rous viral antigens with particulate and non-particulate (homogeneous) distributions in a focus of sarcoma cells. × 1500.



(Mellors and Munroe: Rous sarcoma virus

PLATE 82

FIG. 3. Wing tumor. Rous sarcoma cell with fine and coarse particulate distribution of viral antigens in cytoplasm. × 1500.

FIG. 4. Wing tumor. Rous sarcoma cell with coarse particulate distribution of viral antigens in cytoplasm near nuclear membrane. × 1500.

FIG. 5. Wing tumor. Rous sarcoma cell with fine particulate and indistinctly granular distribution of viral antigens in cytoplasm. × 1500.

FIG. 6. Wing tumor. Rous sarcoma cell with small and large aggregates of viral antigens in cytoplasm. × 1500.

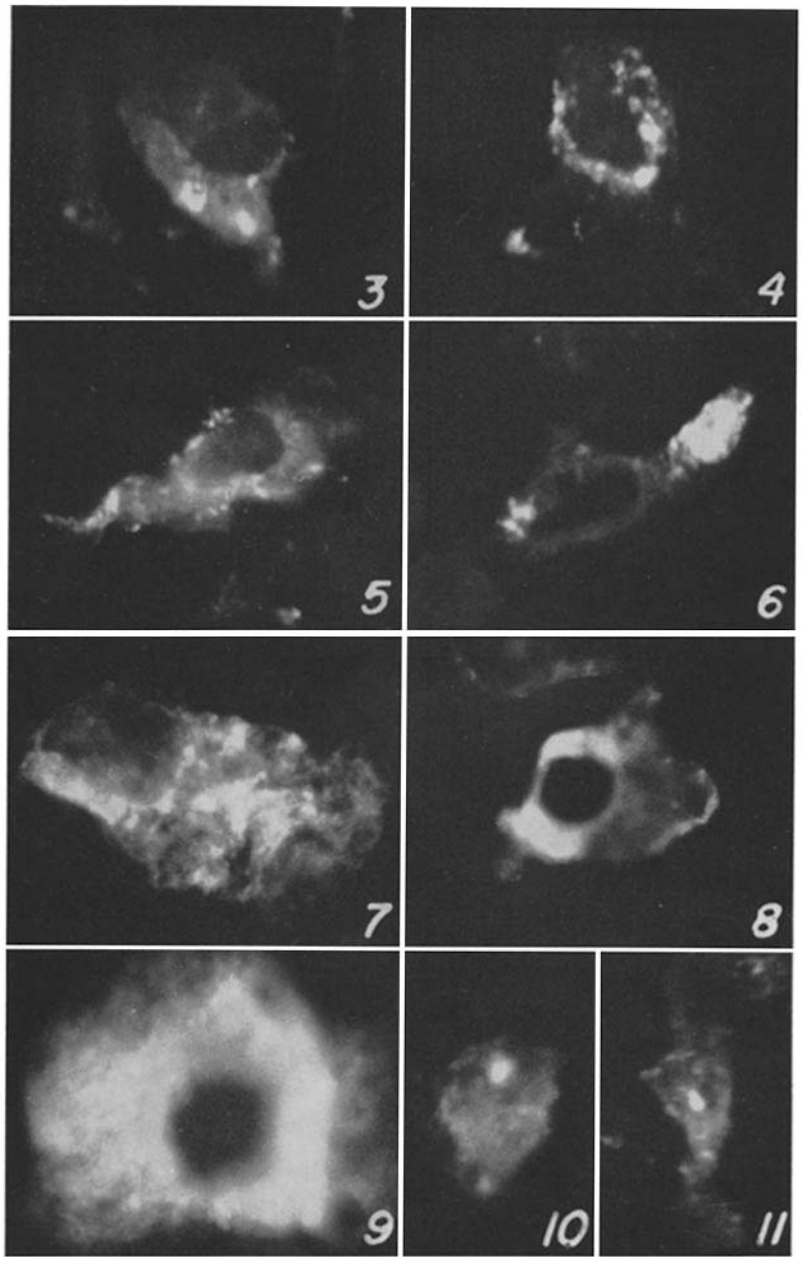
FIG. 7. Wing tumor. Rous sarcoma cell with indistinctly particulate viral antigens occupying the major area of the cytoplasm. × 1500.

