mucosa. They indicate, however, that the chromatographic evidence provided thus far is inadequate to demonstrate such complexes and appears to result from the formation of Fe^{59}-Versene in the experimental procedure. Versene also alters the binding of Fe^{59} to mucosal particulates and both Versene and perchloric acid influence Fe^{59} binding to soluble proteins. Thus it seems necessary to avoid these reagents in procedures designed to study mucosal iron compounds of physiological significance.‖

‖ After preparation of this manuscript a report by Charlton *et al.*(5) described similar findings which indicate that the supposed Fe^{59}-amino acid complex is a complex of iron and Versene Fe-3 Specific.

Summary. Prior chromatographic evidence implicated the participation of Fe^{59}-amino acid complexes in intestinal absorption of Fe^{59}. Present studies show that the chromatographic evidence is inadequate to demonstrate such complexes and appears to result from the formation of Fe^{59}-Versene in the experimental procedure.

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Hemadsorption and Immunofluorescence of Friend Virus in Cell Culture.* (30410)

TOYORO OSATO, EDWIN A. MIRAND, AND JAMES T. GRACE, JR.
New York State Department of Health, Roswell Park Memorial Institute, and State University of New York at Buffalo, N. Y.

We previously reported propagation of Friend virus in mouse embryo cell culture (1). This article concerns an extension of the study with special reference to a hemadsorption reaction which was correlated with viral synthesis as revealed by immunofluorescence.

Materials and methods. The origin and method of preparation of Friend virus inoculum has been detailed elsewhere(1,2). The titer of a 10% infected spleen extract was approximately $10^{5.0}ID_{50}$/ml when inoculated intraperitoneally into Ha/ICR Swiss mice 4-5 weeks old obtained from the Roswell Park Memorial Institute breeding colonies.

The cell cultures used were secondary cultures of Ha/ICR Swiss mouse embryo tissues. Coverslip cultures were prepared using Eagle's basal medium containing 10% heat-inactivated calf serum as growth medium. Eagle's medium with 2.5% heat-inactivated horse serum was used as the maintenance medium.

The monolayer coverslip cultures were inoculated with $10^{5.0}$ mouse ID_{50}/ml of virus and incubated at 37°C for 2½ hours. After thorough washing, the cultures were incubated at 37°C overnight with growth medium followed by maintenance medium.

The indirect staining method was used in the immunofluorescent investigations(1,3). At appropriate intervals coverslips were removed from Leighton tubes, washed with phosphate buffered saline (PBS), dried at room temperature and then fixed in acetone for 10 minutes. Rabbit antiserum to Friend virus was placed on the coverslips and allowed to stand for 40 minutes at 37°C. This serum was previously absorbed twice with mouse liver powder, twice with normal spleen cells, and then twice with secondary mouse embryo cells. The coverslips were then rinsed 3 times with PBS and fluorescein isothiocyanate-conjugated anti-rabbit globulin antibody (Baltimore Biological Laboratory)

