inoculation or adrenalectomy again indicates that some relationship exists between the two treatments. The decreased mortality in adrenalectomized mice receiving a histamine challenge of 4.0 mg in comparison with those receiving 1.0 mg is noteworthy. It is similar to the biphasic response reported by Munoz *et al* for *B. pertussis*-treated(8) and adrenalectomized mice(5), challenged with 8.0 mg histamine as opposed to those receiving only 0.5 to 2.0 mg. It suggests that mice may possess an inactive protective mechanism which can be activated with the proper stimulus. Possibly, extra-medullary chromaffin tissues may form part of this mechanism. It can be postulated that there are quantitative differences in the functional capability of these tissues among strains of mice and among ages within the same strain. The observations made by Gauthier *et al*(6) that one strain of mice failed to become sensitized to histamine after adrenalectomy, while becoming sensitive when inoculated with pertussis vaccine, might be explained on the presence, in that strain, of well developed extra-medullary chromaffin tissue. This is the most logical explanation at present.

*Summary.* The Rocky Mountain Laboratory strain of mouse which is resistant to the effect of histamine sensitizing factor from *Bordetella pertussis* (HSF) at 3 to 5 weeks of age became susceptible to this effect by the age of 8 weeks. The $LD_{50}$ of histamine in HSF inoculated 3-week-old mice was 6.8 mg per mouse (524 mg/kg), while in 9-week-old mice it was less than 0.5 mg per mouse ($<$18.5 mg/kg). The $LD_{50}$ of histamine in the 3-week-old control mice was 18.4 mg per mouse (1417 mg/kg), while in 9-week-old animals it was 19.3 mg per mouse (715 mg/kg). Development of histamine sensitivity after adrenalectomy was also found to be affected by age: $LD_{50}$ of histamine for 4- to 5-week-old mice was 11.3 mg per mouse while it was only 0.7 mg for 10- to 12-week-old mice. Some physiological change related to aging must occur in these mice to bring about the change in susceptibility to histamine sensitization after HSF inoculation. This change appears to be related to the adrenal hormones, but the relationship is not clearly understood.

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## Hemadsorption to Sections of Virus-Infected Tissues. (29593)

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Some viruses cause red blood cells (RBC) to adhere to the surface of infected cells in tissue cultures. An extension of this hemadsorption phenomenon to microtome sections of infected tissues would possess certain advantages both for diagnostic and investigative use. This paper describes the application of a slide-chamber technique to this problem, using tissues infected with the viruses of vaccinia, Newcastle disease (NDV), and influenza.

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*Materials and methods.* Vaccinia infected tissues were prepared as follows: Vaccinia virus originally derived from commercial calf lymph vaccine (Lederle) was maintained in serial passage in primary cultures of Rhesus monkey kidney cells. Twelve-day-old chicken embryos were inoculated by the chorioallantoic route with the virus in a dose determined by preliminary titration to be capable of producing nearly confluent lesions of the chorioallantoic membrane within 72 hours. The embryos surviving this dose after 3 days of incubation at 37°C were sacrificed and their livers were harvested. These livers were studded with yellow pocks and contained $10^{7.8}$ tissue culture $ID_{50}$ per gram wet weight of tissue, as determined by titration in primary cultures of Rhesus kidney cells.

NDV-infected tissues were prepared using virus originally derived from the VIC strain and maintained in this laboratory by serial passage in the chicken embryo. One-tenth ml of a 1:100 dilution of virus-containing allantoic fluid was inoculated into the allantoic cavities of 10-day-old chicken embryos. After 2 days' incubation at 37°C, the surviving embryos were sacrificed. They showed generalized petechial lesions. Their livers were harvested and aliquots were titered in primary cultures of Rhesus kidney cells. By this method, the tissue was found to contain $10^{7.5}$ tissue culture $ID_{50}$ per gram wet weight.

Influenza infected tissues were prepared in the following manner: An egg-adapted PR-8 strain of Influenza A was maintained by serial propagation in the allantoic cavities of chicken embryos. A 1:10 dilution of virus-containing allantoic fluid was made, and 0.05 ml was given intranasally to lightly anesthetized adult Swiss albino mice. After 4 days the surviving mice were sick with ruffled fur and labored respiration. They were sacrificed and their lungs were harvested. Aliquots of lung tissue were titered by allantoic inoculation in chicken embryos. Using the development of hemagglutinating activity for chicken RBC as the criterion of infection, it was established that the infected tissue contained more than $10^{4.7}$ egg $ID_{50}$ per gram wet weight of tissue.

