mice, defective impulse transmission is associated with increased ACh sensitivity of the muscle membrane (*6*). Conversely, electrical stimulation of a severed but still functioning motor nerve has been reported to restrain the development of ACh hypersensitivity in denervated muscle (*7*).

As judged by the present evidence, it seems likely that the role of neurally released ACh in regulating the sensitivity of the muscle membrane is mainly, if not entirely, that of triggering the chain of events which constitutes muscle activity. This provides support for the concept (*13*) that ACh is important in mediating neurotrophic influences on muscle.

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Iodine-125–Labeled Antibody to Viral Antigens: Binding to the Surface of Virus-Infected Cells

Abstract. *The specific binding of iodine-125–labeled antibody to viral antigens can be used to detect newly synthesized viral antigens and determine the time of appearance of these antigens on the surface of infected cells. Incubation of infected cells with unlabeled antibody to viral antigens specifically blocks the attachment of labeled antibody, and by this inhibition technique the titer of unlabeled antibody to viral antigens can be calculated. The attachment of the labeled antibody to virus-infected cells offers an objective and sensitive method of detecting viral antigens and measuring antibody to virus.*

Immunofluorescence has been used to detect viral antigens in the cytoplasm and nucleus of infected cells (*1*), and membrane immunofluorescence has been used to detect viral antigens on the surface of infected cells (*2*). Although these techniques have proved useful for many virus studies, they are difficult to quantitate and are time-consuming. Moreover, interpretation is subjective and often hampered by high background activity. In theory, certain of these difficulties could be overcome by the use of isotopically labeled antibody. We now report that the binding of ^{125}I-labeled antiviral antibody to the surface of virus-infected cells could be used for detecting viral antigens and measuring antibody to virus.

Monolayers of primary rabbit kidney (PRK) cells and vaccinia virus (strain CVI-79) were prepared (*3*, *4*). Serums from rabbits hyperimmunized with vaccinia were the source of antibody to virus (*5*). The serum titers at which plaque formation was reduced by 50 percent were approximately 1:2000. Serums from unimmunized rabbits served as controls, and all serums were heated at 56°C for 30 minutes before use. Serums from immunized and control rabbits were fractionated on Sephadex G-200 (*3*), and the immunoglobulin G (IgG) fractions were labeled with ^{125}I by the chloramine-T method (*6*).

In a typical experiment, confluent monolayers containing approximately $10^{6.0}$ PRK cells were inoculated with infectious virus (virus/cell ratio, 2.0). Except where stated, the virus was allowed to adsorb to the cells for 2 hours. The inoculum then was removed, the monolayers were washed three times, and fresh medium was added. Uninfected cells served as controls. At given times thereafter, the medium was removed, and the monolayers were washed three times and then incubated for 1 hour at 37°C with 0.5 ml of a 1:64 or 1:100 dilution of [^{125}I]IgG from immunized or unimmunized animals. The medium then was aspirated and the monolayers were washed six times. The amount of ^{125}I that remained bound to the cells was determined by removing the cells and assaying them for radioactivity (Packard Auto-Gamma spectrometer). Data are expressed as the number of counts per minute (count/min) bound to the monolayers, and each number represents the average for three monolayers.

The first experiment was designed to study the time of appearance of viral antigens on the surface of vaccinia-infected cells. Within 1 hour after inoculation of the virus, three to four times more antibody had attached to infected cells than to uninfected cells (Fig. 1A). In the next several hours the binding of antibody increased gradually, but 5 hours after infection the binding of antibody began to increase precipitously. At 12 hours after infection, 33 times more antibody had attached to infected cells than to uninfected cells. In contrast, [^{125}I]IgG from unimmunized animals did not bind to either infected or uninfected cells. These results suggest that the binding of labeled antibody early in the infection was due to the attachment of antibody to virus particles that had adsorbed to the surface of cells, whereas the binding of antibody later was due to the appearance of newly synthesized viral antigens (*7*).