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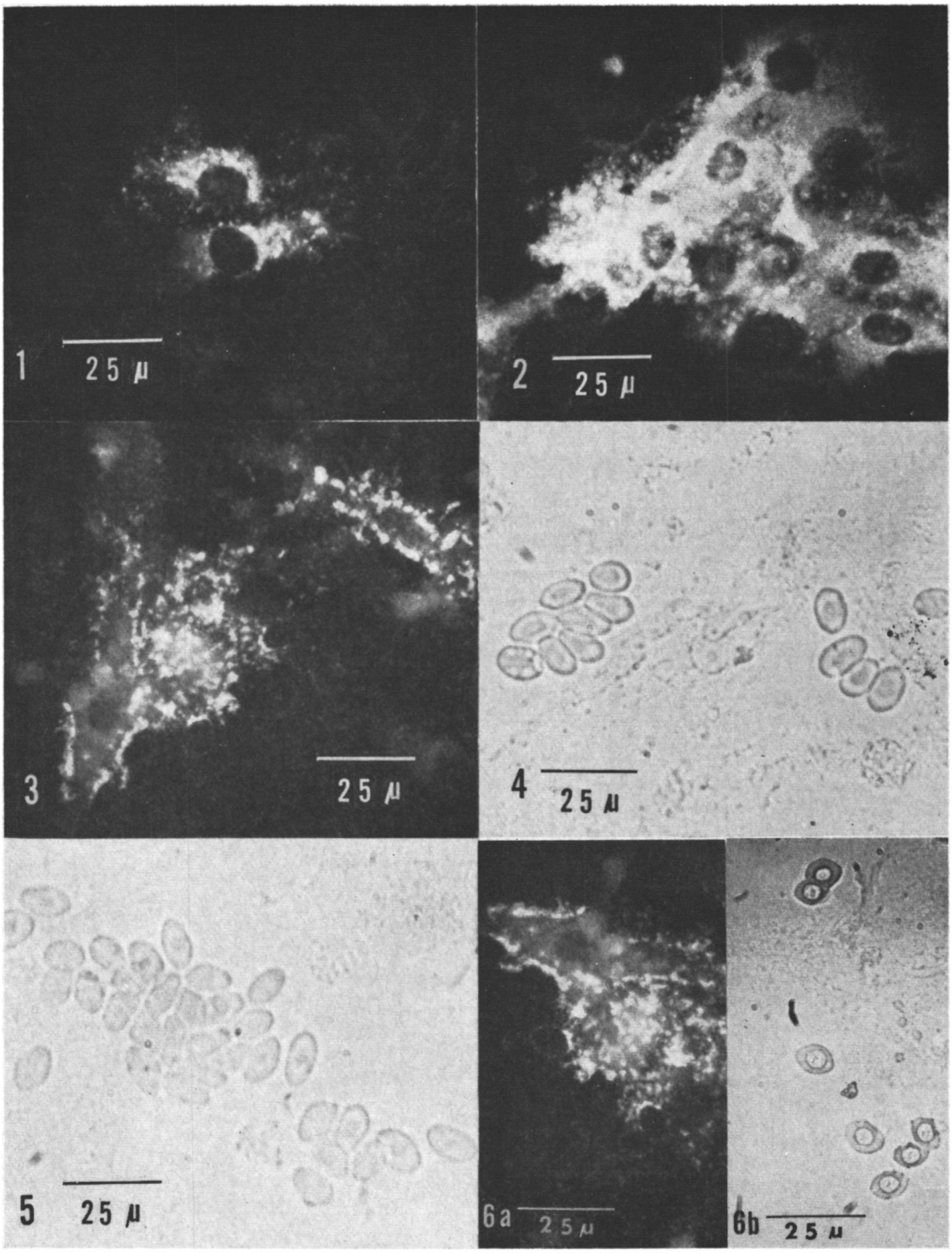

FIG. 1. Mouse embryonic cells one day after infection with Friend virus. Note strong perinuclear fluorescence and fine fluorescent granules distributed in cytoplasm.

FIG. 2. Mouse embryonic cells 6 days after infection with Friend virus. Note large fluorescent focus and fluorescence throughout cytoplasm.

FIG. 3. Mouse embryonic cells 14 days after infection with Friend virus. Note strong peripheral fluorescence.

was applied. The conjugated antibody was also previously absorbed 3 times with mouse liver powder and twice with secondary mouse embryo cells. Following additional washing with PBS, the coverslips were mounted in 20% buffered glycerol. They were examined with an American Optical Fluorolume illuminator, Model 645, with a Corning No. 5113 exciter filter. A Schott GG-1 filter served as the barrier filter and the light source was an Osram HBO-220 mercury vapor arc lamp. Photomicrographs were taken with a 35 mm Kodak camera and Anscochrome 200 color film (ASA 200). The characteristic yellow-green fluorescence was used as the indicator of viral antigen localization. Specificity of the immunofluorescence was established by several controls previously described(1).

