

age of 27 and 26 days respectively, prevented the rise of blood pressure normally observed in intact rats of this age; lesions placed at the age of 59, 75 and 140 days did not show this effect. 2. Rats that had been lesioned at 59, 75 and 140 days of age ingested more sodium than their respective intact controls; this was more pronounced in the female rats than in the male animals. 3. The increased sodium intake due to hypothalamic hyperphagia may be responsible for the alleviation of the hypotensive effect of the lesions resulting from neuroendocrine deficiencies. 4. The fact that ventromedial lesions prevented blood pressure and adrenal weight from attaining magnitudes appropriate for intact controls in weanling rats only, *i.e.*, during a phase of most active growth, suggests that the lesions interfered with the finer neuroendocrine regulation of adrenocorticotrophic hormone and perhaps growth hormone.

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Mycoplasma Isolates from Primary Cell Cultures and Human Diploid Cell Strains.* (30649)

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Contamination of tissue cultures with mycoplasmas has been well documented(1-8). The presence of cytopathic effects and the influence on cell growth by these organisms have varied from negligible or none(3,5,6,8-10) to retardation of growth(11) and widespread cytopathic effects(12-17). With the exception of one recent report(14) mycoplasmas found in uninoculated tissue cultures

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were identified as known human species.

In our studies, agents capable of producing transmissible cytopathic effects were isolated from control cultures of human diploid cell strain WI-26, primary African grivet monkey kidney and primary chick embryo. The cytopathic effect was accompanied by an increase in the acidity of the culture medium. Results of serodiagnosis with several viral reference antisera were negative but mycoplasmas were isolated from the supernatant fluids of these cultures. The complement fixation antigens prepared from these organisms did not react with antisera prepared against the known human species of mycoplasmas, and these cytopathic mycoplasmas probably belong to a previously unclassified group. This report will characterize these agents and describe their effects *in vitro*.

Materials and methods. Tissue cultures. Human diploid cell strains (HDCS) WI-26 and WI-38 were propagated as described(18, 19). Primary cultures of African green monkey kidney (GMK) (*Cercopithecus aethiops*) were prepared and maintained(20), or purchased from Flow Laboratories, Rockville, Maryland. Cultures of a continuous passage line of GMK, BSC-1(21), and primary human embryonic kidney (HEK) were purchased from Flow Laboratories. These were maintained with Basal Medium Eagle (BME) containing 2% agamma calf serum. Primary chick embryo cell cultures (CE) were prepared(22) from 10-day-old embryonated eggs (Kimber Farms) and maintained in BME containing 5% calf serum. Cultures were incubated at 36°C.

Antibiotics. Unless indicated otherwise, all tissue cultures were maintained in medium containing penicillin as the only antibiotic (100 units/ml final concentration). Chlor-tetracycline (Aureomycin, intravenous, Lederle, Pearl River, N. Y.) obtained as a dry powder, was suspended in water and stored at -20°C as a stock solution containing 10 mg/ml. When required for use, the stock was diluted with the experimental media.

Mycoplasma harvests. Tissue cultures were frozen and thawed once, centrifuged at 1500 rpm/10 minutes to remove cell debris, and

the supernatant fluids were tested for the presence of mycoplasmas.

Mycoplasma cultivation techniques. The mycoplasma agar and broth formulae as well as the techniques used for isolation and identification of mycoplasmas(23,24) were similar as used for isolation of *Mycoplasma pneumoniae*, but modified to contain 1% glucose, and in some experiments, 0.002% phenol red was added.

Attempts to isolate mycoplasmas from laboratory personnel producing the contaminated tissue cultures were performed with throat washings collected by gargling with mycoplasma broth. Three preparations were collected from each person at weekly intervals. These specimens were treated with penicillin (200 units/ml) at 37°C for 30 minutes before inoculation into tissue culture. Subsequently, the specimens were supplemented with 1:2000 thallium acetate before cultivation on agar medium.

The mycoplasmas isolated from supernatant fluids of tissue cultures or from throat washings were subcultured on mycoplasma agar and in broth medium. Aliquots of broth cultures were stored at -70°C in glass sealed ampules.

Production of hyperimmune sera in rabbits. The source of prototype mycoplasma strains of man and the preparation of antisera in rabbits to these strains, as well as to the recently classified isolates, *M. orale* types 1 and 2, have been described elsewhere(25-27). The prototype human mycoplasmas were cloned 3 times and subcultured for at least 4 passages in rabbit infusion broth supplemented with rabbit serum(25). This procedure was used to eliminate nonspecific reactions against constituents in the broth medium when these preparations were employed for immunization of rabbits.