FIG. 8. Wing tumor. Rous sarcoma cell with homogeneous distribution of viral antigens in portion of cytoplasm. × 1500.

FIG. 9. Wing tumor. Large Rous sarcoma cell with abundance of viral antigens in cytoplasm. × 1500.

FIG. 10. Wing tumor. Rous sarcoma cell with viral antigens in eccentric oval focus in the nucleus and also in the cytoplasm. × 1000.

FIG. 11. Wing tumor. Rous sarcoma cell with viral antigens in nucleus and cytoplasm. × 1500.



(Mellors and Munroe: Rous sarcoma virus)

PLATE 83

FIG. 12. Wing muscle at 4 days after virus inoculation. Microscopic focus of Rous sarcoma cells containing viral antigens. × 560.

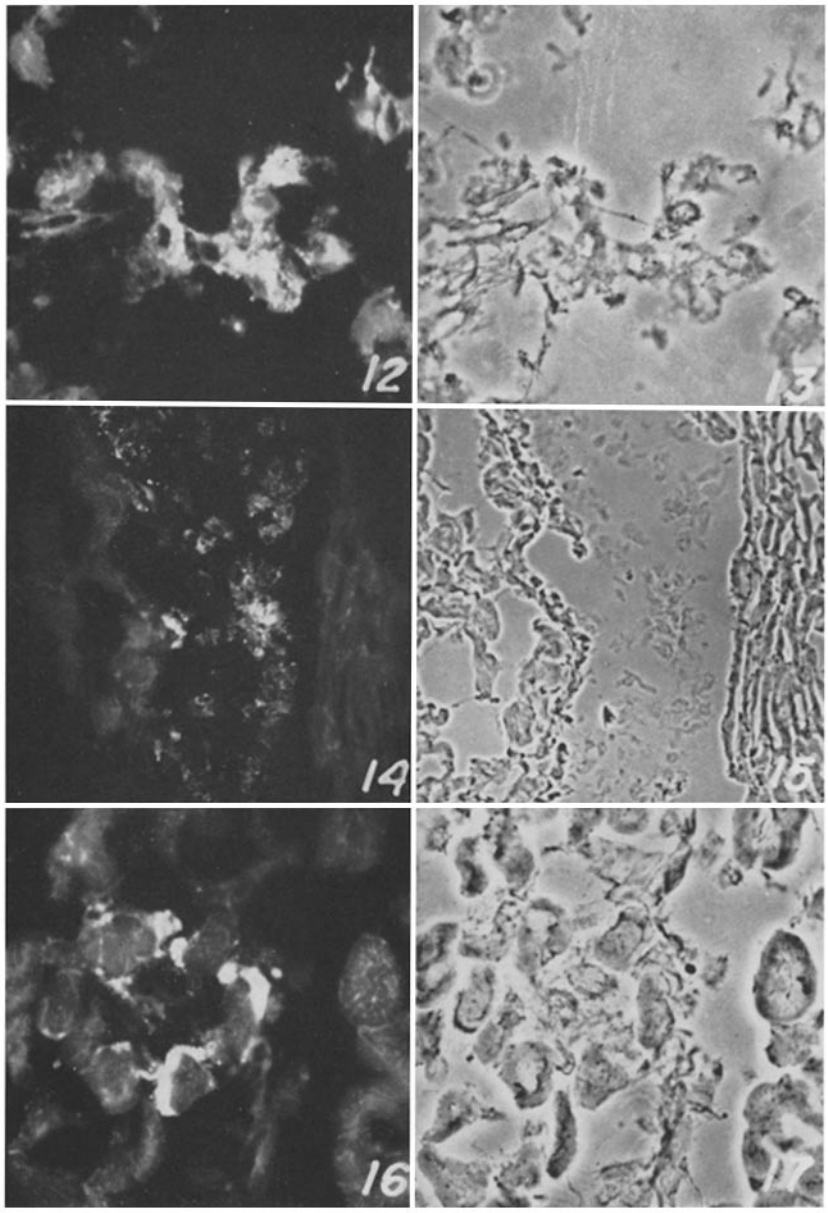
FIG. 13. Same field as Fig. 12 photographed in phase contrast. × 560.

