

total plutonium body burden is much less dramatic than is its effect on skeletal deposition. By a comparison of the total injected dose retained in the Pu controls and the early zirconium treated animals it appears, however, that this plutonium retained in soft tissue may be lost from the body at a considerably faster rate than plutonium deposited in the skeleton. It also seems evident that the soft tissue plutonium is not redistributed to the skeleton after termination of the zirconium citrate treatment (rats 7 and 8).

The late zirconium citrate treatment had perhaps a slight effect both in decreasing the amount of skeletally deposited plutonium and in increasing the amount in the residual carcass. Further studies would be required to establish the significance of these effects. No differences of particular significance were observed in the plutonium content of livers and spleens of rats in the different groups.

Summary. Serial zirconium citrate injections during the month immediately following the administration of plutonium were found to effect a 6-fold reduction in the skeletal

deposition of plutonium and to increase the retention of plutonium in the soft tissues. Therapy with zirconium, commencing a month after the plutonium injection, was found to have little, if any, effect on the distribution of plutonium.

Sincere appreciation is extended Dr. Joseph Katz, Mrs. Willa D. Oakley and the Radiochemistry Laboratory for their assistance during the course of this work.

1. Schubert, J., *J. Lab. Clin. Med.*, 1949, v34, 313.
2. Schubert, J., and White, M. R., *J. Biol. Chem.*, 1950, v184, 191.
3. Kavin, B., Copp, D. H., and Hamilton, J. G., Univ. of Calif. Radiation Lab. Unclassified Document No. 812.
4. Schubert, J., Finkel, M. P., White, M. R., and Hirsch, G. M., *J. Biol. Chem.*, 1950, v182, 635.
5. McClinton, L. T., and Schubert, J., *J. Pharm. Exp. Therap.*, 1948, v94, 1.
6. Katz, J., Manuscript in preparation.
7. White, M. R., and Schubert, J., *J. Pharm. Exp. Therap.*, 1952, v104, 317.

Received June 30, 1953. P.S.E.B.M., 1953, v33.

Use of Baby Chicks for Isolation of Leptospires. (20469)

WARREN G. HOAG, WILLIAM S. GOCHENOUR, JR., AND ROBERT H. YAGER.

From the Veterinary Division, Army Medical Service Graduate School, Washington, D. C.

Guinea pigs(1), embryonated hens' eggs (2), Syrian golden hamsters(3), and laboratory white mice(4) have been employed in the isolation of leptospire from blood, tissues, and other body fluids. Their employment for this purpose has been limited by factors of cost, availability, and facilities appropriate for their maintenance. The successful cultivation of leptospire in embryonated hens' eggs suggested the possible utilization of baby chicks for recovery of these organisms.

Methods. Two-day-old chicks were inoculated intraperitoneally with 0.5 ml of 5-day-old culture of *L. pomona*, S91.(5) in Fletcher's semi-solid medium(6). Subsequent culture generations were lethal for 20 g golden

hamsters. Heart's blood cultures were made in both Schuffner's modification of Verwoort's medium(6) and Fletcher's semi-solid medium on the 4th, 7th, 8th, 9th, 10th, 11th, 14th, and 21st days post-inoculation.

Groups of chicks were sacrificed on the 9th, 11th, 14th, and 21st days post-inoculation. Kidney and liver emulsions in 0.85% NaCl solution were cultured separately on both leptospiral mediums. These cultures were incubated at 30°C for 28 days and examined by darkground microscopy at 7-day intervals.

Serums were obtained by clot-squeeze separation from blood drawn by cardiac puncture on the 9th, 11th, 14th, and 21st days post-inoculation. These serums were examined for presence of leptospiral antibodies by the

TABLE I. Results of Heart's Blood Cultures.