Complement-fixation technique. To grow large quantities of organisms for preparation of CF antigens, the mycoplasmas were subcultured at 3-6 day intervals in the rabbit infusion or mycoplasma broth medium. After several serial passages, a CF antigen was prepared by concentrating organisms 10- to 40-fold by centrifugation at 18,000 rpm for 45 minutes in a Model L (21 rotor) Spinco

ultracentrifuge. The CF antigens of the tissue culture mycoplasma isolates were prepared from organisms grown in 500 ml of mycoplasma broth medium, containing glucose and phenol red. The glucose-fermenting mycoplasmas appeared to grow better in 2 liter Povitsky bottles (Diphtheria Toxin Bottles, Phipps & Bird, Richmond, Va.) than in the 100 or 500 ml Kimler screw-capped bottles routinely used for culturing the non-fermenting strains. The former have a large surface area which offers a shallow layer of culture medium. Antigens and antisera were standardized by block titrations. The method used for complement fixation has been described elsewhere(25,28). Mycoplasma CF antigens were tested against 4 units of rabbit antisera prepared against 7 serotypes of human mycoplasmas: *M. hominis* type 1, strain V2785; *M. hominis* type 2, strain Campo (human origin questionable); *M. pneumoniae*, strain FH; *M. fermentans*, strain G; *M. salivarium*, strain Buccal; *M. orale* type 1, strain CH19299; and type 2, strain CH-20247.

The growth inhibition test used was the one described by Clyde(29). Specific antisera were absorbed onto filter paper discs which were placed in agar plates seeded with the test organism. When homologous serum was employed, zones of inhibition were observed after incubation at 36°C for 3 to 6 days.

Hemolysin production. The hemolysis of guinea pig RBC by discrete mycoplasma colonies growing in mycoplasma agar was tested as described elsewhere(30).

Sugar utilization. One-tenth ml of a mycoplasma broth suspension was added to 10 ml of mycoplasma broth medium containing 1% glucose and 0.002% phenol red. These cultures and uninoculated broth medium were incubated at 36°C. *M. pneumoniae* and *M. salivarium* were used as positive and negative controls, respectively. A color change indicative of acid production was interpreted as being due to sugar fermentation. Aliquots from these broth cultures were placed on agar and the colonies counted to determine whether the organisms had propagated.

Ether and pH sensitivities. Tests for ether (31) and pH sensitivity were performed as

described for classification of virus isolates (32).

Mycoplasma strains. Only the 3 mycoplasma isolates which were studied in some detail will be documented. These organisms were isolated from tissue cultures propagated at a commercial laboratory: Wistar-1 strain from tissue cultures prepared from a 10-day-old Kimber chick embryo; Wistar-2 strain from uninoculated primary GMK cultures; Wistar-3 strain previously reported as strain 83810(33), was isolated from uninoculated cultures of HDCS WI-26 at subculture passage 27. The chick embryo and GMK cultures, though uninoculated, had degenerated "spontaneously." CPE was not present in the HDCS cultures but fluids from these cultures caused degeneration of GMK cells.

The GDL strain was supplied by Dr. R. H. Leach(14).

Results. Isolation from HDCS of the Wistar-3 strain. In the laboratory of origin of HDCS, mycoplasmas were at no time detected in WI-26 and WI-38, even when cultures were tested throughout their entire life span(18,19). As part of a routine surveillance program, uninoculated cultures of a substrain of HDCS WI-26, propagated in another laboratory, had been examined and found free of mycoplasmas at subculture passages 15 and 18. However, mycoplasmas were isolated by passage 27 although CPE was not observed. The positive HDCS culture fluid had been inoculated into cultures of primary GMK tissue, and a CPE (Fig. 1) with marked decrease in the pH of the medium was observed in the recipient cultures. These coincidental observations led to the premise that the CPE might have resulted from contaminating mycoplasmas. Experiments designed to investigate the role of the mycoplasmas indicated that: a. the original HDCS culture fluid contaminant (Wistar-3) gave uniformly reproducible CPE after re-inoculation into several lots of GMK cells; b. the original isolate passed 20 times on mycoplasma agar medium and inoculated into susceptible GMK cells produced a similar CPE; thus, both a mycoplasma and a cytopathic virus were not being carried simultaneously; c. cultures of HDCS reconstituted

from frozen storage at passage levels prior to that at which isolation of mycoplasma was possible did not produce CPE on passage into

GMK; d. the CPE was inhibited by chlor-tetracycline (20 γ /ml) whereas penicillin was not effective in reducing either CPE or the