FIG. 14. Wing muscle at 4 days after virus inoculation. In a needle track, a microscopic focus of Rous sarcoma cells containing viral antigens. × 200.

FIG. 15. Same field as Fig. 14 photographed in phase contrast. × 200.

FIG. 16. Wing muscle at 4 days after virus inoculation. Rous viral antigens localized on or near the sarcolemma of a few of the muscle fibers. × 560.

FIG. 17. Same field as Fig. 16 photographed in phase contrast. × 560.



(Mellors and Munroe: Rous sarcoma virus)

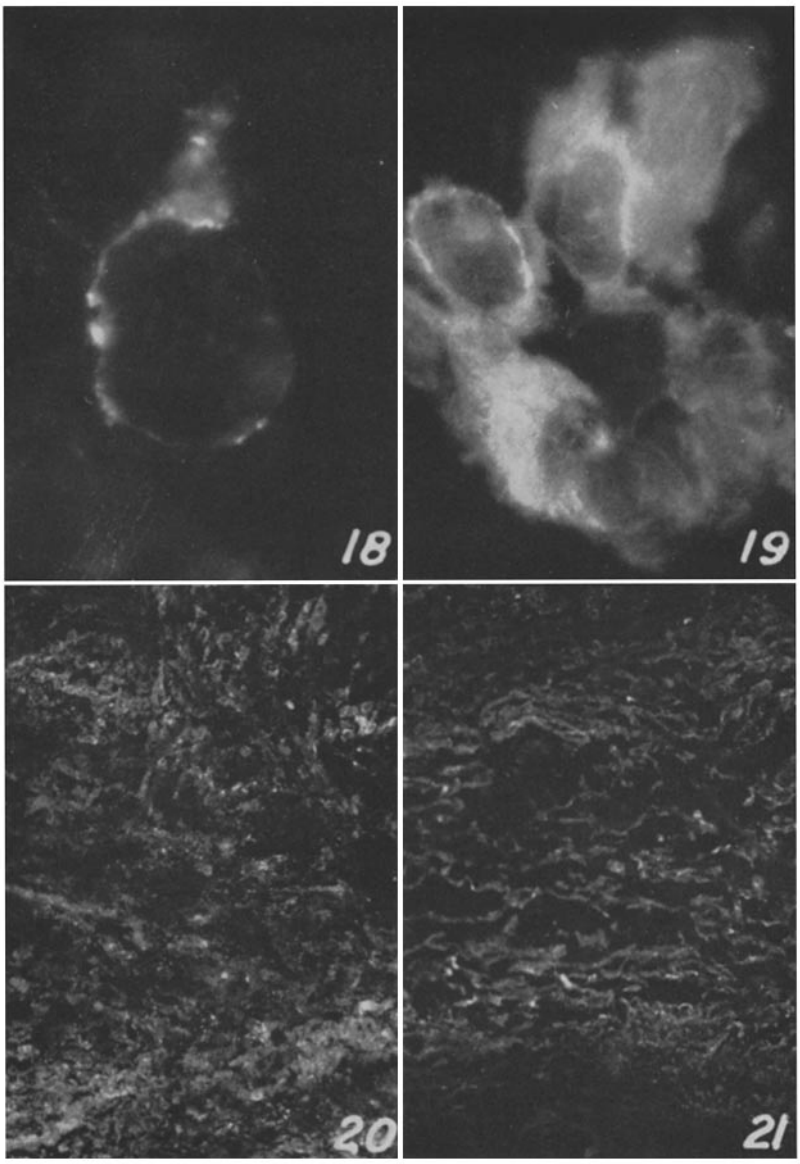
PLATE 84

FIG. 18. Wing muscle at 3 days after virus inoculation. Rous viral antigens localized as fine particulates on the sarcolemma of one muscle fiber. × 1500.

FIG. 19. Muscle in fully established wing tumor. No identifiable sarcoma cells are present in this field. Rous viral antigens localized on the sarcolemma of a few muscle fibers. × 1500.

FIG. 20. Lung tumor. Rous viral antigens localized in sarcoma cells. × 200.

FIG. 21. Chorioallantoic membrane tumor. Rous viral antigens localized in cytoplasm of sarcoma cells. × 200.



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