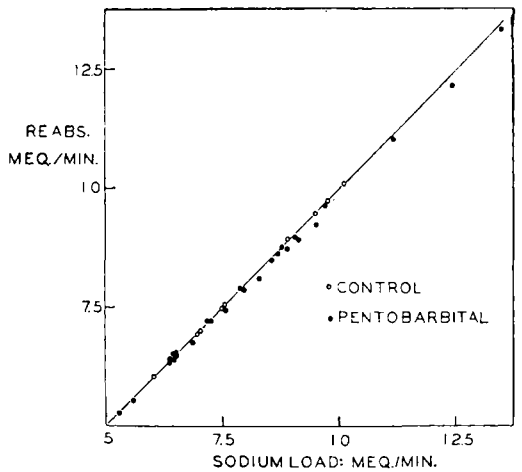

FIG. 1.

Showing the relationship of sodium reabsorption to load (filtration rate × plasma sodium conc.) in 9 exp. in 3 dogs. Duration of anesthesia was approximately 5 hr.

restrained on a padded dog board.

Creatinine clearance was used to measure glomerular filtration rate. Its concentration was measured in tungstate filtrates of the plasma and diluted urine by the alkaline picrate method. All analyses were in duplicate. Sodium was measured in diluted plasma and urine with an internal standard flame photometer. A correction factor of 0.95 was applied to the plasma sodium concentration for the Donnan effect.

Results. Emphasis is placed in Table I on the influence on tubular reabsorption of sodium as given in the last column in terms of the percent of the filtered load which is reabsorbed by the tubules before and during anesthesia. During the control periods an average of 99.46% (S.D. ± 0.31) is reabsorbed in all experiments. With pentobarbital, reabsorption averages 98.75% (S.D. ± 0.78) in all animals. Fig. 1 graphically illustrates the relation of reabsorption to load for each stage of observation. The solid line shows the trend of the control periods.

When the data on sodium excretion in Table I are examined critically it is seen that in every animal a perceptible, though variable, increase results during anesthesia. On the average, this represents an increase in excretion of 66 micro-equivalents over the control average of 41 micro-equivalents per minute. This increase in excretion results from the less than one percent decrease in reabsorption noted above. When this difference in reabsorption was analyzed statistically, it proved to be significant (P of $<.01$). However, in terms of physiological alterations generally observed during experimental procedures, *e.g.*, sodium loading(4) such small variations are considered unimportant.

Summary and conclusions. The influence of prolonged light surgical anesthesia with sodium pentobarbital on the renal tubular sodium reabsorptive ability was investigated in dogs initially given 50 cc per kg of isotonic saline by stomach tube. During time intervals averaging 5 hours, the tubules continued to reabsorb an average of 98.75% of the filtered load. The conclusion is reached that this anesthetic agent has a negligible effect on sodium reabsorption.

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Propagation of Pathogenic Fungi in the Yolk Sac of Embryonated Eggs.* (18455)

JOHN W. BRUECK AND G. JOHN BUDDINGH.

From the Department of Microbiology, Louisiana State University School of Medicine, New Orleans.

Investigations now in progress in this laboratory show that the yolk sac of embryonated eggs provides superior nutritional and environmental conditions for the propagation of pathogenic fungi. That the chick embryo method should prove to be useful for the study of

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fungi was first suggested by Goodpasture(1). The observations reported by Moore(2) on the infection of the chorio-allantois with several fungi represent the only significant contribution to this problem up to the present time. The preliminary results of studies now in progress will be presented in the present report.

Methods. The present studies began with an attempt to produce infection of the chorio-allantois with *Actinomyces bovis*. The extra embryonic membranes were found to be indifferently suited for this purpose. Chance inoculation of the yolk sac resulted in the abundant growth of *A. bovis*. This observation led to a more thorough investigation of the possibilities of this method of inoculation for the propagation of pathogenic fungi and the study of mycotic infections. Embryos of 7 to 9 days incubation proved to be most suitable for this purpose and were used throughout. The microorganisms were introduced into the yolk sac of living embryos through a small slit in the eggshell by means of a tuberculin syringe and 22 gauge needle. The initial inoculum in the case of most of the fungi studied consisted of a crude saline suspension of the microorganism cultured on media appropriate for it. Embryos were sacrificed at daily intervals following inoculation. The course of infection was observed by studying appropriately stained smears prepared from aspirated yolk or from small portions of yolk membrane. Subcultures to artificial media suited to each type of fungus were made at regular intervals. The entire yolk of selected embryos were fixed in Zenker's (10% acetic acid) solution from which suitably sized blocks were cut for embedding and microscopic section. As indicated below most of the fungi studied were propagated in series in the yolk sac through few to numerous transfers. Subinoculations for passage to fresh embryos were usually made on the 5th or 6th day following inoculation.