Chicken erythrocytes were primarily used for hemadsorption studies. Following removal of maintenance medium from the Leighton tubes, the coverslips were rinsed with PBS and 1% suspension of chicken erythrocytes in cold PBS (pH 6.8) was allowed to stand in contact with the cells for 20 minutes. The coverslips were then gently washed 10 times with PBS (pH 6.8) to produce a zero background in the control cells. The cells were then examined microscopically for hemadsorption. Specificity of the positive hemadsorption reactions was established as follows: (a) Lack of hemadsorption in uninfected cells, in normal spleen extract-inoculated cells, in heat-inactivated virus-inoculated cells, and in neutralized virus-inoculated cells; (b) Ruling out the presence of certain indigenous contaminating viruses (polyoma, K, adenovirus, Sendai, PVM, and hepatitis) in our mice by examination of sera for antibodies against these viruses; (c) Lack of cytopathic effect in Friend virus-infected cell cultures and in the subsequently passaged cell cultures, based on the non-cytopathogenicity of Friend virus in mouse embryo cells(1); (d) Lack of inhibition of hemadsorption by polyoma virus specific antibody; (e) Inhibition of hemadsorption by Friend virus specific antibody.

Results. Specific immunofluorescence first appeared in a small number of virus-infected cells 24 hours after infection and was limited largely to the perinuclear area of the cytoplasm (Fig. 1). By 48 hours, about 20-30% of the cells showed specific fluorescence which involved most of the cytoplasm. With continued incubation, progressively more cells stained specifically and at 6 days about 50% of the cells were involved, forming large discrete foci (Fig. 2). At 10 days after infection, the fluorescence began to increase in intensity at the cell periphery and at 14 days there was bright fluorescence at the cell surface with only a minimal amount inside the cell (Fig. 3). At this time fine fluorescent filaments could be observed to protrude from the cell membrane. At no time was there evidence of specific nuclear fluorescence in the infected cells.

Specific hemadsorption was first noted about a week after infection (Fig. 4). The hemadsorbing cells were initially arranged in discrete foci and corresponded to the foci showing specific immunofluorescence. As the cells were incubated longer, progressively more cells adsorbed erythrocytes specifically. Fig. 5 shows cells which were infected 14 days previously. The hemadsorption positive cells are still clustered in larger discrete foci and now account for about 20-30% of the total cell population.

In some experiments infected cells were studied concomitantly by immunofluorescence and hemadsorption. Erythrocytes were added to cells and the cultures studied by immunofluorescent staining. The cells which showed hemadsorption revealed viral antigen in the cytoplasm and at the cell surface. Fig. 6a and 6b show the same microscopic field and demonstrate the presence of viral antigen and

FIG. 4. Mouse embryonic cells 7 days after infection with Friend virus. Note hemadsorption positive focus.

FIG. 5. Mouse embryonic cells 14 days after infection with Friend virus. Note large hemadsorption positive focus.

FIG. 6. Several mouse embryonic cells 14 days after infection with Friend virus. (a) Immunofluorescence; (b) Hemadsorption (photographed after immunofluorescent staining). Note attachment of erythrocytes to the same surfaces of Friend virus infected cells.

adsorbed erythrocytes on the same cell surface.

Discussion. These findings indicate our earlier observations of the *in vitro* growth of Friend virus(1) are clearly confirmed with the additional evidence that a virus-induced modification of the infected cell surface occurs which resembles myxovirus infections (4-6).

The results of immunofluorescent studies obtained here were basically similar to those of our earlier observations(1). However, viral synthesis progressed much faster as a result of a higher multiplicity of infection; thus, bright fluorescence was observed at the cell surface 10 days after infection. We could not observe such a fluorescence in our earlier studies in which virus growth was very slow due to the use of a low virus inoculum. Moreover, it was difficult to keep infected coverslip cultures intact for a month. The deep fluorescent border from which fine threadlike fluorescent projections reach out into the surroundings, as illustrated in Fig. 3, is characteristic of the process of viral maturation and release as in myxovirus infections(7,8). In fact, culture medium after 10 days of the viral infection showed a high leukemogenic activity(9).

In view of these observations and a striking resemblance of growth characteristics of Friend virus to those of parainfluenza virus (1), it seems interesting that the Friend virus-infected cells absorbed erythrocytes. Hemagglutination by Friend virus was previously reported in which erythrocytes from various species were equally effective at 4°C, at room temperature, and at 37°C and at pH 5.5-5.6 (10). In our study, in addition to chicken erythrocytes, mouse and guinea pig erythrocytes were used. Like chick erythrocytes, mouse erythrocytes were most sensitive; however, occasionally non-specific adsorption with mouse erythrocytes was observed. Guinea pig erythrocytes seemed less sensitive than chicken erythrocytes. Human type O erythrocytes were not adsorbed.