Chick No.	Days post-inoculation							
	4	7	8	9	10	11	14	21
1	+	+	ND	+				
2	+	+	ND	+				
3	+	+	ND	+				
4	+	ND	+	ND	ND	+		
5	+	ND	+	ND	ND	+		
6	+	ND	+	ND	ND	—		
7	+	ND	ND	ND	+	ND	+	
8	+	ND	ND	ND	—	ND	—	
9	+	ND	ND	ND	+	ND	+	
10	ND	ND	ND	ND	ND	ND	ND	+
11	ND	ND	ND	ND	ND	ND	ND	+
12	ND	ND	ND	ND	ND	ND	ND	—
13	ND	ND	ND	ND	ND	ND	ND	+

* Animals sacrificed.

Key: + = Leptospire recovered. — = No leptospire recovered. ND = Not done.

TABLE II. Results of Culture of Tissue Emulsion.

Days post-inoculation	Liver		Kidney	
	Pos.	Neg.	Pos.	Neg.
9	1	2	1	2
11	2	1	2	1
14	2	1	2	1
21	0	4	0	4

TABLE III. Agglutinating Antibody Response.

Days post-inoculation	Titer against <i>L. pomona</i>		
	Less than 1:100	1:100	Greater than 1:100
9	3	0	0
11*	2	0	0
14*	1	1	0
21	1	2	1 (6400)

* Serum samples available on only 2 chicks.

agglutination-lysis technic(6).

Results. The results of heart's blood cultures are shown in Table I. The results of cultures of tissue emulsions are shown in Table II.

It is apparent from the above that leptospiremia persisted in all chicks examined through the 9th day post-inoculation. It is significant that leptospire could not consistently be demonstrated in cultures of either kidney or liver emulsions.

Table III lists the results of serological examination of the post-inoculation serums of these chicks. With but one exception, serologically demonstrable antibodies were at extremely low levels or absent.

No clinical symptoms of illness were apparent in any of the chicks. All appeared healthy at the time of sacrifice and no gross

changes were evidenced at autopsy.

Comments. Previous work on experimental leptospirosis in chickens resulted in few recoveries of inoculated organisms(7). However, older birds were employed in these studies. The uniform development of leptospiremia following inoculation with infective material suggests the possible utilization of baby chicks in the recovery of leptospire from material unsuitable for direct cultural methods. It is, therefore, suggested that such materials be inoculated intraperitoneally and that heart's blood cultures in suitable leptospiral mediums be made on the 4th and 7th days post-inoculation. Replicate cultures should be made in order to detect leptospire which may be present only in limited numbers. Neither clinical illness nor serological response can be used as criteria of infection in chicks of this age. It is noteworthy that only an inconstant and transient renal infection was observed. The possible role of fowls in the epidemiology of leptospirosis requires further investigation.

Summary. Two-day-old chicks uniformly developed leptospiremia persisting for at least 5 days following intraperitoneal inoculation of leptospire. No clinically detected illness resulted from this infection nor was there constant significant serologically demonstrable antibody response. Employment of chicks for recovery of leptospire from materials contaminated with other microorganisms and hence unsuitable for direct culture is suggested.

1. Van Thiel, P. H., *The Leptospiroses*, Universitaire Pers Leiden, 1948.
2. Morrow, G., Syverton, J. T., Stiles, W. W., and Berry, G. P., *Science*, 1938, v88, 384.
3. Randall, R., and Cooper, H., *Science*, 1944, v100, 133.
4. Larson C. L., *Public Health Rep.*, 1941, v56, 1546.
5. Gochenour, W. S., Jr., Johnston, R. V., Yager, R. H., and Gochenour, W. S., *Am. J. Vet. Res.*, 1952, v13, 158.
6. Gochenour, W. S., Jr., Yager, R. H., Smadel, J. E., Wetmore, P. W., and Hightower, J. A., *Am. J. Pub. Health*, 1953, v43, 405.
7. Bernkopf, H., *Proc. Soc. Exp. Biol. and Med.*, 1948, v67, 148.

Received June 30, 1953. P.S.E.B.M., 1953, v83.