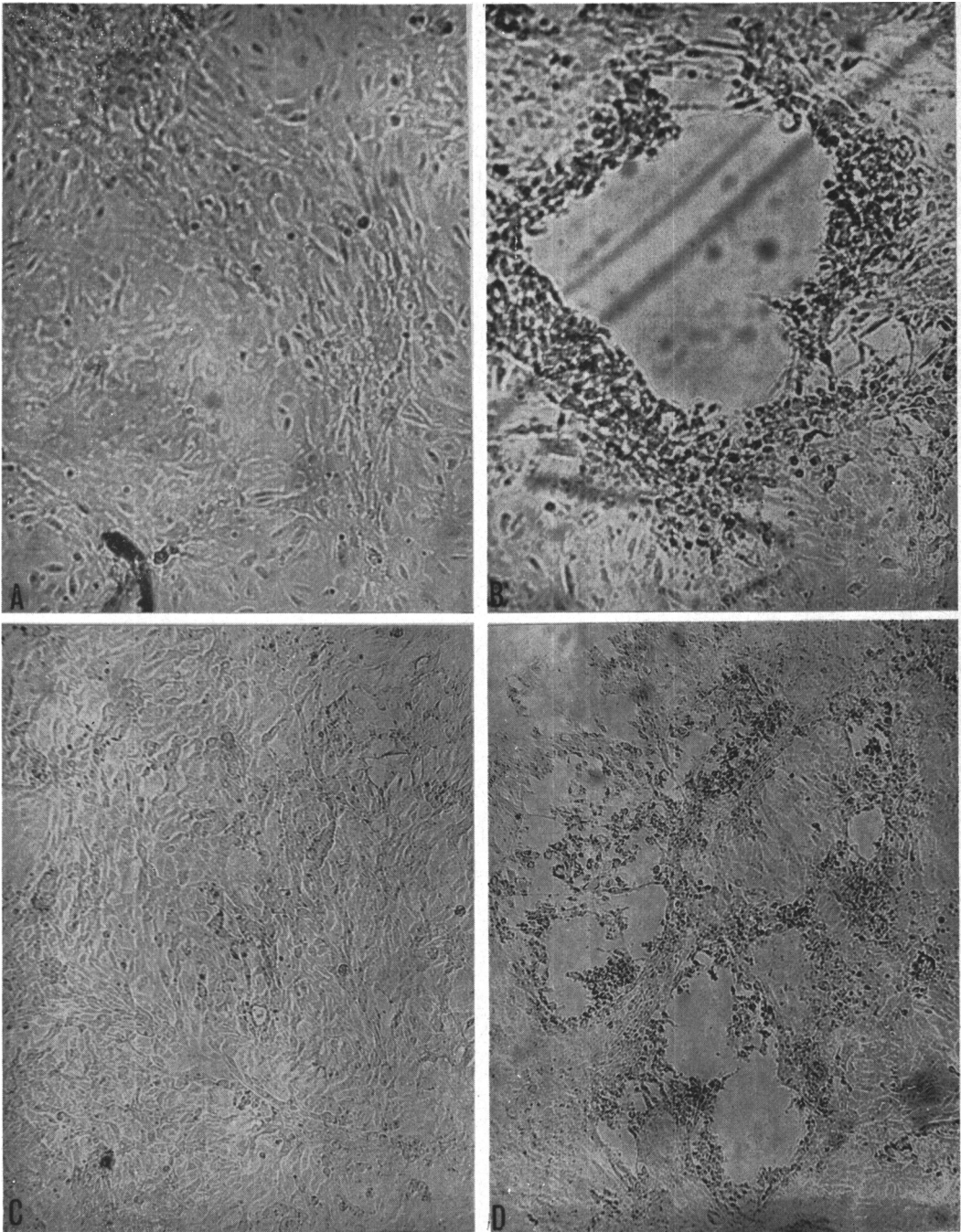


FIG. 1. (a) Normal HEK; (b) HEK infected with W-3 mycoplasma, showing plaque of CPE; (c) Normal GMK; (d) GMK infected with W-3 mycoplasma. Live unfixed cells in stationary cultures; 80 $\times$.

TABLE I. Growth of Wistar Strain 3 in Mycoplasma Broth and in Tissue Cultures.

Day Post Inoculation	Degree of CPE and Colony Forming Units/ml in Harvests from Broth or Cell Cultures*						
	Mycoplasma Broth CFU/ml**	GMK		HEK		CE	
		CPE	CFU/ml.	CPE	CFU/ml.	CPE	CFU/ml.
3	10 ⁵	0	10 ⁶	0	10 ⁶	1+	10 ⁷
4	—	1-2+	—	1-2+	—	3	—
7	10 ⁶	3	10 ⁶	3-4	10 ⁷	4	10 ⁶
8	—	4	—	4	—	—	—
10	10 ^{2.6}	—	10 ^{6.8}	—	10 ^{5.2}	—	10 ^{6.25}
14	10 ^{1.5}	—	10 ^{6.97}	—	10 ^{3.5}	—	10 ^{5.2}
Controls	0	0	0	0	0	0	0

* Inoculum = 100 colony forming units (CFU) per ml of culture fluid or broth.

** CFU determined by plating serial dilutions on mycoplasma agar medium.

growth of the organism; streptomycin gave irregular results; e. the CPE was transmitted serially through GMK cultures and at each passage mycoplasmas were isolated.

