of isolation it was possible to obtain purified crystals showing similar characteristics as those reported by Foster. It appears therefore that during the growth of tubercle bacillus riboflavin may be converted into lumichrome. This would furnish an explanation, at least partly, for the high incidence of riboflavin deficiency among tuberculous patients.

The finding that addition or increased concentration of riboflavin in the medium did not influence the growth of tubercle bacillus would lead us to believe that there is no danger of promoting the growth of the organism by therapeutic administration of riboflavin.

Summary. A slightly virulent strain of human tubercle bacillus was cultured in a vitamin free medium or in media to which riboflavin with or without other vitamins and biotin were added.

It was found that the presence or absence of riboflavin made no difference in the growth of the tubercle bacillus. On the other hand during the growth of the organism there was found a slow but steady disappearance of riboflavin from the medium. The presence of lumichrome crystals in the media containing riboflavin after growth of the tubercle bacillus indicated that the loss of the vitamin was due to conversion to lumichrome.

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17002

Recovery of Psittacosis Virus from Chicks Hatched from Inoculated Eggs.

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Investigations[1] of psittacosis infections in psittacine and other birds have shown that active infection with the virus (*Miyagawanella psittaci*) is encountered in young birds, even nestlings. This has suggested that infection is transmitted from latently infected adults either directly to the nestling or congenitally through the egg.[1] Furthermore the virus has been reported to have been recovered from ovaries and egg yolk in the oviduct of parakeets.[1] The possibility exists therefore, that congenital transmission may be important in dissemination of the virus among psittacine birds, pigeons, and other susceptible species.

In this laboratory an effort was made to secure experimental evidence concerning transmission of *M. psittaci* by the egg in avian species. Isolations of virus were attempted from chicks hatched from eggs which had been inoculated during various stages of embryonic development.

Although psittacosis infection in chickens is not known to be widespread this species has been found naturally infected[2] and the ready accessibility of chicken eggs and familiarity with technics for handling the infection in them made this species a logical choice for preliminary work.

The strain of virus used, No. 4, had been isolated in this laboratory from a psittacine bird (*Aratinga* sp.) and maintained by serial egg passage for at least 14 transfers. It was approximately 10^4 times more lethal for white mice by intracranial inoculation than by the intraperitoneal route and was well adapted to growth in allantoic cavity and yolk sac of the developing chick embryo.

[1] Meyer, K. F., *Medicine*, 1942, **21**, 175.

[2] Meyer, K. F., and Eddie, A., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 522.

TABLE I.
Recovery of *Miyagawanella psittaci* from Chicks Hatched from Eggs Inoculated after Various Periods of Incubation.

Days, incubation	No. eggs inoculated in yolk sac	No. eggs inoculated in allantoic cavity	No. chicks hatched	Recovery of virus from chicks by age in days*					
				0-1	2-9	10-19	20-29	30-39	Total
0	83		14	0/1	0/5				0/6
4	56		12	0/4	0/8				0/12
7	53	32	12	0/4	0/2				0/6
8	126	48	8	1/8†					1/8
9	67		12	0/4	0/5	0/2			0/11
10	65		35	0/10	0/9	0/7	0/8		0/34
11	31		16	0/4	0/5	0/3			0/12
12	57		14	0/5	0/2	0/2			0/9
14	202		88	14/20	8/20	2/13	1/15	0/10	25/78
15	109		82	4/15	1/15	2/17	0/10	0/13	7/70
16	58		39	0/2	3/7	1/10	0/18		4/37
18		31	26	1/5	0/5	0/3	0/13		1/26
Total	907	111	358	20/85	12/83	5/57	1/64	0/23	38/304 (12.5%)

* No. chicks infected/No. chicks examined.
† Infected chick hatched from egg inoculated in allantoic cavity.

The eggs were held at 38° C throughout the entire time of incubation. After hatching the chicks were kept in a brooder for at least one week.

After being incubated for various periods from 0 to 18 days the developing embryos were inoculated into the yolk sac or allantoic cavity with .25 ml of a dilution of virus in physiological saline found by previous titration to kill approximately one half the embryos. Nearly all chicks which hatched were autopsied and virus isolations attempted at various time intervals from 1 to 39 days after hatching. The spleen, liver, and kidney of each chick were ground together in a mortar, suspended in physiological saline and injected intracranially into 5 mice. In some instances these organs were suspended and inoculated separately. The microscopic demonstration of typical clusters of elementary bodies in impression smears taken from the brains of mice dying from 3 to 7 days after inoculation and stained by the Machiavello method was taken as evidence of the presence of *M. psittaci* in the chicks.