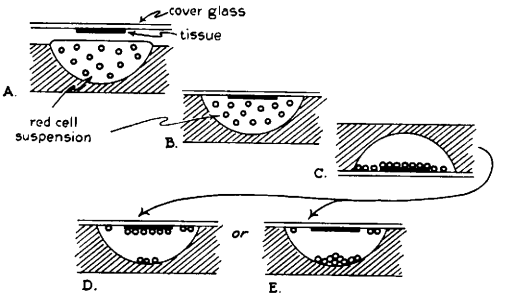


FIG. 1. Schematic diagram of slide-chamber procedure. Explanation in text.

All tissues were frozen immediately after harvesting and stored at —70°C. Prior to use, they were sectioned at 4-10 $\mu$ intervals using an International freezing microtome. The sections were then attached to 22 × 40 mm coverslips by thawing and air drying. Then they were stored at —70°C until used. It was possible to store the sectioned tissues in this manner for up to 2 weeks without affecting the reliability of results.

Sheep RBC used in the test were obtained commercially in Alsever's solution. Blood from other species was taken from apparently healthy donors, using either ACD solution or heparin. Prior to use, the RBC were washed 3 times in 0.9% saline and then diluted in phosphate buffered saline, pH 7.4 to a 1% concentration.

The method for demonstration of hemadsorption on tissue sections was adapted from that devised by Tönder, Milgrom and Witebsky(1) for the mixed agglutination reaction with tissue sections. Thick microculture slides with concave wells about 2 cm in diameter and 0.3 ml in volume were prepared by applying a small amount of petrolatum around their wells and then overfilling the wells with the RBC suspension. The coverslips with tissue sections were then placed over the wells so that the sections contacted the RBC suspensions without entrapping air bubbles (Fig. 1A and 1B). The assembled apparatus was then inverted and incubated in a moist chamber for 45 minutes at 37°C. During this time the RBC sedimented onto the coverslip and the attached tissue section (Fig. 1C). Then the apparatus was reverted and incubation continued another 15 minutes at 37°C. When

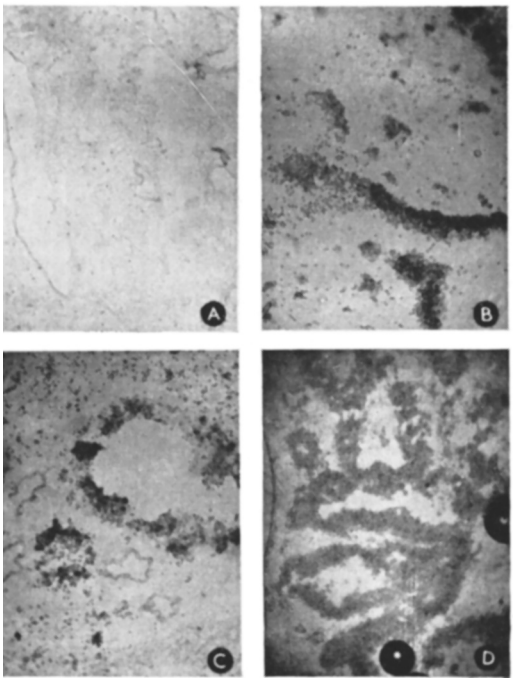

FIG. 2. Adsorption of Human "O" RBC to various tissues. A. Normal tissue. A negative pattern. B. NDV-infected chicken embryo liver. A positive pattern, showing focal aggregation of RBC. C. Influenza-infected mouse lung. Focal aggregation is strongest about the bronchioles. D. Normal mouse spleen. Strong hemadsorption in serpiginous patterns. (Original magnification: A, B and C, 105 ×; D, 35 ×.)

hemadsorption occurred, the RBC remained attached to the tissue section (Fig. 1D). In the absence of hemadsorption, the cells settled to the bottom of the chamber (Fig. 1E).

The slides were examined microscopically at low magnification (Fig. 2). Adherence of cells to wrinkled or folded areas of the tissue was ignored, as such areas displayed nonspecific adherence. Red cells often remained attached to the peripheral areas of the coverslip but, in properly prepared slides, the area immediately surrounding the tissue was clear; lack of this finding indicated the need for further incubation before the slide could be evaluated. The degree of hemadsorption was graded from negative to three plus, using the scheme described by Tönder, Milgrom and Witebsky(1).