Sensitivity of cell cultures. Cell cultures other than GMK were tested for their sensitivity to the Wistar-3 strain. Table I compares the growth and development of CPE in simultaneous experiments in broth medium and in primary cultures of GMK, HEK, and CE cells. The initial concentration of mycoplasmas in each system was 100-colony forming units (CFU) per ml. In 3 to 7 days, the Wistar-3 strain had grown to high concentrations in all cultures and the growth was accompanied by progressive CPE. A cell line from GMK, BSC-1, as well as primary human amnion, could support the growth of these mycoplasmas and showed the CPE; however, quantitative studies were not performed.

Description of cytopathic effect. In GMK and HEK cultures, the morphologically altered cells were readily detached from the glass and consequently good fixation and staining were difficult. Photographs (Fig. 1-4) of living cultures and fixed and stained preparations show the effect of the Wistar-3 strain. Cells appeared granular, shrunken and highly refractile, and were easily dislodged by gentle shaking. If stationary cultures were not moved until several days after the CPE was initiated, a large focus of CPE was seen

as a streak or plaque in the center of the cell sheet (Fig. 1). In contrast, tube cultures which were incubated in a roller drum, or those which were examined frequently, showed the initial CPE throughout the cell sheet (Fig. 2). In either case, complete destruction of the cell sheet occurred and the effect was transmissible.

In HDCS cultures, the initial effect consisted of diffuse granularity (Fig. 3) and though the CPE progressed more slowly than in HEK or GMK, complete destruction ensued. One striking feature in infected HDCS was the appearance of "leopard" nuclei apparently resulting from chromatin clumping. These nuclei could be distinguished from normal cells in prophase (Fig. 4). The latter appear as cells with normal cytoplasm and the chromosomes' integrity is retained; "leopard" nuclei often appear with little or no cytoplasm attached, may sometimes be collapsed, and the clumped chromatin does not appear as normal chromosomes. Experiments to determine the specific nature of this lesion in relation to mycoplasma infection are in progress.

Stability of the Wistar-3 strain. The isolate was ether sensitive and was inactivated at pH 3.0, when tested by methods described previously (31,32).

Effect of Wistar-3 strain on growth of HDCS. Routinely, HDCS were subcultured by a 1:2 split ratio at 3- or 4-day intervals,

at which time a confluent cell sheet developed. Cultures could be held without subcultivation as long as 2 months without CPE or detachment of the cell sheet. The cells had a finite

life span of 40 to 50 passages after which senescence and death resulted(18,19). However, these characteristics changed when the cultures were inoculated with mycoplasmas.