Although there were striking resemblances of growth characteristics of Friend virus to myxoviruses, Friend virus-infected cells seemed to absorb erythrocytes weakly even at 4°C and at pH 6.8. Erythrocytes detached more easily from infected cells at room temperature and at pH 7.2 conditions, but with myxovirus infections at these conditions hemadsorption was still stable(11). As described here, approximately 50% of the cells were specifically fluoresced; however, 20-30% of the cells adsorbed erythrocytes. This may be due partly to the weak binding of erythrocytes by infected cells.

The specific fluorescence involved most of the cytoplasm by the second day, whereas hemadsorption positive cells first appeared a week after infection. This indicates a slow growth characteristic of Friend virus as previously suggested(1). Considerable amounts of viral matrix substances which react with specific viral antibody are synthesized rapidly, while relatively small amounts of viral hemagglutinins are produced and liberated slowly. Relative insensitivity of hemadsorption reaction may be due largely to these circumstances. In spite of demonstrating hemadsorption reaction, infected culture fluids showed no hemagglutination reaction.

Rapid titration of Friend virus and viral antibody has been developed recently *in vivo* (12,13). Our results suggested a possibility that the virus and viral antibody may rapidly and precisely be titrated *in vitro* by using either immunofluorescent or hemadsorption reaction.

Summary. *In vitro* propagation of Friend virus in mouse embryo tissue cultures has been observed by immunofluorescence and hemadsorption. Hemadsorption of chick erythrocytes was demonstrated. Parallel studies with immunofluorescence showed that the appearance of hemadsorption coincided with a build-up of viral antigen at the cell surface and in the cytoplasm. Even though no gross changes were observed in the infected cells, a virus-induced modification of the Friend virus infected cell surface was indicated. Hemadsorption can now be used for quantitation of the virus in culture, thus circumventing the cumbersome bioassay in mice.

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Growth Hormone Releasing Factor of a Guinea-Pig Hypothalamic Extract: Its Activity in Guinea Pig and Rat.* (30411)

E. MÜLLER, A. PECILE (Introduced by G. Pozza)
Department of Pharmacology, University of Milan, Milan, Italy

The involvement of central nervous system in the secretion of pituitary trophic hormones has obtained wide experimental support particularly for corticotropin, thyrotropin and gonadotropins(1-3).

Only recently evidence of the existence of a nervous regulation also for growth hormone secretion has been accumulated. Growth is poorly supported in hypophysectomized animals by pituitaries grafted in the kidney capsule(4,5); conversely it can continue almost normally if the pituitary is transplanted in the hypophysiotropic area of the hypothalamus(6). Rats with experimentally produced hypothalamic lesions fail to grow(7). A growth hormone releasing activity of hypothalamic extracts has been demonstrated in the rat by the experiments *in vitro* of Deuben and Meites(8) and by the *in vivo* studies of Pecile *et al*(9).

The existence of a nervous control of growth hormone secretion in the rat has prompted us to investigate whether growth hormone releasing activity is present also in other animals. Our aim was to provide not only new and larger evidence of the physiological role of nervous regulation in growth processes, but also to evaluate if and to what extent, growth hormone releasing activity of the hypothalamus of an animal might reproduce its biological effect in animals of different species. Thus in the present work growth hormone releasing activity of guinea-pig hypothalamic extracts was investigated by injecting the extracts in guinea pigs as well as in rats.

Materials and methods. Thirty-day-old female guinea pigs were used. The stalk median eminence region (SME) and the cerebral cortex (CC) of 80 guinea pigs were removed, weighed, pooled and homogenized with 0.1 N HCl. The diluted extract was centrifuged at 3,000 rpm for 15 minutes at 4°C. The pH of the supernatant was adjusted to 7.4 by addition of $NaHCO_3$. The final volume was made up so that the acid extract from 5 mg of guinea-pig SME or CC was contained in 0.2 ml of medium. As recipient animals, groups of 15 (30-day-old) female guinea pigs and (30-day-old) Sprague-Dawley female rats were used. All recipient animals were injected in the carotid with 5 mg/100 g bw of SME or CC. Fifteen minutes after injection recipient guinea pigs and rats were killed by decapitation; the pituitaries of each experimental group of ani-

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