To study further the interaction of [^{125}I]IgG with viral antigens that had adsorbed to the surface of cells, we inoculated monolayers with partially purified virus or soluble viral antigens (*8*). Approximately 25 times more virus was used in this than in the first experiment. Lysates from uninfected cells served as controls. Within 5 minutes after addition of the virus, binding of antibody to monolayers exposed to virus was more than 6000 count/min, compared to 500 count/min on monolayers that had not been exposed to virus (Fig. 1B). The large increase in uptake of labeled antibody in the first 40 minutes, compared to the relatively small increase in the next 80 minutes, suggests that most of the virus had adsorbed to the monolayers shortly after inoculation. Soluble viral antigens also adsorbed rapidly to the monolayers but, in contrast to infectious virions, the noninfectious soluble viral antigens did

not result in the late appearance of viral antigens on the cell surface (data not shown).

In addition to detecting viral antigens, the attachment of labeled antibody to the surface of infected cells can be used to determine the titer of antibody to viral antigens. Serial twofold dilutions of [^{125}I]IgG against vaccinia were incubated with uninfected cells and cells that had been infected for 6 hours. At each dilution, significantly more labeled antibody attached to infected than to uninfected cells (Fig. 2A). Even at a dilution of 1:1024, considerably more antibody had attached to the infected (600 count/min) than to uninfected cells (25 count/min). The fact that the amount of labeled antibody that attached to the infected cells had not reached a plateau, even at the highest concentration of antibody used (1:16), suggests that viral antigens on the surface of the infected cells were in great excess over antibody to virus.

Unlabeled antiserum to vaccinia can specifically block the attachment of [^{125}I]IgG against vaccinia, and the inhibition of attachment of labeled IgG can be used to measure the concentration of unlabeled antibody (Fig. 2B). Cells that had been infected for 6 hours were incubated with serial twofold dilutions of unlabeled antiserum to vaccinia and then incubated with a standard dilution (1:64) of [^{125}I]IgG against vaccinia. As the concentration of unlabeled serum was increased from 1:2048 to 1:2, the attachment of labeled IgG to the cells was inhibited by more than 90 percent. Other experiments suggest that even greater sensitivity can be achieved if the number of infected cells is decreased and the incubation conditions are altered.

We used isotopically labeled antibody to detect cell-surface antigens induced by other viruses (*9*). Cells were infected with herpes simplex virus (HSV), influenza, or vaccinia. After 8 hours the monolayers were incubated with labeled antibody to HSV, to influenza, or to vaccinia. Antibody to HSV reacted only with cells infected with HSV. Similarly, antibody to influenza, or to vaccinia reacted only with cells infected with the respective virus. These and other results (*5*) suggest that each virus induced different antigens on the surface of infected cells and that these antigens were recognized only by antibody specific to that virus.

Our studies indicate that isotopically labeled antibody can be used to determine the time of appearance and relative amount of virus-induced antigens on the surface of infected cells (*10*). The demonstration by membrane immunofluorescence that less than 1 percent of cells infected with vaccinia were permeable to fluorescein-labeled antibody, even as late as 8 hours after infection (*11*), supports the contention that the majority of the labeled antibody had attached to antigens on the surface and not within the cytoplasm of infected cells. Moreover, our data indicate that this technique is sensitive enough to detect virion and soluble viral antigens that simply have adsorbed to the surface of cells. In comparison, membrane immunofluorescence revealed few if any viral antigens on the surface of infected cells during the early phase of the infection (that is, within 2 hours after virus inoculation