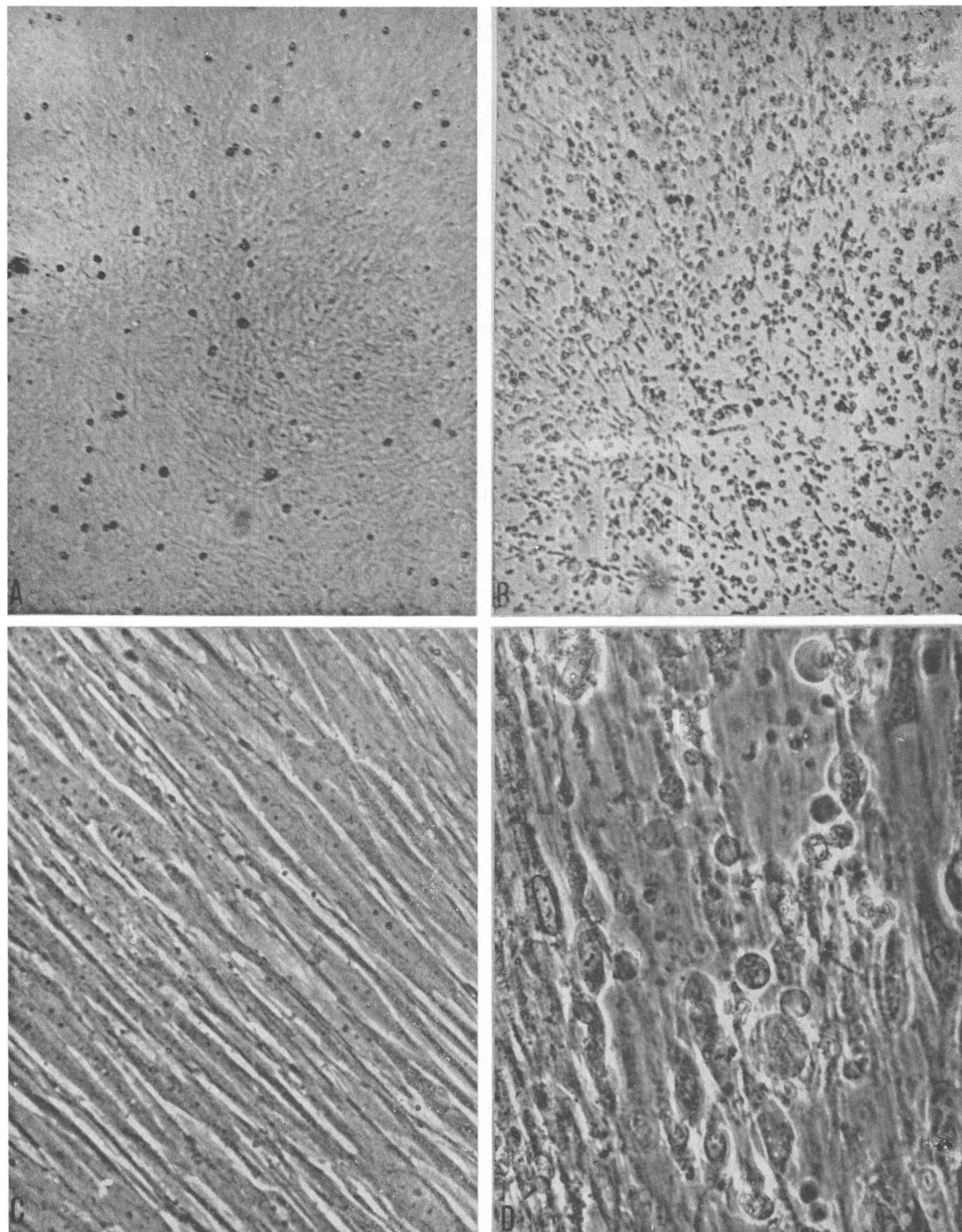


FIG. 2. (a) Normal CE 70 $\times$; (b) CE infected with W-3 mycoplasma 70 $\times$; (c) Normal WI-38 cells, phase contrast 350 $\times$; (d) WI-38 infected with W-3 mycoplasma, phase contrast 350 $\times$. Live unfixed cells in cultures incubated in roller drum, showing widespread CPE.

Cultures infected at passage 18, although not eliciting a rapid CPE, were forced into "early senescence" at about passage 25 to 30.

Furthermore, cultures held without subcultivation at various passage levels after inoculation with mycoplasmas could not be main-

