histamine. Its failure to show a greater drop in pressure to large doses of histamine may be a factor involved in the resistance of the unanesthetized chicken to endotoxin.

Summary. Hemodynamic and lethal effects of endotoxin in the chicken were studied. It was noted that the unanesthetized bird is highly resistant to endotoxin, all animals surviving intravenous doses up to 40 mg/kg. Systemic arterial hypotension and a slight degree of hemodilution were observed in the anesthetized chicken given 10 mg/kg endotoxin. The possible role of histamine is discussed.

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Respiratory Syncytial Virus: Properties of Strains Propagated in Monkey Kidney Cell Cultures.* (28941)

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Respiratory syncytial (RS) virus is unstable, undergoing rapid inactivation under usual conditions for maintaining viruses. Inactivation can be slowed by storage of virus stocks in chicken sera, or in gelatin, but with these, decay continues, although at reduced rates. During recent studies on respiratory diseases we recovered 24 RS viruses from 18 infants and children. During our work we found that RS viruses may be preserved in 50% glycerin for at least 9 months.

Another aspect of the study deserves mention. During the outbreak in the spring of 1962 RS viruses were detected more often in cultures of monkey kidney than in other cell lines. Although it has been known that monkey kidney cell cultures support the growth of RS virus, several writers(1-3) have found these cells less satisfactory than those of human origin. Our studies subsequently led us to define several properties of RS viruses propagated in monkey kidney cell cultures.

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Materials and methods. Strains of virus. Procedures used for isolating these viruses have been described(4). "Long" strain of RS virus was received from Chanock in 1960. This strain has been propagated 18 times in HEp-2 cells, and 20 to 25 times in monkey kidney cell cultures in our laboratory. The "87" strain of RS virus which was isolated during the 1962 studies was passed 20 to 25 times in monkey kidney cell cultures. Infectivity titers for both strains were 10^{-5} to $10^{-5.5}$ $TCID_{50}$ per ml when titrated in monkey kidney cell cultures.

Growth curves. Monkey kidney cells were grown in monolayers in tubes; cells were inoculated either with "Long" or "87" strains of RS virus. The input of infectious virus, using undiluted stocks, was such that about every hundredth cell became infected. Cultures were incubated at 37°C on a "roller drum." Three hours after inoculation the fluid overlay was drawn, the cell sheet was washed twice with PBS and fresh medium was put on the cell sheet. Fluid overlays from 4 tubes were collected thereafter at

different intervals; after one quick freeze (—70°C) and thaw, fluids were pooled and cells separated by centrifugation. The supernatant was harvested and titrated in 0.5 log steps using 5 tubes per dilution.

Stability tests at different temperatures. Freshly harvested fluid overlays containing strains "Long" and "87" were used for obtaining strain-specific stocks. Harvests were centrifuged and the supernatant distributed into either Wasserman tubes (stored at +50°C and +37°C) or in glass sealed vials (stored at temperatures below 37°C). Prior to sealing, the following components were each added to a series of tubes containing the virus suspension: 1 M $MgCl_2$(5); 20% chicken serum; 50% glycerin; and gelatin, to fluid concentration of 0.5%. Control cultures contained distilled water instead of $MgCl_2$. The control for the last 3 additional substances consisted of stock suspended in original maintenance fluid (HACEM). Samples drawn at different intervals were titrated in monkey kidney tissue cultures in 0.5 log steps with 4 cultures for each dilution.

Staining methods. For staining purposes infected monkey kidney cells grown on coverslips in Leighton tubes were used. Staining of the cells was done with hematoxylin-eosin.

Results. Recovery of RS virus in primary monkey kidney tissue cultures (MKTC's). Twenty-four RS viruses were recovered from 18 children using MKTC's. The virus was detected in both the nose and throat of 6, in the nose of 10, and only in the throat of 2 children. The same samples tested in HEp-2 cells yielded only 5 isolates; in primary human amnion cells, none was revealed using cytopathic effect (CPE) as index. In our hands MKTC's were much (approximately 80%) more sensitive than HEp-2 cells for revealing RS viruses.

Cytopathic changes were observable in a small fraction (13%) by the 3rd day, and in additional 58% between the 4th and 6th day post-inoculation. Fluid overlays from cultures which failed to show CPE in first passage were harvested and inoculated on fresh cultures. When the first passage was negative, no virus was ever recovered in the second passage.

