varies considerably throughout the length of the tract. Moreover, measuring potassium escape from isolated strips as an index of drug activity can be very misleading, particularly since it has been found that the amount escaping is a function of the amount already available in the tissue. It would appear more realistic to evaluate the drug effect on the isolated intestinal strips in terms of the ratio of available tissue potassium and escape potassium.

Summary. 1. Tissue potassium concentration in the small intestine of the guinea pig differs appreciably along the length of the tract. 2. The escape of potassium from isolate strips is a function of the available tissue potassium.

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Interaction of Newcastle Disease Virus with Megakaryocytes in Cell Cultures of Guinea Pig Bone Marrow.* (28771)

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Influenza and Newcastle Disease Virus (NDV), members of the myxoviruses, agglutinate red blood cells(1), leukocytes(2) and blood platelets(3). NDV is also hemolytic(1). We have suggested(3) that the platelets and red blood cells have similar surface receptors for myxoviruses. Electron micrographs indicated that following adsorption myxoviruses were incorporated into platelets(4). Both influenza and NDV affect the coagulation and clot retraction activities of blood platelets(5), but only NDV alters the shape of the platelets, presumably because of its lytic properties(6). Since platelets are fragments of megakaryocytes, the interaction of NDV with megakaryocytes seemed worthy of investigation.

Materials and methods. Bone marrow suspensions. Bone marrow was obtained from femurs of guinea pigs weighing 300-350 g. After dissection of the femurs from the surrounding tissue they were cut lengthwise, and the marrow aseptically removed into 17 ml of Tyrode solution containing antibiotics (250 units/ml penicillin and 125 μg/ml streptomycin) in a siliconized tube. The tube was gently shaken for 2 minutes, incubated for 15 minutes at 37°C, the Tyrode supernate changed and the marrow tissue was agitated by means of a syringe to a homogenous suspension. The suspension was washed in Tyrode by centrifugation at 4°C for 5 minutes at 800 rpm. Half of the supernate was discarded, the remainder gently shaken, 8.5 ml of Tyrode added and the cells washed again for 5 minutes at 800 rpm. The cells were then suspended in 2 ml of the remaining supernate; it contained 0.3-0.4% of megakaryocytes. It was adjusted to 2×10^7 total cells/ml.

Cultures. Bone marrow cells were diluted 1:20 in nutrient medium and cultures prepared on cover glasses attached to aluminum slides(7). The nutrient medium consisted of 2/3 Tyrode's solution with antibiotics and 1/3 chicken plasma (with heparin as anticoagulant).

Virus. NDV and control allantoic fluids were prepared as previously described(5). 0.25 ml of virus suspension (hemagglutinat-

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ing titer 1:2000), or allantoic fluid, were added to 0.25 ml of the cell suspension in siliconized test tubes. After incubation at 4°C for 30 minutes the supernate was removed, the cells were washed, and diluted 1/10 in Tyrode for smear slides or 1/10 in nutrient medium for preparation of cultures. The supernate was titrated by hemagglutination. From this titer the percentage of adsorbed virus was calculated.

The fluorescent antibody staining the preparation of NDV-infected bone marrow cell cultures was as follows: 2×10^6 cell/ml in culture medium were seeded in slide cultures and incubated for 18 hours at 37°C. The supernate was removed and 0.1 ml of NDV (or control allantoic fluid) was added; adsorption proceeded for 30 minutes at 4°C, whereupon the supernate with virus or allantoic fluid was removed. The culture was then washed with Tyrode solution, fresh nutrient medium was added and the cultures were incubated for 1, 2, 3, 4, and 15 hours at 37°C. At the end of these incubation periods the cover glass with the cells was washed in Tyrode, dried, fixed and stained with fluorescent antibodies.

Preparation of fluorescent antibody. NDV antiserum was prepared in guinea pigs: 3 weekly intramuscular injections of NDV with adjuvant were given. The blood was collected 2 weeks after the last injection. The separated serum had a hemagglutination-inhibition (HAI) titer of 1:640. Globulin was prepared from the serum by standard procedure(8), the final solution was adjusted to 2.5% protein and this was coupled to fluorescein isothiocyanate at the rate of 1 mg of dye to 17 mg of protein. The conjugate was diluted to give 1% protein content. The final solution was adsorbed once with guinea pig liver powder and twice with guinea pig bone marrow powder (100 mg/ml). *Fluorescent antibody staining.* Dry slides with bone marrow cultures or suspensions were fixed with acetone for 20 minutes at room temperature, washed for 15 minutes in phosphate buffered saline (pH = 7.2), and dried at room temperature. The slides were then stained with fluorescein conjugated anti-NDV globulin (0.05 ml/slide) for 30 minutes in a moist chamber at room temperature. The slides were then washed 3 times for 30 minutes in PBS, dried and mounted for microscopic observation(9).