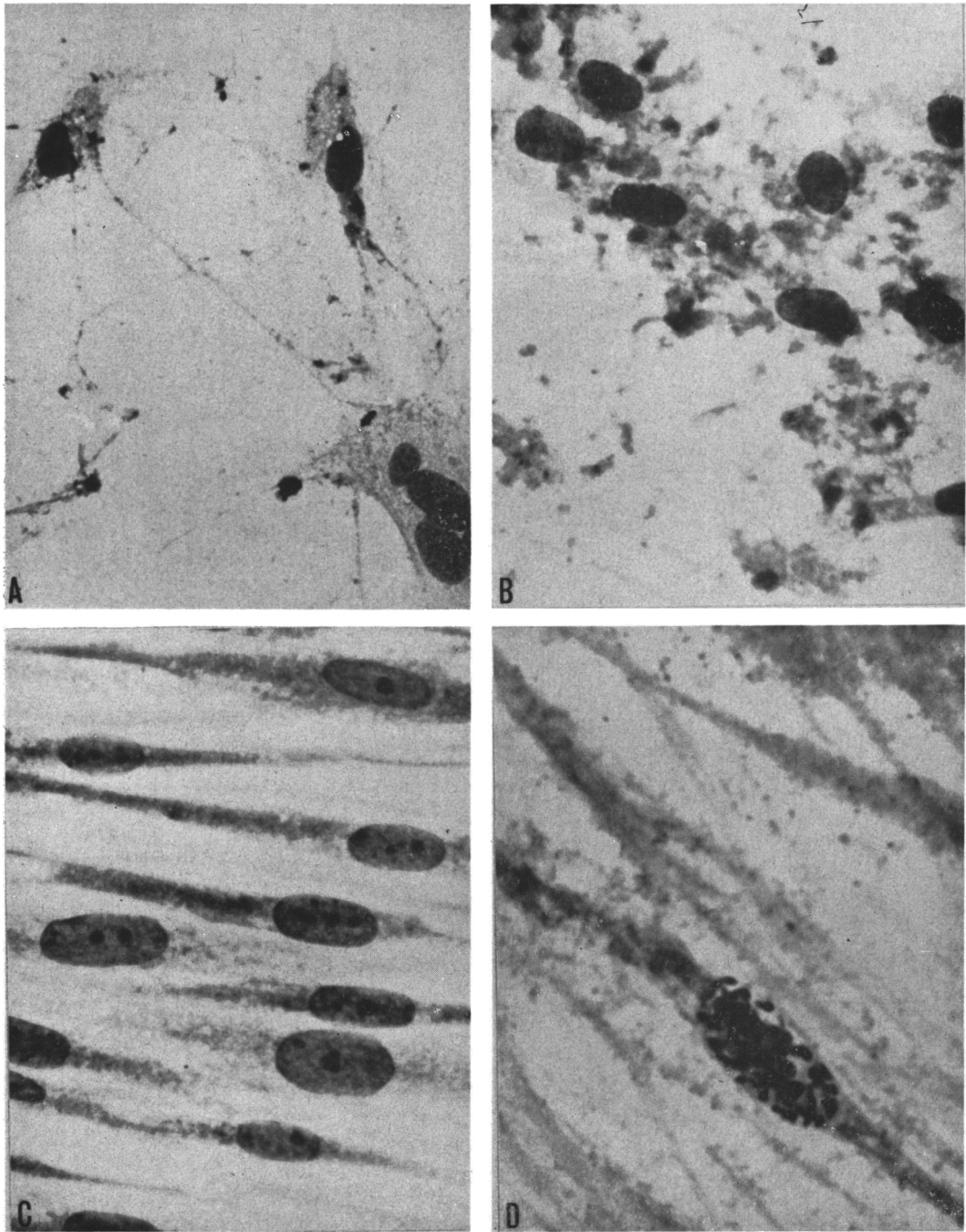


FIG. 3. (a) HEK infected with W-3 mycoplasma, Giemsa, 250 $\times$; (b) GMK infected with W-3 mycoplasma, Giemsa, 625 $\times$; (c) Normal WI-38, Giemsa, 625 $\times$; (d) WI-38 infected with W-3 mycoplasma, showing fragmented cells, granular debris and "leopard" nucleus, Giemsa, 1350 $\times$.

tained in optimal conditions beyond two weeks.

Since early senescence resulted in the HDCS cultures infected intentionally with mycoplasmas, other substrains of HDCS which had experienced premature death "spontaneously" when propagated in a commercial laboratory were tested for mycoplasma contamination. Two substrains of HDCS, WI-26 and WI-38, had shown early senescence; one substrain could not be subcultivated beyond passage 27, and the other beyond passage 28. Both preparations yielded mycoplasmas which produced CPE upon inoculation of GMK cultures.

Antibiotic sensitivity. Chlortetracycline at a concentration of 20 γ /ml suppressed the growth and CPE produced by the Wistar-3 strain. However, CPE was noted in some instances in cultures held beyond 1 to 2 weeks without refeeding. Renewal of the culture medium and re-establishment of a satisfactory antibiotic level prevented extension of the CPE and apparently normal cells repopulated denuded areas of the cultures.

Chlortetracycline at 5 γ /ml gave a slight suppression of CPE.

Other cytopathic mycoplasmas. Strains of mycoplasmas producing "spontaneous" CPE were obtained from uninoculated control cultures of primary chick embryo (Wistar-1 strain) and grivet monkey kidney (Wistar-2 strain). These organisms were isolated in two laboratories not associated with studies of the Wistar-3 strain and their isolation probably did not represent cross-contamination with this strain.

Certain tests described with the Wistar-3 strain were performed with the 2 isolates with essentially similar results; all strains produced similar transmissible CPE in the same cell systems and were sensitive to the effects of chlortetracycline, ether, and low pH. Both strains caused "early senescence" of HDCS WI-38.

Other strains of mycoplasmas were isolated on mycoplasma medium from several individual lots of GMK and CE cells which showed spontaneous CPE. However, strain properties other than those indicating ability



FIG. 4. (a) Normal WI-38 in prophase; (b) WI-38 infected with W-3 mycoplasma showing "leopard" nuclei with clumped chromatin globules; (c) same as (b) with collapsed "leopard" nucleus, Giemsa, 1350 $\times$.

TABLE II. Complement Fixation Studies with the Wistar Mycoplasma Strains.

Rabbit Serum Prepared Against:	Reciprocal of CF Antibody Titer With Antigens of:				
	Wistar-1	Wistar-2	Wistar-3	GDL	7 Human Species
Wistar-3	640	640	1280	2560	<20; 40
GDL	1280	1280	1280	2560	<20; 80*
7 Human Species (5 Prototypes, 2 New Oral Types)	<20; 20	<20; 40	<20	<20; 40; 160**	Note Below†

* 1:80 titer was obtained with standard reference antigen prepared from *M. salivarium*.

** 1:160 titer was obtained with serum prepared against *M. orale* type 1.

† Specific homologous reactions were obtained with homologous titers ranging from 1:640 to 1:10,240, except for *M. fermentans* which had an homologous titer of 1:160.

to produce CPE in tissue culture and chlor-tetracycline sensitivity were not studied.

Serologic studies In CF tests (Table II), rabbit antiserum prepared against the Wistar-3 mycoplasma strain reacted to approximately the same titer with the Wistar-1, 2, and 3 isolates. Antisera to Wistar-3 did not react, or reacted to a very low level, with antigens prepared against the human "prototype" mycoplasmas or against the newly identified mycoplasmas, *M. orale* type 1 and type 2. Similarly, sera prepared against the 7 species of mycoplasma of man showed little if any reaction with antigens prepared from the 3 Wistar strains. These findings suggested that all 3 isolates were closely related to each other, and have little antigenic resemblance to the known human mycoplasmas. However, an antiserum prepared against another tissue culture isolate, the GDL strain(14), reacted to the same high titer with antigens of the 3 Wistar strains as with those of the homologous organisms. Similarly, antiserum prepared against the Wistar-3 strain reacted to high titer with antigens prepared from the GDL strain.

