piratory depression or stimulation until continuous arterial blood gas analyses become more practical.

Summary. 1. The alveolar O_2, CO_2 tension and R.Q., minute volumes and respiratory rates have been recorded continuously in animals and man under pentothal sodium anesthesia administered by single and intermittent injection.

2. When the alveolar gas concentrations are plotted with the aid of a CO_2-O_2 diagram a typical anesthesia pattern can be shown indicating the regions of depression, compensation and recovery.

3. With intermittent injections of pentothal sodium this typical pattern is repeated.

4. The theoretical significance of this respiratory pattern and its utilization for analysis of quantitative changes in respiration in the study of anesthetic agents is discussed.

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A Technic for the Recovery of Viruses from Crude Feces.

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During the course of studies on the isolation of psittacosis and other viruses from feces, an attempt was made to develop a method, which would allow for direct inoculation of a crude fecal suspension into eggs, for the recovery of a variety of viruses by the use of anti-bacterial agents. Several different test agents were used to determine the applicability of the technic to known viruses in the hope that it might prove useful in the isolation of as yet unknown viruses from the gastrointestinal tract of man and animals. Since penicillin and the sulfonamides are known to inhibit certain viruses of the psittacosis-lymphogranuloma venereum group, they were eliminated from consideration.[1,2] Streptomycin has been shown to have no effect on them[1] nor on several other virus agents[3-6] so that it was useful for study. However, streptomycin alone is not efficacious against all gram positive organisms[7,8] so that it was necessary to find another agent to aid in the suppresion of the gram positive organisms in the feces.

The anti-bacterial agents tested were streptomycin, tyrothrycin in alcoholic solution, tyrothrycin in propylene glycol solution and crystal violet.

Since specimens are frequently submitted for virus isolation studies from patients receiving sulfonamide and/or penicillin therapy, it was of interest to determine whether psittacosis virus strains, known to be susceptible to their action, could be recovered from virus-fecal suspensions containing sulfadiazine or penicillin by the use of antagonistic or inactivating compounds such as p-aminobenzoic acid or cysteine hydrochloride.

The yolk sac route was chosen for the

* Part of this work was carried out under the tenure of a Senior Fellowship in Medical Sciences of the National Research Council while working at the Thorndike Memorial Laboratory, Boston City Hospital.

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inoculation of eggs since (a) it allows for inoculation of a large volume of material and (b) it has been shown to be the route of choice for the inoculation of psittacosis virus as well as an excellent method for the cultivation of a wide variety of viruses.[9-11]

Three viruses were selected as models for this study: psittacosis (strain 6 BC), influenza A (PR8) and mumps.

Material and methods. A 10% suspension of human feces in infusion broth was prepared by homogenization in a Waring blendor. Large sized particles were removed by centrifugation at 800 rpm for 3 minutes. The suspension had a bacterial plate count of 2.1×10^7 organisms per ml. An aliquot of the fecal suspension was sterilized in the autoclave. To separate aliquots of the fresh fecal suspension and of the sterile fecal suspension, an active preparation of one virus was added to give a virus dilution of 1:10. Each different suspension was placed in a separate series of glass ampoules, sealed and stored in the dry icebox. The virus preparations added were: (a) a suspension of yolk sacs infected with psittacosis virus, or (b) allantoic fluid infected with PR8 influenza virus, or (c) allantoic fluid infected with mumps virus.

In testing each of the viruses with a diluent containing different types and amounts of inhibiting agents, serial 10 fold dilutions of the following were prepared:

(a) Virus-sterile feces diluted in plain beef heart infusion broth

(b) Virus-sterile feces diluted in beef heart infusion broth containing the antibacterial agents

(c) Virus-fresh feces diluted in plain beef heart infusion broth

(d) Virus-fresh feces diluted in beef heart infusion broth containing the antibacterial agents

The dilutions were allowed to stand at room temperature (*i.e.* 20–24°C) for 1-2 hours following which 0.25 ml amounts of each were injected into the yolk sacs of 12 embryonated eggs 6 or 7 days old. The eggs were placed in an incubator at 35°C and were candled daily. Eggs inoculated with 10 fold dilutions of the virus + fresh feces in plain broth up through 10^{-6} died within 24-72 hours with gross evidence of contamination while eggs inoculated with the suspension diluted in broth containing the inhibitors died in from 2 to 10 days depending on the particular virus and the degree of its dilution. As the latter group of eggs died: (a) smears were made of the yolk sacs, stained and examined to detect bacteria, and, in the case of eggs injected with psittacosis virus, were stained by Machiavello's method to demonstrate the typical elementary bodies, (b) in some instances, the yolk sacs were cultured on blood agar plates and in thioglycollate broth to test the reliability of the smears as an indicator of bacterial contamination, (c) in the case of the eggs inoculated with suspensions containing influenza or mumps viruses, the yolk, allantoic and amniotic fluids of the egg were tested for their ability to agglutinate chicken red cells as an index of the presence of virus.