Results. Adsorption of NDV on bone marrow cells. Following incubation of the bone marrow cell suspension with NDV at 4°C for 30 minutes, about 60% of the virus disappeared from the supernate. Fluorescent antibody staining of such suspension at the end of the adsorption period showed fluorescent material adsorbed on the surface of the bone marrow cells and megakaryocytes.

Effect of NDV on thrombopoiesis by megakaryocytes in tissue culture. Following incubation of bone marrow cells with NDV or control allantoic fluid the development of megakaryocytes was observed and photographed in phase contrast, starting at 24 hours after setting up the cultures (Fig. 1). Young megakaryocytes treated with uninfected allantoic fluid (Fig. 1 a-d) were round or oval, their margins were refractile and the nucleus well demarcated. After 48 hours of incubation in culture, the plasma became granular, the contours lost their refractibility and their round shape. A heavy granulation appeared and few platelets emerged at the margins of the megakaryocytes. After 72 hours of incubation the megakaryocytes lost completely their regular shape, and masses of platelets appeared next to them. After 96 hours of incubation only remnants of megakaryocytes were discernible; all cells turned into aggregates of platelets.

In contrast to this course of events, cultures of bone marrow cells infected with NDV (Fig. 1 e-g) showed morphological changes within 24 hours of incubation. The megakaryocytes in an infected culture (Fig. 1 e) were round and only a part of their margin was refractile; the granulation in the cytoplasm was not even. Fig. 1 f shows progressive damage of megakaryocytes 48 hours after infection. After 72 hours of incubation only ghosts of megakaryocytes, containing fine granulation, remained (Fig. 1 g). In 20 experiments detailed observation of 100 infected and 80 uninfected megakaryocytes was made. Only about 20% of the infected megakaryocytes released a few platelets,