Attempts to identify antigens of the 3 Wistar strains with standard reference sera prepared with animal mycoplasma species were unsuccessful. In reciprocal tests, antigens prepared from mycoplasmas of animal origin did not react with 4 units of serum prepared against the Wistar-3 strain. The animal mycoplasma strains tested were: *M. arthritis*, *M. spumans*, *M. canis*, *M. maculosum*, *M. gallinarum*, *M. neurolyticum*, *M. iners*, and *Simian 696*. In addition similar tests performed with antigens of *M. laidlawi*

A and B, and the L-1 of *Streptobacillus moniliformis* and *Proteus "L"* were negative. Thus, the Wistar strains appear to belong in a specific antigenic group, distinct from either the recognized human mycoplasmas or from many of the known animal strains, but related to one unclassified isolate obtained from uninoculated tissue cultures(14).

Sugar fermentation. All 3 Wistar strains produced acid in glucose-enriched broth cultures. Typical pH values obtained with the Wistar-3 strain showed an initial medium of pH 7.7 and a final value at 9 days of pH 5.9.

Hemolysin production. Plaques of beta hemolysis were observed after 15 to 48 hours with colonies of the 3 Wistar strains growing on mycoplasma agar overlaid with a guinea pig RBC-agar mixture. The hemolysis was characteristic of that produced by *M. pneumoniae*, rather than of the incomplete (alpha) hemolysis which develops around colonies of the other human mycoplasmas.

Growth inhibition test. The 3 Wistar strains were not neutralized in the growth inhibition tests with sera prepared against *M. hominis* type 1, *M. hominis* type 2, *M. pneumoniae*, *M. fermentans*, *M. salivarium*, and *M. orale* which are known species of human mycoplasmas. Also, sera prepared against the animal species *M. mycoides* var. *capri*, *M. gallisepticum*, *M. gallinarum*, *M. pulmonis* and sera prepared against 3 avian pathogenic types did not inhibit growth of the Wistar strains.

Source of mycoplasmas. Nine mycoplasma isolates were recovered from throat washings of laboratory personnel when mycoplasma agar and broth media were employed but

these throat washings did not produce CPE in HEK and GMK cultures. None of the isolates produced CPE in GMK or HEK cultures, even after 3 blind passages. The isolates, in contrast to Wistar strains 1, 2, and 3 did not produce plaques of beta hemolysis(30). Mycoplasmas related to the Wistar strains were not isolated from culture media, cell lines, or primary cell cultures of various tissue sources other than GMK or chick embryo.

Discussion. The Wistar mycoplasma strains 1, 2, and 3 have the following properties in common: (a) they were isolated from human or animal cells *in culture*; (b) they produce CPE in cell cultures of several strains; (c) their growth is accompanied by acid production; (d) they produce a beta hemolysin; (e) serologically, they are closely related, if not identical; (f) these organisms share with other mycoplasma species the properties of sensitivity to chlortetracycline, low pH, and ether treatment.

The serological studies performed on the 3 mycoplasma strains indicate that they are distinct from any of the known human mycoplasma species. The tests performed with antisera to some known animal mycoplasma species were also negative, but these isolates cannot be completely excluded as being mycoplasmas of animal origin for which antisera were unavailable. However, the isolation of mycoplasmas other than those of human origin from cell cultures has not been documented.

The Wistar mycoplasma isolates were similar serologically to the GDL strain of Butler and Leach(14). Furthermore, serum prepared with the Wistar-3 strain reacted with antigens of mycoplasma strains Cincy 7, F 11, and F 12 isolated from tissue cultures used in investigations of human tumors(34,35), but did not react with antigens of strain 880(36) or Negroni(37) (Somerson, Fox, Walls, and Chanock, unpublished results). Mycoplasmas recently isolated from HEP-2 tissue cultures purchased from several commercial sources were found to be serologically related to the Wistar isolates (Smith, Somerson and Chanock, unpublished data), as were isolations from other cell lines (Hayflick and

Stanbridge, unpublished data). Furthermore, a similar serotype has also been isolated directly onto agar from a human bladder tumor(38,39).

