cation" from which it recovered with difficulty. There is the impression that massive doses of vit. A were of some help. (This therapy was suggested by the observation of signs of vit. A deficiency, such as a scaly skin, sublingual ulcers, blindness, reduced sensitivity of the skin, etc.).

Two dogs (HC-27 and HC-28, Table II) in a state of hepatic coma produced by ligation of the common hepatic artery, combined with a classical Eck fistula(4) showed albumin percentages of 31.7 and 37.7, *i.e.*, not markedly low values. One animal, HC 34 (Table II), which had recovered from hepatic coma and was still alive after 13 months, showed a normal pattern.

Summary. Successive surgical interference with the blood supply to the liver is followed by a decrease in the total proteins. The greater the interference with the circulation, the lower is the albumin level. The higher globulin level is due to an increase in the $\beta + \phi + \gamma$ fractions. It is suggested that a longer lapse of time after the experimental reduction of the blood supply permits the establishment of an adequate collateral circulation and tends to reestablish the normal production of proteins by the liver. In acutely induced fatal hepatic coma no large changes in the electrophoretic patterns of the dog plasmas were observed during the short survival time.

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Isolation of *M. tuberculosis* by Inoculation of the Yolk Sac of Embryonated Eggs.* (19701)

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Previous investigators(1,2) have demonstrated that *M. tuberculosis* proliferates with great rapidity in the yolk sac of embryonated eggs. These observations were made on embryos inoculated into the yolk sac with pure cultures of the microorganism. Careful search of the literature gives no indication that the yolk sac has been utilized for the rapid isolation of *M. tuberculosis* from materials collected from patients for the purpose of a more rapid laboratory diagnostic procedure. Investigations which are being performed in this laboratory indicate that inoculation of the yolk sac of chick embryos provides a rapid means for establishing the etiological diagnosis with materials from patients with suspected tuberculosis. A preliminary report of the results obtained thus far appears to be warranted.

Methods. Age of embryos and method of inoculation. Embryos of 5 to 8 days preliminary incubation are suitable. The yolk sac is inoculated through a small drill hole in the shell by means of a syringe and 20- or 22-gauge needle. After injection the drill hole is covered with melted paraffin.

Preparation preliminary to inoculation of materials tested. Only such materials collected from patients were tested in which a thorough search by the established methods, including concentration procedures, failed to reveal the presence of acid fast bacilli by microscopic examination. Six embryos each were inoculated into the yolk sac with each specimen.

Spinal fluid. Spinal fluid collected under aseptic precautions requires no preliminary treatment. The amount of fluid available determines the size of the inoculum which can vary from 0.2 to 0.5 ml per yolk sac.

Pericardial and pleural exudates. A preliminary test by inoculating 0.2 ml amounts

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on a blood agar plate and into thioglycollate broth is run to assure bacteriological sterility. The size of the inoculum varies from 0.2 to 0.5 ml per yolk sac. Bacterial contaminants are controlled by the addition of appropriate antibiotics.

Gastric washings and sputum. These are digested in equal parts 4% NaOH at 37°C until all mucus and visible particles are dissolved and then centrifuged at 2000-3000 r.p.m. for 30-60 minutes. The supernatant is discarded and the sediment is carefully titrated to normal with 6% H_2SO_4. The sediment is then suspended in 1 to 2 ml of saline which contains 1000 units of penicillin per ml. Usually 0.1 to 0.2 ml of this suspension per yolk sac is used as inoculum.

Tissues and exudates. Tissues are ground with sterile alundum and suspended in saline containing 1000 units of penicillin per ml. Exudates too thick for aspiration are diluted in saline containing penicillin. 0.1 to 0.2 ml amounts are inoculated into each yolk sac.

Other materials. No opportunity for testing urine or stool samples has thus far presented itself. The sediment from the centrifuged urine specimens should present no difficulty. The presence of Gram negative bacterial contaminants may be controlled with the addition of chloromycetin. Stool specimens would require the judicious addition of antibiotics.

Incubation and examination of inoculated embryonated eggs. Inoculated eggs are incubated at 35 to 37°C. They are candled daily and embryos which die during the first 4 days are discarded. Beginning the fourth day after inoculation one embryo from each group is examined daily. The yolk sac is transferred with aseptic precautions to a sterile Petri dish.