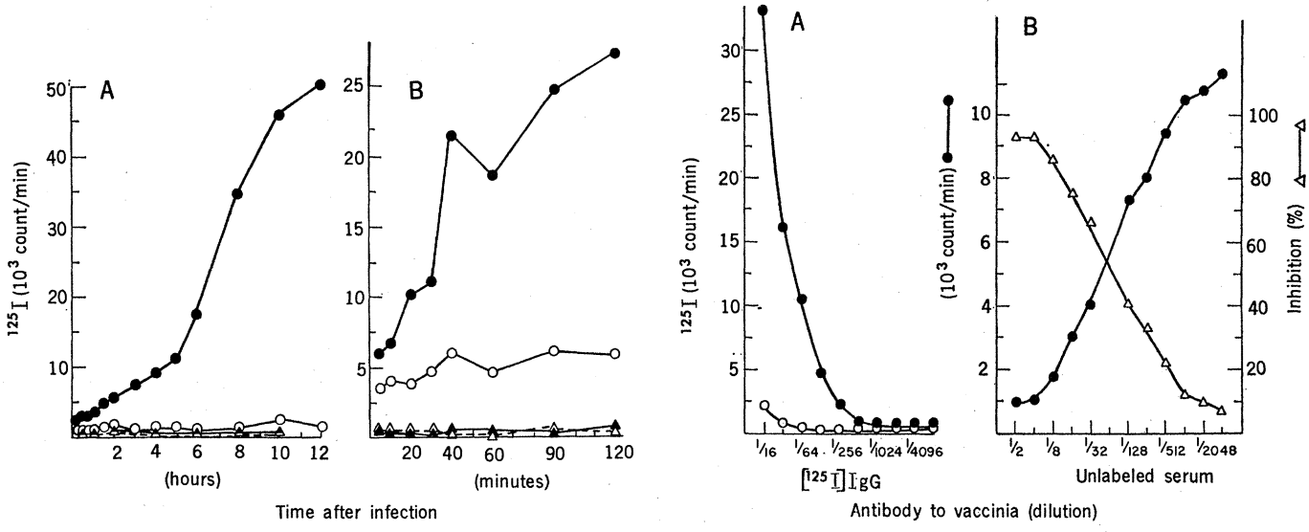

Fig. 1 (left). Time of appearance of viral antigens on the surface of infected cells. (A) Monolayers were inoculated with vaccinia (virus/cell ratio, 2.0). Virus was allowed to adsorb to the cells for various times up to 2 hours. When the inoculum was removed before 2 hours, the monolayers were washed and then treated immediately with a 1 : 64 dilution of [^{125}I]IgG. When the inoculum was removed at 2 hours the monoloyers were washed and then incubated with fresh medium for appropriate intervals before treatment with [^{125}I]IgG. Symbols are ●—●, infected cells plus [^{125}I]IgG against vaccinia; ▲—▲, infected cells plus normal [^{125}I]IgG; ○—○, uninfected cells plus [^{125}I]IgG against vaccinia; △—△, uninfected cells plus normal [^{125}I]IgG. (B) Monolayers were inoculated with partially purified vaccinia (virus to cell ratio, 50) (●—●) or with noninfectious soluble viral antigens (○—○). Lysates from uninfected cells, prepared in the same way as the partially purified virus and soluble viral antigens, served as controls (▲—▲, resuspended pellet; △—△, supernatant). At various times after inoculation the monolayers were washed and incubated with a 1 : 64 dilution of [^{125}I]IgG against vaccinia. Fig. 2 (right). (A) Binding of [^{125}I]IgG against vaccinia to cells infected with vaccinia. Cells were infected with vaccinia (virus to cell ratio, 2.0). Six hours later the cells were incubated with serial twofold dilutions of [^{125}I]IgG against vaccinia, and the amount of ^{125}I bound to the cells was determined. Symbols are ●—●, infected cells; ○—○, uninfected cells. (B) Inhibition of binding of [^{125}I]IgG against vaccinia by unlabeled antiserum to vaccinia. Cells were infected with vaccinia (virus to cell ratio, 2.0) and incubated at 37°C for 6 hours. Then the cells were incubated for 12 hours at 4°C with serial twofold dilutions of unlabeled antiserum to vaccinia. The monolayers then were incubated for 12 additional hours at 4°C with a 1 : 64 dilution of [^{125}I]IgG against vaccinia, and the amount of ^{125}I bound to the cells was determined and the percentage of inhibition was calculated.

(*11*). Other experiments showed that labeled antibody can also be used to detect vaccinia antigens within the cytoplasm of infected cells if the cells are fixed with acetone before they are incubated with [^{125}I]IgG against vaccinia (*11*).