Results. A brief summary of the results obtained with each of the fungi investigated in this manner will be given as follows.

Actinomyces bovis. A saline suspension prepared from a blood agar shake culture† was used to initiate infection of the yolk sac of 8- to 9-day embryos. Within 48 to 72 hours numerous microorganisms typical of *A. bovis* were demonstrable in Gram stained smears of aspirated yolk. Transfers to fresh embryos were made on the 5th or 6th day following inoculation. The inoculum consisted of 0.1 ml of infected yolk emulsified by shaking in a flask with sterile glass beads. Thirty-two successive uninterrupted passages were maintained before the series was purposely terminated. Microscopic sections of the infected yolk sacs show numerous clumps of the microorganisms with typical "ray fungus" appearance. Control studies indicate that *A. bovis* is maintained in its typical morphological and cultural characteristics by passage in the embryonic yolk sac. Embryos survive yolk sac infection until hatching. Hatched chicks develop normally although *A. bovis* can be recovered from the absorbed yolk sac in 1-month-old chicks inoculated during incubation. Other such chicks kept until 6 to 8 months of age showed no evidence of infection and were free of *A. bovis*.

Nocardia asteroides. This microorganism grows very readily in the chorioallantois as well as in the yolk sac. In both of these sites *N. asteroides* proliferates so rapidly that the embryo is overwhelmed and dies within 3 to 4 days following inoculation. It apparently can be maintained indefinitely in serial passage in the membrane or within the yolk sac. The limited observations made indicate that much can be learned regarding the behavior of this group of microorganisms by a study of its effect on the chick embryo.

Nocardia intracellularis.‡ Unlike *N. asteroides* this microorganism thrives very poorly in the chorio-allantois but grows readily in the yolk sac. Within 48 to 72 hours following inoculation numerous microorganisms can be demonstrated in Gram or acid fast stained smears prepared from the yolk. It has been maintained through 5 successive transfers.

1. Goodpasture, E. W., *South. Med. J.*, 1933, v26, 418.

2. Moore, M., *Am. J. Path.*, 1941, v17, 103.

† Obtained from Dr. Norman F. Conant.

‡ Obtained from Dr. Norman F. Conant.

Further detailed studies are required for an elucidation of the behavior of this interesting microorganism.

Sporotrichum schenkii. Infection of the yolk sac of 8-day-old embryos was initiated with a 10% saline suspension prepared from a subcutaneous nodule removed surgically from a patient with typical sporotrichosis. Within 72 hours after inoculation Gram stained smears show the yolk to contain both the cigar-shaped bodies and mycelial elements of the microorganism in abundance. Microscopic sections reveal that *S. schenkii* localizes in the immediate vicinity of the cells of the yolk membrane. It remains to be determined whether or not continued passage will result in the exclusive adaptation of the cigar-shaped bodies or of the mycelial form. Studies of its spread from the yolk to the embryo should prove to be rewarding.

Histoplasma capsulatum.§ Infection of the yolk sac of 7- to 9-day-old embryos was readily initiated with 0.1 ml of a slightly turbid saline suspension of the yeast form of this fungus. Within 48 to 72 hours and from then on until hatching heavily encapsulated forms are seen in abundance in smears stained by Wright's or Giemsa's method. Embryos survive to hatching without evidence of infection. The yeast form has been maintained by serial passage at 8 to 10 day intervals over a period of 6 months. Attempts to establish infection of the chorio-allantois with the yeast form obtained from blood agar slant cultures were consistently unsuccessful. Inoculation with yolk from the 10th passage produced grossly visible lesions in the membrane within 72 to 96 hours. Smears from the lesions show the encapsulated microorganism in abundance. Sections indicate a widespread intracellular invasion of the fibroblasts and monocytes of the mesoderm. Inoculation of the yolk sac with a suspension of the mycelial phase produces a rapid and complete transformation into the yeast phase within 48 to 72 hours.