For hemadsorption inhibition, immune sera were prepared in rabbits. The vaccinia antigen was virus-containing fluid from hamster kidney cell cultures; the NDV antigen was virus-containing allantoic fluid. Immunization was accomplished by intradermal inoculations of antigen in Freund adjuvant combined with intravenous injections. The antisera were diluted 1:5 and repeatedly absorbed with powdered dried stroma of chicken RBC. After absorption, further serum dilutions were prepared. Tissue sections on coverslips were overlaid with a drop of antiserum at the appropriate dilution and then were incubated in a moist chamber at 37°C for one hour. The sections were then washed in 0.9% saline before being used in the manner described above for the slide chamber hemadsorption technique.

In performing hemagglutinin titrations, round-bottomed 13 mm tubes were used. Serial 2-fold dilutions of virus-containing allantoic or tissue culture fluids were prepared in 0.9% saline. To a volume of 0.2 ml per tube was added 0.1 ml of a 1% suspension of the appropriate RBC and the tubes were incubated at 37°C. This temperature was chosen in order to allow comparison of the results with those of the hemadsorption tests. Care was taken to read the agglutination patterns(2) as soon as they had formed. The highest dilution of the stock virus suspension showing a definitely positive pattern was taken as the hemagglutinating titer of the virus.

*Results. Vaccinia.* (Table I). RBC not agglutinable by vaccinia in the test tube failed to hemadsorb to infected tissues in the slide test. However, when the test was performed using RBC from a chicken whose

TABLE I. Vaccinia. Comparison of viral hemagglutination with hemadsorption to infected tissue sections.

| Origin of RBC | Reciprocal of test tube HA titer | Hemadsorption in slide test: Normal chick embryo liver | Hemadsorption in slide test: Vaccinia-infected chick embryo liver |
|---|---|---|---|
| Man | <10 | — | — |
| Sheep | <10 | — | — |
| Mouse | <10 | — | — |
| Guinea pig | <10 | — | — |
| Dog | <10 | — | — |
| Chicken 1 | 20 | — | ++ |
| " 2 | <10 | — | — |

TABLE II. NDV. Comparison of viral hemagglutination with hemadsorption to infected tissue sections.

| Origin of RBC | Reciprocal of test tube HA titer | Hemadsorption in slide test | |
|---|---|---|---|
| | | Normal chick embryo liver | NDV-infected chick embryo liver |
| Man | 40 | — | ++ |
| Sheep | <10 | — | — |
| Mouse | 80 | — | ++ |
| Guinea pig | 160 | — | ++ |
| Dog | 160 | — | + |
| Chicken | 160 | — | ++ |

RBC were agglutinable by vaccinia, hemadsorption was noted. The positive reaction consisted of massive agglutinates covering almost the entire tissue. Prolonged incubation of the slide after reversion to the upright position, or jarring or tapping the slide prior to reading sometimes resulted in sedimentation of the large agglutinates, producing a less strongly positive pattern. Nevertheless, a sufficient number of scattered RBC remained adherent to the tissue to allow interpretation of the result as positive.

*NDV.* (Table II and Fig. 2B). Sheep RBC did not hemadsorb to infected tissue sections, nor were they agglutinated by NDV at the temperature used. RBC of several other species were tested; these all were agglutinable by NDV and also hemadsorbed to the infected tissue. In positive tests, there were scattered foci of adherent RBC agglutinates. The RBC were firmly attached to the tissue and did not dislodge after several hours incubation at room temperature.

*Influenza.* (Table III and Fig. 2C). Similar patterns to those seen with NDV-infected tissue were seen with influenza-infected mouse lung tissue. The agglutinates were most massive around the bronchioles. Again, the phenomenon was limited to RBC agglutinable by the virus in question, as shown by parallel tube titrations of hemagglutinating activity and slide tests using RBC derived from various species.