The labeled antibody technique can also be used to quantitate the amount of antibody to viral antigens in a particular serum. This can be done either by labeling the serum immunoglobulin with ^{125}I and measuring radioactivity bound to infected cells (Fig. 2A) or by quantitating the amount of unlabeled serum required to block the attachment of a standard amount of labeled IgG (Fig. 2B). The advantage of the latter approach is that a single preparation of labeled IgG can be used to measure the concentration of specific antibody in an unknown serum. An even simpler approach might be the use of isotopically labeled antibody to immunoglobulins (anti-immunoglobulin) (*12*). We showed that the binding of labeled anti-immunoglobulin to vaccinia or HSV infected cells was directly related to the concentration of virus-specific antibody that had attached to the cells (*13*). By this procedure a single reagent (for example, labeled goat antibody to human immunoglobulins) might be used to measure the binding of virus-specific antibody to cells infected with different viruses.

The binding of labeled antibody to infected cells appears to have certain advantages over several of the widely used diagnostic procedures for detecting viral antigens and antibody to virus. For example, the binding of labeled antibody would not be affected by factors that inhibit complement fixation (anticomplimentary factors). Moreover, the radiolabeled antibody technique could be used to detect virus-specific antibody that does not fix complement. In comparison to immunofluorescence, the radiolabeled antibody technique is objective, quantitative, and unaffected by autofluorescence. Moreover, samples can be counted rapidly in an automated gamma counter. The attachment of isotopically labeled antibody to virus-infected cells also has potential advantages over immunoprecipitation procedures in which labeled viral antigens are used. These antigens are difficult to prepare and purify, but antibody to virus is plentiful and easy to label. In addition, labeled immunoglobulins that do not bind to infected cells can be rapidly separated from cell-bound antiviral antibody by simply washing the monolayer. By adapting the isotopically labeled antibody technique to microplates and by using fixed cells which are stable when stored, it should be possible to develop simple, rapid, and sensitive techniques for screening and quantitating large numbers of serums for antibody against different viruses.

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10. From the specific activity of the [^{125}I]IgG and from the number of counts bound to the monolayers (10^6 cells), it is possible to estimate the number of antibody molecules attached to each cell. In initial experiments, more than $7.5 \times 10^{5.0}$ molecules of IgG attached to cells infected for 12 hours. From this type of information an estimate can be made of the number of virus-induced antigenic sites on the surface of each cell.
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Delayed Fertilization and Chromosome Anomalies in the Hamster Embryo

Abstract. *Polyploidy and aneuploidy in hamster embryos are produced as manifestations of pregnancy wastage in surviving young when the interval between the estimated time of ovulation and copulation and hence fertilization is progressively increased. Significant delays resulted in triploidy in runted embryos, and the sporadic occurrence of mosaic aneuploidies.*

We report here the experimental production of polyploidy and aneuploidy in hamster embryos by lengthening progressively the intervals between copulation and the estimated time of ovulation, hence fertilization. At 9 hours after ovulation, sexual activity diminished and if mating did occur, it did not result in fertilization. Even as short a delay as 3 hours after the estimated time of ovulation resulted in a measurable increase of pregnancy wastage and, more importantly, in demonstrable polyploid and aneuploid chromosome patterns of developing embryos and fetuses.

Others have reviewed background work on the impact of delayed fertilization on the subsequent development of eggs in amphibians and mammals (*1–3*). Important contributions include the demonstrations of triploid embryos stemming from aging ova from dispermy (diandry) in rats and from suppression of second polar body sequences (digyny) in mice and rabbits. Not so well documented or confirmed are the occasional observations of monosomy, trisomy, and other aneuploidies (*1–3*) that develop under similar conditions and are suggestive of a cause and effect relation.

Large elements of uncertainty exist about the origin of these less frequently observed anomalies because of the number of critical variables involved, including species variation, circadian rhythms, ovulation and aging of ova, mating behavior, fertilization times, differences in preimplantation development, and chromosomal patterns. Such variables make confirmation or even comparison of rare observations difficult and inconclusive.