difference between the 2 groups was not statistically significant ($p=.5$). Both groups were quite hypertensive, since normal male Wistar rats of the same age have an average blood pressure of approximately 105 mm Hg.

Conclusions. These results indicate that in rats under these conditions, presence or absence of adrenal tissue has no effect on the severity of hypertension produced by desoxycorticosterone administration.

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Hemadsorption in Monkey Kidney Cell Cultures of Mycoplasma (PPLO) Recovered from Rats.* (27013)

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The hemadsorption test for viral agents in cell cultures has been widely utilized(1). This report presents evidence that a PPLO isolated from the respiratory tract of laboratory rats causes hemadsorption and cytopathic changes (CPE) in monkey kidney cell (MKC) cultures. The isolations were made during etiological investigation of chronic respiratory disease (CRD) in these animals(2).

Materials and Methods. Preparation of rat specimens. Three 2-3 month old Sprague-Dawley rats exhibiting signs of CRD were furnished by Dr. Benjamin N. Berg(3). Gross examination revealed abnormal amounts of upper airway secretion and pulmonary consolidations. Lung and tracheal tissues were ground in diluting fluid consisting of Hanks' balanced salt solution (BSS) containing 0.5% lactalbumin hydrolysate (LAH). penicillin, streptomycin and mycostatin. Supernatant fluids were collected after centrifugation and inoculated directly into cultures or stored at −60°C. Airway washings were inoculated immediately.

Cell cultures. Primary rhesus MKC cultures were initially nourished with BSS containing 0.5% LAH and 2-10% calf serum. Prior to use, cultures were refed with Eagle's basal medium (4) prepared with BSS. Penicillin, steptomycin and mycostatin were added to both media. Incubation was at 36-37°C.

Assay and detection in MKC cultures. Serial 10-fold dilutions of material to be tested were prepared in diluting fluid. Four cultures were inoculated with 0.1 ml of each dilution. Cultures were observed for CPE and were tested usually after 4 to 5 days for hemadsorption of guinea pig erythocytes according to the technic of Vogel and Shelokov(1) as modified by Chanock *et al*(5). Titers were calculated by the Reed-Muench method and expressed as the log TCD_{50}/0.1 ml of inoculum.

PPLO broth and agar cultures. Test material was inoculated into Bacto-PPLO broth(6). After 2-4 days aliquots were sub-inoculated in broth or spread over the surface of Bacto-PPLO agar(6). Both broth and agar contained 3% PPLO serum fraction(7), penicillin and streptomycin. Incubation was at 36-37°C. Agar plates were observed microscopically for typical PPLO colonies, recognition of which was periodically verified by the stained agar technic of Dienes(8). No bacterial growth

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was observed on several occasions when fluids known to contain PPLO were inoculated into thioglycollate broth.

Virus. Sendai virus strain D/Sendai/52 was obtained from Dr. Keith Jensen. Material from a MKC culture passage was used.

PPLO. A strain (HPPLO) isolated from HeLa cell cultures and passed 205 times in broth and a strain (MPPLO) isolated from mouse lung and passed 4 times in broth were furnished by Dr. John B. Nelson. The strains isolated from rats in this work will be referred to as rat PPLO (RPPLO).

Experimental. Three changes occurred in MKC cultures inoculated with respiratory tract material from each of the 3 rats. First, after one week CPE was observed which was characterized by sharply demarcated holes bordered by pyknotic and fragmented cells (Fig. 1c). Examination after staining with hematoxylin and eosin revealed numerous tiny eosinophilic, cytoplasmic granules similar to those previously noted in PPLO infected cell cultures(9). Ultimately the entire sheet was destroyed. Second, coincident with CPE, culture fluids while remaining clear became distinctly more acidic than in controls. Third, hemadsorption tests were positive 9 days after inoculation (Fig 1a). In subsequent subcultures these changes appeared within 4-5 days. It was shown in the following experiment that RPPLO can induce these effects.

A specimen from a single rat was inoculated into MKC and PPLO broth cultures. In the second broth transfer RPPLO was demonstrated on agar from which colonies were removed to initiate 10 more broth passages. Material from the final passage was RPPLO positive on agar and in MKC cultures exhibited characteristic CPE. The log infectivity titer of this material determined by hemadsorption was 6.5 per 0.1 ml. In cell cultures the transmissible effects mentioned above were evident. After 3 subcultures the log infectivity titer of the fluid determined by hemadsorption was 6.0 per 0.1 ml and RPPLO was recovered on agar from this final passage material. Fluids from uninoculated MKC cultures were passed in parallel and hemadsorption was never observed nor was RPPLO recovered.