Certain similarities shared by some viruses and cytopathic mycoplasmas indicate the importance of testing for the presence of the latter, before classifying microorganisms as viruses, especially as myxoviruses. In addition to producing a transmissible cytopathic effect, some viruses and mycoplasmas share properties such as size, filterability, morphology in electron microscopy, sensitivity to ether and low pH, ability to hemagglutinate and cause hemadsorption, ability to interfere with virus replication, resistance to inhibition by the commonly used antibiotics, penicillin and streptomycin, and inhibition of growth by homologous antiserum. Obviously, the absence of bacteria and molds is an unreliable criterion for identification of unclassified isolates as viruses, and attempts should be made to isolate mycoplasmas in an artificial medium. However, in some instances tissue cultures may prove to be more reliable for the *initial propagation* of certain strains of mycoplasmas. Thus, following filtration of 2 isolates from human diploid cell strains, mycoplasmas were easily propagated from the filtrates in cultures of HEK and GMK, whereas these filtrates were negative when routine tests were performed using mycoplasma agar and broth media(40). However, the culture fluids from the positive indicator HEK and GMK cultures yielded mycoplasmas when subcultured on artificial medium. Perhaps the tissue culture system has a greater sensitivity in meeting the initial nutrient requirements of some mycoplasmas. An alternate explanation is that the filterable minimal reproductive units of certain species of mycoplasmas might require a living cell for their "maturation" to a form which can grow on artificial medium (40). The only reliable method available *at present* for final unequivocal identification of the mycoplasmas is their growth on agar medium.

Summary. Mycoplasmas were isolated from "uninoculated" cultures of human diploid cell strain (HDCS) WI-26, and primary cultures of African green monkey kid-

ney and chick embryo. These 3 Wistar mycoplasma strains were characterized by their ability to produce CPE in cultures of HEK, GMK, CE, and HDCS; ferment sugars; and cause beta hemolysis of guinea pig erythrocytes. HDCS cultures subcultured routinely after infection with these strains could not be propagated for their usual finite life span and "early senescence" occurred 10 to 20 passages before that in uninoculated control cultures. Complement fixation and growth inhibition studies revealed that these mycoplasma strains were serologically similar to each other, if not identical, and were related to the unclassified GDL strain of Butler and Leach(14). However, the Wistar strains were not related to the known human prototypes of mycoplasmas nor to 16 animal serotypes for which standard typing reagents were available.

Though the source is still obscure, their presence in uninoculated cell cultures and certain of their characteristics shared with cytopathic viruses stress the need for careful evaluation before classification of new isolates as viruses.

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Neonatal Cortisol Administration: Effect on Growth, the Adrenal Gland and Pituitary-Adrenal Response to Stress.* (30650)

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The infant rat does not secrete ACTH in response to stress in the same manner as the adult until about the eighth postnatal day (1-3). The basis for this neonatal stress-non-responsive period probably lies within the central nervous system. The available evidence indicates, however, that the steroid feedback mechanism is operating during fetal (4) and early postnatal life (5-6) so that central mechanisms responding to changing internal signals (plasma cortical hormone levels) appear to be functioning before external signals (stress or noxious stimuli) are relayed to the pituitary gland. These observations are compatible with the view that endogenous hormones, acting in the classic feedback manner to produce a steroid blockade, may prevent stress from activating the neonatal pituitary. The "set" of the "cortico-stat" (that region of the central nervous system, presumably within the diencephalon, upon which adrenal cortical hormones act to moderate the secretion of ACTH) may render it more sensitive to cortical hormones during the early postnatal period.

This report compares the adrenal atrophy produced in infant and adult rats to graded doses of cortisol. In addition several observations were made concerning the effect of one injection of cortisol upon growth and the pituitary-adrenal response to stress.

Methods. Sprague-Dawley rats bred in the laboratory were used in all experiments. A. Effect of multiple injections of cortisol upon adrenal weight. Litters born the same day were paired to 8 pups each and from days

2-5 or 30-33 received daily I.P. injections of 0.10-2.5 mg/100 g cortisol acetate (cortef-Upjohn). On day 7 or day 35 all rats were killed, including a control group receiving saline, and the adrenals weighed individually. B. Effect of single doses of cortisol upon growth and the adult response to stress. Litters were paired to 8 pups at day 2 and half of each litter received a single subcutaneous injection of 1 mg cortisol acetate. They were weaned at 21 days of age and on day 44 some of each group were rapidly anesthetized with ether, decapitated, trunk blood collected and the adrenals removed and weighed. Others were anesthetized with ether and 20 minutes later decapitated and blood and adrenals also saved for corticosterone analysis (7). The thymi and brains were removed from some rats in each group, weighed and brain cholesterol (8) was determined. C. Effect of single dose of cortisol upon fertility. From day 55-75 daily vaginal smears were performed on 12 female rats which had received cortisol as infants. To assess fertility four cortisol treated females were placed with proven males for 20 days. Pregnancies occurred in all instances.

Results. A. Effect of cortisol upon adrenal weight: Fig. 1 illustrates the changes in adrenal weight that occurred in infant and 30-day-old rats on graded doses of cortisol. The changes are based upon comparisons with saline treated controls. At all but the highest dose level there was a greater effect upon the adrenals of the infants than upon the adults. B. Effect upon growth, fertility and the response to stress. Following a single injection of 1 mg cortisol into the 2-day-old

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