As eggs in the group inoculated with the virus-sterile feces suspension diluted in plain broth or broth containing the bacteriostatic agents died, they were tested as noted above for the presence of the specific virus injected. These two groups served as controls for any possible deleterious action of the bacteriostatic agents on the virus itself.

In order to see whether virus could be recovered from specimens containing penicillin or sulfonamides, the following experiment was carried out: (a) 1000 U penicillin were injected into each egg of a series along with the suspension of the virus in sterile feces. Then 15 mg of cysteine hydrochloride[12] were injected into different groups of these eggs at intervals up to 24 hours. (b) A second series of eggs was injected with a suspension

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of virus in sterile feces along with 10 mg of sulfadiazine. At intervals up to 24 hours, 1 mg of p-aminobenzoic acid[13,14] was injected into different groups of these eggs. In all instances, the eggs died after incubation at 35°C for several days. Smears of the yolk sacs were stained and examined for the presence of psittacosis virus elementary bodies.

Results. Using a diluent of beef heart infusion broth containing 10 mg of streptomycin and 2 mg of tyrothrycin per ml (containing 1% glycerine to give a stable suspension of the alcoholic solution of tyrothrycin added to broth), it was possible to recover the viruses of psittacosis, influenza or mumps which had been added to a fresh suspension of feces without any evidence that the bacteriostatic agents used in the diluent for the suppression of bacterial contaminants had any deleterious effect on the virus. In fact, in many instances, even the eggs injected with a 10^{-1} dilution of the fecal suspension of virus (10^{-2} dilution of virus infected fluid) could be shown to contain virus free of detectable bacteria even though this dilution was shown to contain at least 2.1×10^6 aerobic bacteria per ml. High dilutions (*i.e.* 10^{-2} or 10^{-3}) almost always gave virus free from bacterial contaminants. This technic was found useful in the isolation of psittacosis virus from the ground-up intestines and feces of mice infected with the virus by the intravenous route.

In some experiments, a solution of tyrothrycin in propylene glycol was used but this solvent was found to inhibit the growth of the viruses. Therefore, the alcoholic solution was used subsequently with the addition of 1% glycerine to stabilize its suspension in broth.

It is important to point out that tyrothrycin can be used as a bacteriostatic agent in materials to be tested in eggs only if the yolk sac route of inoculation is used since it is toxic in 0.2 mg amounts in the allantoic or amniotic sacs.

Crystal violet in amounts up to 0.25 mg was tolerated when injected into the yolk sac of embryonated eggs but was not effective in controlling the gram positive bacteria in fecal suspensions tested.

It was found that the injection of (a) 15 mg of cysteine hydrochloride or (b) 1 mg of p-aminobenzoic acid into the yolk sac any time up to 24 hours after the injection of as much as (a) 1000 U of penicillin or (b) 10 mg of sulfadiazine respectively in the virus-feces inoculum would permit the recovery of psittacosis virus (strain 6 BC) which was known to be sensitive to 250 U of penicillin[1] or 0.5 mg of sulfadiazine.[14]

Summary. Using an infusion broth diluent containing 10 mg streptomycin and 2 mg tyrothrycin per ml, it was possible, by injection into the yolk sacs of 6-7-day-old embryonated eggs, to recover free from bacteria: psittacosis, influenza or mumps viruses from suspensions of fresh feces to which they had been added. The diluent had no apparent injurious effect on these viruses.

P-aminobenzoic acid was found to counteract the inhibitory action of sulfadiazine allowing for the recovery of psittacosis virus when the virus was suspended in materials containing the sulfonamide and injected into eggs. The injection of the p-aminobenzoic acid could be delayed up to 24 hours when injected in 1 mg amounts after a virus suspension containing 10 mg of sulfadiazine. In a similar series of experiments it was found that 15 mg cysteine hydrochloride would overcome the inhibitory action of 1000 U penicillin on psittacosis virus when given as long as 24 hours after the virus suspension containing this antibiotic. These antagonistic compounds made it possible to recover psittacosis virus from materials containing large quantities of sulfadiazine or penicillin in spite of the fact that this strain of psittacosis virus is known to be susceptible to much smaller quantities of each of these therapeutic agents.

This technic was useful in the isolation of virus from the intestines of mice infected with psittacosis virus and may find application in the isolation of viruses from the gastrointestinal tract of other animals or man.

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