Demonstration of acid fast bacilli in the yolk sac. Approximately 1 ml of the yolk is transferred to a 2.5 x 10 cm test tube and 20 ml of 15% phenol is added. The tube is sealed with a rubber stopper, shaken vigorously for 5 minutes and allowed to stand for at least 2 hours. By means of a capillary pipette a small amount of the fatty layer at the surface is carefully removed and smeared extremely thinly on the surface of one or more chemically clean slides. After drying and heat fixation the smears are stained by the Ziehl-Neelsen method and examined microscopically for the presence of acid fast bacilli. In most instances preparations made in this manner from the yolk sacs on the 4th day following inoculations contain typical acid fast bacilli in abundance.

Identification of the acid fast microorganisms as M. tuberculosis. Tuberculin negative healthy guinea pigs were inoculated into the groin and intraperitoneally with 0.5 to 1 ml from at least one yolk sac from the group of embryos used for each test. Three weeks later they were again tuberculin tested. Positive reactors were sacrificed and the presence of typical acid fast bacilli demonstrated in smears from the lesions.

Results. A total of 50 specimens of various types were subjected to yolk sac inoculation. Of these 39 were derived from patients with various types of tuberculous infection. Eleven specimens were obtained from known negative controls. Acid fast bacilli were demonstrable within 4 to 6 days after inoculation in every instance in which positive results were obtained. In each of these cases the acid fast bacilli demonstrated in the yolk sac cultures have proven to be *M. tuberculosis* by guinea pig inoculation. Acid fast bacilli have not been demonstrable in yolk sacs inoculated with materials from the negative controls. Guinea pigs injected with yolk from embryos inoculated with the control material have developed no evidence of tuberculosis. Table I summarizes the results thus far obtained.

Discussion. The yolk sac of the developing chick embryo apparently provides exceptionally favorable nutritional and environmental conditions for the rapid proliferation of *M. tuberculosis.* Within 4 to 6 days after inoculation acid fast bacteria are readily demonstrable from specimens in which these microorganisms are not demonstrable by direct examination. By this means the time period for detecting *M. tuberculosis* is shortened considerably as compared with that required by the established cultural methods and by animal inoculation.

The method has been found to be especially valuable in establishing the diagnosis

TABLE I. Results of Inoculation of Chick Embryo Yolk Sac for the Demonstration of *M. tuberculosis.*

Type of specimen		No. tested	Acid fast bacilli in yolk sac Pos.	Neg.	Inoculation of yolk into guinea pigs Pos.	Neg.
Spinal fluid	Tests	13	13	0	13	0
	Controls	6	0	6	0	6
Sputum	Tests	7	7	0	7	0
	Controls	0				
Gastric washings	Tests	5	5	0	5	0
	Controls	2	0	2	0	2
Pleural fluid	Tests	6	6	0	6	0
	Controls	2	0	2	0	2
Peritoneal fluid	Tests	3	3	0	3	0
	Controls	0				
Pericardial "	Tests	1	1	0	1	0
	Controls	0				
Tissue	Tests	4	4	0	4	0
	Controls	1	0	1	0	1
Total tests		39	39	11		
Controls		11				

in cases of tuberculous meningitis, tuberculous pericardial, pleural, and peritoneal effusions. Limited experience thus far indicates its usefulness in following the effect of therapy in cases of tuberculous meningitis.

No attempts have as yet been made in this laboratory to determine whether or not the method can be adapted to assess the effect of therapeutic agents. It also may prove to be useful in determining the development of drugfastness of strains of *M. tuberculosis.*

These investigations are being continued in order to assess more accurately the usefulness of this method as a practical laboratory procedure for the more rapid etiological diagnosis of tuberculosis infections.

Conclusions. Inoculation of the yolk sac of 5- to 8-day embryonated eggs provides a rapid method for demonstrating the presence of *M. tuberculosis* in materials collected from patients with suspected tuberculous infections. Acid fast bacilli are readily demonstrated in the yolk of embryos 4 to 6 days after inoculation.

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Influence of Niacin and Priscoline on Ketonuria, Liver Glycogen and Liver Lipid.* (19702)

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It has been established that when niacin is given in relatively large amounts to diabetic rats or normal fasting rats a marked ketonuria is exhibited by many of the animals(1,2).

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