The CPE differed markedly from that seen in HEp-2 cells, or other "stable" cell lines. Initially CPE consisted of rounding and great clustering of cells. At no time was the whole cell sheet affected. After several passages the "87" strain destroyed 90% of the cell sheet; multinucleated giant cells, later found to contain eosinophilic inclusions, constituted remnant cells adhering to the glass. In addition, elongated spindle-shaped cells remained adherent to the glass.

Multiplication of RS virus in MKTC's. The growth curves of "Long" and "87" strains of RS virus are plotted in Fig. 1. Both strains had about the same passage histories in MKTC's. Cultures containing approximately 500,000 cells were inoculated with 0.1 ml of virus suspension containing $10^{-3.5}$ $TCID_{50}$.

Within 3 hours, as determined by titration in MKTC's (see Materials and Methods section), 96% of "Long" and 98% of "87" RS virus was adsorbed to the cells. Infectious virus was detected first 7 hours after inoculation, but no CPE was seen. Small areas containing rounded cells could be observed not earlier than 30 hours after infection with both strains. At this time the virus titer was 10^{-2} per ml. Cells infected with "Long" and "87" strains shed infectious virus at similar rates during the early cycles of infection, even with a more pronounced CPE from "Long" virus. At maximal CPE (72 to 96 hours) strain "87" yielded slightly larger amounts of infectious virus than "Long," probably because the cell sheet was destroyed less rapidly. The "87" virus never destroyed the whole cell sheet; about 10% of the cells were left on the glass and these probably were still able to yield virus.

At the time of maximal CPE (Fig. 1) the remaining cells of cultures infected with "Long" consisted of larger multinucleated syncytia containing large eosinophilic inclusion bodies. In contrast, cultures infected with "87" virus consisted of smaller syncytia containing smaller inclusion bodies; but, in addition many spindle-shaped cells adhered to the glass surface. These elongated cells while possibly capable of supporting virus