The table presents data concerning 1018 eggs inoculated in the yolk sac or allantoic cavity after various periods of incubation. Three hundred fifty-eight chicks were hatched. Of these 304 were examined and the presence of *M. psittaci* was demonstrated in 38 of them (12.5%). The table also shows the number of chicks infected with virus in relation to the number examined for various ages in days. A few more than half of the isolations were from chicks autopsied within 24 hours after hatching; some of these chicks were moribund. The proportion of infected chicks to those examined decreased with the advancing age of the chick. The oldest chick from which virus was isolated was 22 days old, and the egg from which it had hatched had been inoculated on the 14th day of incubation.

With the exception of the moribund recently hatched chicks, all appeared in good condition and the only abnormality noted at autopsy was an enlarged spleen in a few instances which was not correlated with the recovery of virus. Virus was recovered from separate suspensions of the kidney, spleen, or liver of 7 and 14 day old chicks. Sixteen embryos dying from the 18th to 21st day of development which had been inoculated on the 8th day were examined and virus was recovered from the internal organs of 9 of them.

Summary. The data here presented indicates that chicks will hatch from eggs which have been infected with *M. psittaci* during the course of embryonic development and will survive in apparently good condition while harboring the virus in the organs for at least

22 days after hatching. While these experiments do not furnish evidence for the congenital transmission of the virus in the chicken, it is possible that in a more susceptible species the virus could be carried more effectively through the developmental and hatching period after either experimental or congenital infection.

17003 P

Studies of the "Thrombin" Effect of Fresh Serum.*

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A factor capable of producing prothrombin conversion has been described in a previous report.[1] This factor can be easily demonstrated after thromboplastin is added to serum which contains no thrombin and only traces of prothrombin. The resultant mixture of serum and thromboplastin causes rapid coagulation of a 0.01 M oxalated plasma.† Suitable control studies revealed that this factor (designated "prothrombin-converting factor"), is not thrombin, but a substance which requires prothrombin to mediate a coagulation effect on fibrinogen. Furthermore, the coagulation of 0.01 M oxalated plasma by the serum-thromboplastin mixture could not be explained as a separate action of either component.

A study has been made of the coagulation effect of *fresh serum alone* on 0.01 M oxalated plasma. It has been assumed by other workers,[2,3] that the residual clotting action of fresh serum is related to thrombin. After conversion of fibrinogen to fibrin, thrombin was believed to combine with albumin to form inactive metathrombin,[2] or that thrombin was destroyed by an enzyme.[3] Our results reveal that the residual coagulating action of fresh serum is almost entirely due to "prothrombin-converting factor". This data confirms and extends the observations of Bordet and Gengou[4] who showed that serum coagulated whole oxalated plasma much more rapidly than it did oxalated plasma from which prothrombin was removed by adsorption with tricalcium phosphate.

The coagulation effect of fresh serum was studied by the following methods. Blood was withdrawn from human donors and placed in glass tubes with internal dimensions of 1.0 x 10.0 cm. Ordinary care was used in collecting the specimens to avoid contamination with tissue thromboplastic substances. Within 15 minutes of collection, the tubes containing the blood were placed in a 26°C water bath. Approximately 50 minutes later the serum was separated from the clot. One-tenth cubic centimeter of serum (kept at 26°C) was pipetted into 0.1 cc of a 0.01 M oxalated plasma and the coagulation time determined.

It was observed that serum, which coagulated plasma rapidly when tested within 70 minutes of the time of collection from the donor, was incapable of coagulating a buffered‡ fibrinogen solution (200-300 mg %

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1 Jacox, Ralph F., to be published in *J. Clin. Invest.*

† All plasma used in these experiments was prepared by adding 9.0 cc of whole blood to 1.0 cc of 0.1 M potassium oxalate solution.

2 Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

3 Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.*, 1940, **24**, 169.

4 Bordet, J., and Gengou, O., *Ann. Inst. Pasteur*, 1904, **18**, 98.

‡ Collidine buffer (pH 7.3) was used in all experiments.[5]