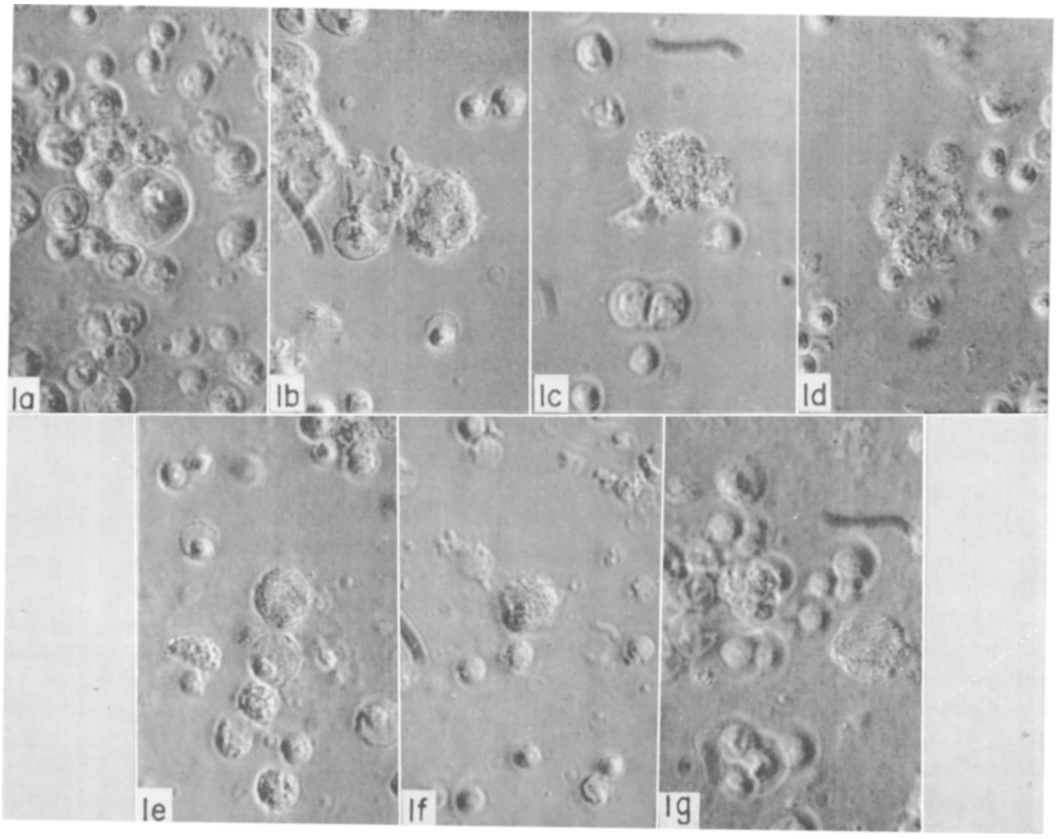

FIG. 1. Interaction of NDV with megakaryocytes in guinea pig bone marrow cell culture. Phase contrast × 325. a-d: Development of megakaryocyte and thrombopoiesis in uninfected control culture. a, 24 hr; b, 48 hr; c, 72 hr; d, 96 hr. e-g: Megakaryocyte from infected bone marrow cell culture. e, 24 hr; f, 48 hr; g, 72 hr.

while in the control cultures 75% of megakaryocytes developed well and showed normal thrombopoiesis.

Fluorescent antibody studies. Bone marrow cells incubated with NDV at 4°C for 30 minutes showed a bright ring of fluorescence located at the periphery of the cells. No intracellular staining was observed in cells immediately at the end of adsorption period. Control suspensions incubated with uninfected allantoic fluid were not stained. In bone marrow cells incubated in slide cultures following infection with NDV, no antigen was detectable by fluorescent antibody staining for 1-3 hours after setting up the cultures. First traces of viral antigen appeared only after 4 hours. The fluorescence was finely granular. After 15 hours of incubation in culture all cytoplasm of megakaryocytes was filled with dense fluorescent granules (Fig. 2). The nucleus was not stained at 4 and 15 hours of incubation. Control cultures were negative.

Discussion. NDV adsorbs to guinea pig bone marrow cells, including megakaryocytes, similarly to its adsorption to blood cells. The morphological sequence demonstrated in Fig. 1 e-g suggests that infection with NDV interferes with the thrombopoietic function of megakaryocytes. This interference may be due to destruction of the granules that precede the appearance of platelets(10), or to inhibition of platelet release from the megakaryocytes.

The presence of viral antigen has been demonstrated in this study by fluorescent antibody staining. It is not known whether this viral antigen represents infectious NDV

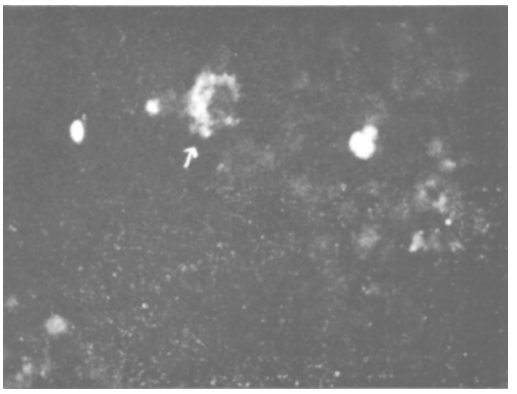

FIG. 2. NDV-infected megakaryocyte in guinea pig bone marrow cell culture after 15 hr incubation. Fluorescent antibody staining × 128.

or incomplete virus; megakaryocytes comprise only a small fraction of the total number of cells in bone marrow culture, therefore it is very difficult to determine by infectivity counts whether the virus multiplied in the megakaryocytes. It is supposed that the antigen found in megakaryocytes in cell cultures is newly formed since the infection with NDV is followed by an eclipse period of about 4 hours when no antigen is demonstrable inside the cells in infected cultures. In the study of Prince and Ginsberg(11), infection of Ehrlich ascites tumor cells with NDV also resulted in formation of new antigen following an eclipse period. They showed the viral antigen to be associated with incomplete NDV.

Whether the suppression of thrombopoiesis by NDV might have a bearing on the mechanism of thrombocytopenia occurring in virus infections is not known.

Summary. Incubation of bone marrow cells with NDV results in morphological changes in megakaryocytes and suppression of thrombopoiesis. Fluorescent antibody staining of infected megakaryocytes in cell culture shows adsorption of NDV, an eclipse period, and formation of new viral antigen starting at 4 hours after infection.

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The Blood Calcium Lowering Effect of Hydrocortisone in Parathyroidectomized Rats. (28772)

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Previously it has been observed that hydrocortisone (Cpd. F) lowers the blood Ca in hypoparathyroid patients(1) and in parathyroidextomized (PTX) animals of several species(2,3). The same treatment has no effect on the blood Ca of patients or animals with functioning parathyroids. Since the parathyroids are known to maintain Ca-homeostasis by secreting more parathormone (PTH) in response to lowered Ca concentrations, we have suggested that compensatory

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