TABLE III. Influenza PR-8. Comparison of viral hemagglutination with hemadsorption to infected tissue sections.

| Origin of RBC | Reciprocal of test tube HA titer | Hemadsorption in slide test | |
|---|---|---|---|
| | | Normal mouse lung | Influenza-infected mouse lung |
| Man | 160 | — | +++ |
| Rhesus monkey | <10 | — | — |
| Sheep | <10 | — | — |
| Mouse | 80 | — | ++ |
| Guinea pig | 160 | — | +++ |
| Chicken | 160 | — | +++ |

In attempts to extend the above observations to other viral infections in mice, the incidental observation was made that the spleens of some, but not of all, normal mice hemadsorbed chicken, human and sheep RBC. The adsorbing areas formed serpiginous and lobate patterns (Fig. 2D).

To study the reliability of the slide chamber technique in distinguishing various virus infections, blind tests were set up in which the observer was given normal, vaccinia-infected and NDV-infected chick embryo liver sections; he was kept unaware of the nature of the tissues. Two types of RBC were used, human and vaccinia-agglutinable chicken, and identification of the tissue was made on the basis of the species of the hemadsorbing RBC. Tissue showing hemadsorption with neither RBC species was interpreted as normal; tissue showing hemadsorption with chicken cells only was interpreted as vaccinia-infected; tissue showing hemadsorption with both RBC species was interpreted as NDV-infected. The results of the test performed in this manner indicated that the nature of the tissue could be accurately discerned by use of the hemadsorption reaction with various RBC types.

Inhibition of hemadsorption (Table IV) was tried using vaccinia and NDV-infected tissues and vaccinia-agglutinable chicken erythrocytes. Inhibition by the homologous antiserum was observed in both cases; the hemadsorption-inhibition titer of the anti-vaccinial serum was 1:40 and of the anti-NDV serum 1:80.

*Discussion.* Since the demonstration of the viral hemadsorption phenomenon in cell cultures infected with influenza(3), this phenomenon has been applied to detection of viruses in cell cultures infected with a variety of agents, including parainfluenza, vac-

TABLE IV. Inhibition of Hemadsorption to Infected Tissue Sections by Pretreatment with Antiserum.

| Serum and dilution used to pretreat tissue | | Hemadsorption after serum pretreatment A. Vaccinia-infected chick embryo liver | B. NDV-infected chick embryo liver |
|---|---|---|---|
| Normal rabbit | 1:10 | ++ | ++ |
| Anti-vaccinia | 1:10 | — | ++ |
| | 1:20 | — | ++ |
| | 1:40 | — | ++ |
| | 1:80 | ++ | ND* |
| | 1:160 | ++ | ND |
| Anti-NDV | 1:10 | ++ | — |
| | 1:20 | ++ | — |
| | 1:40 | ++ | — |
| | 1:80 | ND | — |
| | 1:160 | ND | ++ |

* Not done.

cinia(4) and measles(5). To our knowledge, this principle has not previously been applied to the demonstration of viruses in microtome sections of infected tissues. This development of the test has certain advantages. A simple method is made available to test tissues for the presence of viruses. Furthermore, the opening of infected cells by the microtome knife may reveal intracellular viruses and thus allow any hemagglutinating virus to be detected, in contrast to hemadsorption on cell cultures which apparently reveal only those viruses which form hemagglutinin on the cell surface.

*Summary.* Frozen tissues infected with vaccinia, NDV and influenza were sectioned. The sections were incubated in a slide-chamber with 1% suspensions of RBC derived from various species. Hemadsorption to infected tissue sections occurred with RBC agglutinable in the test tube by the infecting virus. Other RBC did not hemadsorb. The phenomenon could be specifically inhibited by using anti-virus sera. It was shown that the described procedure can be used as a reliable tool for detecting viral infection.

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## Effect of Quinidine on Plasma Potassium and Glucose in the Intact Dog.* (29594)

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Interest in potassium dynamics and its relationship to quinidine therapy has followed the initial observations by Huggins and Chapman(1) on decreases in plasma potassium as caused by intravenous quinidine lactate. Gertler *et al*(2) found a significant increase in canine intracellular ventricular K with the alkaloid and Armitage(3) and Holland(4) have reported on similar findings in isolated rabbit atrial preparations. During recent studies on the acute reduction of plasma potassium concentration in dogs by vivodialysis it was observed that the electrocardiographic changes seen in this type of hypokalemia were similar to those produced by quinidine. The study reported here was undertaken to ascertain if similarly low potassium levels could be obtained with quinidine sulfate and if such could be related to electrocardiographic changes in the intact dog.

*Methods.* Experiments were conducted in animals of 8-14 kg in weight which were allowed to fast for 24-36 hours and then

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