Cryptococcus neoformans. Infection of the yolk sac was initiated with 0.2 to 0.3 ml of spinal fluid from a clinical case of the disease. This microorganism increased in numbers with marked rapidity within the yolk sac as demonstrated by examination of fresh mounts or India ink preparations. Exceedingly large capsules are elaborated. Invasion of the embryos occurs without apparently adversely affecting its development. Encapsulated yeast cells are readily demonstrated in the liver of chicks hatching under these circumstances.

Coccidioides immitis. Inoculation of the embryonic yolk sac with a suspension of the mycelial growth of a culture of *C. immitis* results in its rapid and almost complete reversal into the spherule phase. Detailed studies will be required to elucidate all of the steps which can readily be observed in this transformation. This microorganism is best observed by means of fresh mounts of the infected yolk. Studies are now in progress in an effort to adapt it to the yolk sac by continuous passage.

Discussion. These preliminary observations are presented with the purpose of indicating the great potentialities of the chick embryo method for the propagation of pathogenic fungi and for the study of the behavior of these microorganisms. As in the case of other pathogens, *e.g.*, the rickettsiae, the yolk sac, rather than other parts of the embryonated egg, has been found to be the site of choice for investigative work with pathogenic fungi.

The method has proven of considerable aid for the diagnostic isolation of some of these agents. By the addition of 50 units of penicillin and 50 mg of streptomycin per cc to exudates or suspensions of lesions obtained from 3 different patients, *Actinomyces bovis* was isolated and identified by yolk sac inoculation. *Sporotrichum Schenkii* has been thus far isolated and identified by this means in 2 separate instances. It should prove of considerable value in the isolation of *Histoplasma capsulatum* as well as other mycotic agents of disease. A new approach is also offered for the testing of the effectiveness of therapeutic and antibiotics by the yolk sac method. The present studies are being pursued further with especial emphasis on the early stages of the pathogenesis of these various mycotic

§ Obtained from Dr. Norman F. Conant.

agents of disease.

Summary. The yolk sac of the developing chick embryo has proved to be highly suitable for the propagation of the following pathogenic fungi: *Actinomyces bovis, Nocardia asteroides, Nocardia intracellularis, Sporotrichum Schenkii, Histoplasma capsulatum, Cryptococcus neoformans* and *Coccidioides immitis.* This method promises to be of great value for the experimental study of these mycotic infections. It is quite likely that many other of the pathogenic as well as non-pathogenic fungi may be studied with profit by infection of the yolk sac of chick embryos.

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Effect of Cortisone on Passively Induced Skin Hypersensitivity in Man. (18456)

GENE H. STOLLERMAN, SIDNEY J. RUBIN, AND CHARLES M. PLOTZ.
(Introduced by David Seegal)
From the Maimonides Hospital of Brooklyn, and The Department of Medicine. State University Medical Center at New York.

Beneficial effects following the use of ACTH and cortisone have been described in status asthmaticus, hay fever, serum sickness, atopic dermatitis and gastrointestinal allergy (1-7). The tuberculin test has also been inhibited by these two agents(8,9) and cortisone has prevented anaphylactic shock in sensitized mice(10). Germuth and Ottinger (11) demonstrated that the concomitant administration of a sensitizing antigen and either ACTH or cortisone to normal rabbits prevented the development of hypersensitivity and suppressed antibody formation. The same animals, however, manifested the Arthus phenomenon if passively sensitized. Although these reports suggest that cortisone and ACTH have an effect on immune responses, certain manifestations of hypersensitivity have not been suppressed by the administration of these hormones. ACTH or cortisone, given prior to the injection of a shocking dose of tetanus antitoxin horse serum in sensitized guinea pigs, failed to alter the subsequent course of anaphylactic shock(8,12,13). The administration of cortisone to sensitized rabbits has also been ineffective in altering the active or passive Arthus phenomenon (8,14).

The present study was undertaken to determine the effect of cortisone on passively

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