Characteristically RPPLO continued to cause hemadsorption after repeated passage in

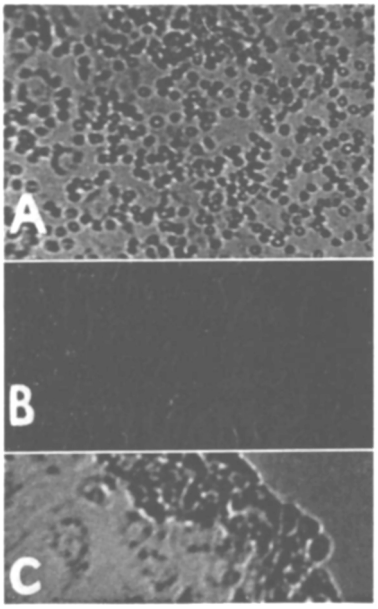

FIG. 1. a) Positive hemadsorption test in monkey kidney cell culture 4 days after inoculation with RPPLO. b) Similarly treated uninoculated culture. c) Typical RPPLO CPE prior to addition of erythrocytes (×140).

MKC cultures followed by serial transfer in broth. In an isolated case, after 8 cell and 18 broth culture passages hemadsorption became irregular.

The following experiments demonstrated that inactivated RPPLO failed to induce the observed changes in cell culture. Broth and cell culture fluids containing 10^3-10^5 TCD_{50}/0.1 ml of RPPLO were either heated for 15 minutes at 50°C, rapidly frozen and thawed 5x or exposed to kanamycin sulfate (125 μg/ml) for 48 hours at 4°C. After each treatment RPPLO could not be recovered on agar nor were CPE or hemadsorption observed following inoculation of MKC cultures. Furthermore, after 5 days of incubation fluids from these cultures were negative for RPPLO when tested on agar. Similar exposure of Sendai virus to kanamycin failed to inhibit the usual development of CPE and hemadsorption in MKC cultures, indicating that kanamycin itself did not prevent the occurrence of hemadsorption in such cultures.

Certain features of the hemadsorption test were studied. Hemadsorption became maximum 4 days following incubation and was strongest 20-30 minutes after incubation with erythrocytes at either 6°C or 36°C. Shaking

detached red cells more easily than in the case of cultures infected with Sendai virus. That hemadsorption is an accurate index of the presence of RPPLO in MKC cultures was shown in one titration when organisms were recovered only from cultures positive for hemadsorption. Rarely heavily inoculated cultures failed to hemadsorb, or did so weakly, while diluted material yielded positive tests.

HPPLO and MPPLO were the only other strains tested for hemadsorption in MKC cultures. The latter was cytopathogenic; however, under the experimental conditions employed neither strain caused hemadsorption.

Discussion. Chronic respiratory disease is endemic in most laboratory rat colonies(3). and is reportedly comprised of 2 pathological entities; namely, infectious catarrh and enzootic bronchiectasis(2). Infectious catarrh has been associated with PPLO(10), whereas there is evidence for a viral etiology of enzootic bronchiectasis(11). Investigation of CRD is complicated by the apparent frequent clinical and pathological overlapping of the two conditions. During attempts to isolate the virus in MKC cultures hemadsorption was observed. This effect was caused by RPPLO which was consistently isolated from inoculated, hemadsorption positive cultures. PPLO was never recovered from control culture fluids.

Evidence that hemadsorption was not due to a latent simian agent(12) is as follows: During the course of each experiment repeated testing of uninoculated MKC cultures prepared from the same monkey revealed no hemadsorption. When fluids from uninoculated cell cultures were passed serially, in parallel with experimental fluids, hemadsorption tests were consistently negative. In addition, fluids from experimental tubes failed to hemagglutinate chick erythrocytes, a property shared by some noncytopathogenic simian agents(12).

Dependency of the observed changes on the presence of an accompanying virus of rat origin was excluded by the demonstration that hemadsorption and CPE were associated with RPPLO following its isolation and serial passage in broth and agar cultures. The organism continued to hemadsorb after as many as 14 cell free subcultures.

Attention is drawn to behavioral similarities shared *in vitro* by certain viruses and PPLO. Both microorganisms may multiply and cause cytopathic effect in cell cultures(13). Both may agglutinate erythrocytes(14), and the present paper describes the ability of a PPLO to cause hemadsorption, a property shared by many viruses.

The ability to cause hemadsorption may prove useful in classifying strains of mycoplasma.

Summary. Hemadsorption of guinea pig erythrocytes by MKC cultures infected with a PPLO from the respiratory tract of laboratory rats is reported.

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