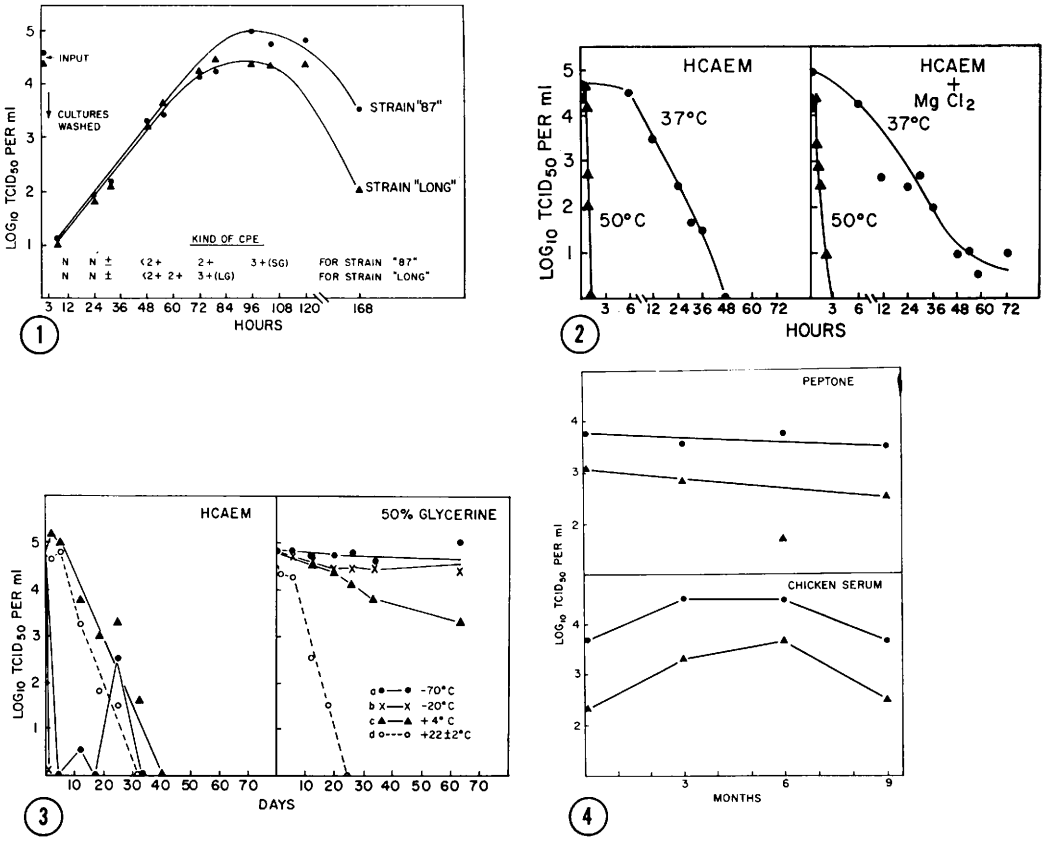

FIG. 1. Multiplication of respiratory syncytial viruses in monolayers of monkey kidney cells. SG, only small giant cells; 10% of the cells are elongated, and still adherent to glass. LG, only large giant cells left on glass. N, negative.

FIG. 2. Rates of inactivation of RS (Long) virus in Altered Eagles' Maintenance (HCAEM) with and without 1 M $MgCl_2$.

FIG. 3. Temperature stability of RS virus, strain "Long," stored in HCAEM or 50% glycerin. Essentially the same results were obtained with the "87" strain of RS virus. Data shown for 2 months' period of observation.

FIG. 4. Preservation of RS virus (Long) suspended in peptone (upper panel) or chicken serum (lower panel). Circles, frozen liquor; triangles, lyophilized liquor. All samples stored in a Dry Ice Box.

growth were apparently morphologically unaffected.

Multiplication of virus in stationary and rotated cultures. Cultures prepared from the same kidneys and inoculated with the same dilutions of virus were incubated at 36 ± 0.5°C while stationary or on a revolving drum. CPE was read on 5th, 7th, and 9th day after inoculation. The results of the last reading are recorded in Table I; an appreciably higher virus titer was achieved from rotating cultures. In addition CPE was much more pronounced following rotation.

Stability of RS virus by 1 M $MgCl_2$. Although 1 M $MgCl_2$ (Fig. 2) provided a measurable stability, a large fraction (≥97%) of RS virus was no longer measurable after 2 hours' incubation at 50°C. In contrast with the prompt heat lability of the controls,

TABLE I. Growth of RS Virus at 37°C. Comparison of stationary and rotated cultures.

Strain	$TCID_{50}$/ml	
	Stationary	Rotated
"Long"	3.1	3.9
"87"	3.3	4.5

Mean titer of 2 experiments, using 0.5 $\log_{10}$ dilution steps, 6 cultures per dilution.

$MgCl_2$ apparently protected a small residue of virus. Essentially similar results were obtained at 37°C (Fig. 2). Under these same conditions 50% glycerin failed to stabilize RS (either "Long" or "87") virus.

Stability of virus at various temperatures. The marked lability of RS virus is shown graphically in the left panel of Fig. 3. Inactivation occurred much more swiftly at —20°C than at room temperature. Viruses "quick" frozen and placed in a dry ice box lost titer rapidly. Very likely the infectious agent is damaged during freezing; the causes of denaturation, however, are not fully understood.

Addition of 50% glycerin failed to stabilize RS virus at 37°C, or at room temperature (Fig. 3). However, virus was stabilized by glycerin during storage in the ice box (4°C) or in the freezer. No loss of virus occurred over a period of 9 months of storage at either —20°C or —70°C. The decay rate at 4°C was also delayed; between the 20th and 60th days, however, a loss of $\geq$90% occurred.

In parallel studies, 20% chicken serum and 0.5% gelatin were much less effective than glycerin for preservation of virus. Virus suspended in both media deteriorated rapidly during the first few days; some virus residual in the population was apparently hardy, since survival with but slow decay occurred during the 9 months of observation. But among tests of samples stored at —70°C the fraction of virus surviving varied, sometimes considerably (Fig. 3).

Stability after lyophilization. "Long" and "87" viruses, suspended in either 5% peptone or 50% chicken serum were transferred to glass ampoules. Half the samples were lyophilized;† those, like the corresponding liquid controls were stored at —70°C. Dried and liquid samples were withdrawn at specified intervals (Fig. 4) and titrated. Lyophilization resulted in an immediate large (90%) virus loss. Decay of the remainder was similar to virus stored in corresponding liquid media. Virus suspended in chicken serum was preserved; virus preserved in peptone decayed at a slow but gradual rate during the 9-month period of observation.

Discussion. RS virus is very unstable. Readily inactivated during freezing, or soon thereafter, the population survives longer when left at room temperature or in the refrigerator (4°C). The exquisite sensitivity of this virus has been noted herein immediately following lyophilization. Because of rapid inactivation this virus has been difficult to maintain, and absolute increments and losses (*e.g.*, growth cycle experiments) of virus difficult to control.

The stabilization of RS viruses, both "Long" and "87" strains by 50% glycerin has been useful to us for preserving prototype strains, as well as new isolates which may have to be put aside temporarily. Glycerin may not be superior to 50% chicken serum, but it is as good and avoids the risk of virus loss from toxicity or serum inhibitors of one type or other. As noted earlier lyophilization does not have an advantage over other methods pertaining to this study.

MKTC's in our study were more sensitive than HEp-2 cells for primary isolation of RS virus. Among the MKTC positive samples, only 20% were positive with HEp-2 cells. Moreover, CPE develops rapidly, occasionally by the 3rd, and almost always (71%) before the 6th day. These findings differ from those of others in two ways: (a) sensitivity of MKTC's as opposed to continuous human cell cultures(1,2) and (b) time of onset of CPE, which is earlier than often reported(3,6).

Incubation of infected MKTC cultures on a rotating drum not only provided a more pronounced CPE, but also a higher virus titer. In growth curve experiments (with both "Long" and "87" strains) infectious virus was detected within 7 hours, with peak virus yield between 78 and 96 hours. The first signs of CPE were noted as early as 31 hours; maximal CPE occurred within 72 to 96 hours. Excepting the slightly longer latent period in HEp-2 cells, our results in respect to virus yield, onset of CPE, and kind of CPE agree closely with those obtained by Jordan(7) and Kisch *et al*(8).

Summary. RS viruses recovered in 1962

† Stokes freeze-drying equipment Model 176A.

were cytopathic on MKTC's. MKTC's were much more sensitive (80%) than HEp-2 cells. Seventeen of 24 isolates were revealed between the 3rd and 6th days post-inoculation. Growth curves in MKTC's revealed virus by the 7th hour, with peak titers at 78 to 96 hours. Rotation yielded a 90% net increase of virus over those obtained in stationary cultures. RS virus is not stabilized by 1 M $MgCl_2$. The virus can be maintained in 50% glycerin for at least 9 months of storage at $\geq -20°C$. Even at 4°C the decay rate in glycerin is markedly delayed. Preservation in glycerin is superior to 0.5% gelatin or 20% chicken serum. Preservation was not enhanced by lyophilization.

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Peroxidation and Lysosomes in Nutritional Muscular Dystrophy of Chicks.* (28942)

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Nutritional muscular dystrophy is characterized by appearance of white striations in the pectoral and, to a lesser extent, in the leg muscles of chicks deficient in both Vit. E and sulfur amino acids(1). Various studies on similar nutritional dystrophies are reported in the literature but the biochemical mechanism of such pathology is not well understood. Oral administration of large amounts of polyunsaturated fatty acids or injection of their peroxides has been found to cause severe symptoms of encephalomalacia in chicks(2,3). Recently, it has been shown in the dystrophic tissues of Vit. E deficient rabbits(4) and in the genetic dystrophy of mice and chickens(5) that a significant increase occurs in all hydrolytic enzymes of the lysosomes. This paper describes the increases which occur in the hydrolytic enzymes of the lysosomes in the muscles of chicks suffering from nutritional muscular dystrophy produced by a dietary deficiency of Vit. E and sulfur amino acids and by feeding excess linoleic acid.

Methods. One-day-old chicks from Vit. E-depleted White Plymouth Rock × Vantress hens were used in the study. Each treatment was replicated and consisted of equal numbers of male and female chicks. They were housed in electrically-heated battery brooders with wire mesh floors. Feed and water were supplied *ad libitum.* The basal diet used in the experiment reported in Table I was the casein-torula yeast diet previously described by Scott and Calvert(6). In all other experiments the casein-gelatin diet of Calvert *et al*(7) was used with modifications as follows: the casein was methanol-extracted, the stripped lard and diphenyl-p-phenylene diamine (DPPD) were omitted and the diet was supplemented with 0.5% hydrogenated coconut oil, 0.1 ppm selenium (as Na selenite) and 0.0125% ethoxyquin.

Chicks were sacrificed at the end of the 4-week experimental period and scored for

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