22. Miller, G. L., and Golder, R. H., *Arch. Biochem.,* 1950, v29, 420.

23. Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F., *Am. J. Clin. Path.,* 1948, v18, 723.

24. Abramson, H. A., Moyer, L. S., and Gorin, M. H., *Electrophoresis of Proteins,* Reinhold Publishing Corp., New York, 1942.

25. Johnson, P., and Naismith, W. E. F., *Faraday Soc. Discussions,* 1953, v13, 98.

26. Perlmann, G. E., *ibid.,* 1953, v13, 67.

27. Longsworth, L. G., *Methods in Med. Research,* A. C. Corcoran, ed., Year Book Publishers, Chicago, 1952, v5, 63.

28. Hoch-Ligeti, C., and Hoch, H., *Biochem. J.,* 1948, v43, 556.

29. Li, C. H., *Amino Acids and Proteins,* D. M. Greenburg, ed., Charles C. Thomas, Springfield, Ill., 1951, 487.

30. Haurowitz, F., *Chemistry and Biology of Proteins,* Academic Press, New York, 1950, 9.

31. Perlmann, G. E., and Kaufman, D., *J. Am. Chem. Soc.,* 1945, v67, 638.

32. Longsworth, L. G., and Jacobsen, C. F., *J. Phys. and Colloid Chem.,* 1949, v53, 126.

33. Armstrong, S. H., Jr., *Plasma Proteins,* Vol. II, J. B. Youmans, ed., Charles C. Thomas, Springfield, Ill., 1950, 22.

Received March 8, 1954. P.S.E.B.M., 1954, v86.

Alteration in Pentose Content of Chick Embryos Infected with Japanese Encephalitis Virus. (21009)

NATHANIEL ROTHSTEIN, C. W. HIATT, AND R. H. YAGER.

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

The developing chick embryo has been used as a medium for the cultivation of a wide variety of obligate intracellular parasites, but the host-parasite relationship involved has received comparatively little attention. Since the embryo undergoes marked changes in size and composition during the growth period of the infectious agent, it necessarily constitutes a complex and dynamic medium.

The nucleic acids of the embryo are constituents of particular interest because of the direct correlation between ribonucleic acid concentration and the rate of protein synthesis (1-3). The invasion of unicellular hosts (*E. coli*) with bacteriophage has been shown by Cohen (4,5) to involve an inhibition of ribonucleic acid synthesis, whereas the synthesis of desoxyribonucleic acid proceeds at an essentially normal rate. Our observations have indicated that infection of chick embryos with the virus of Japanese encephalitis induces changes in tissue composition of comparable magnitude and in the same direction as those found in bacterial cells invaded by phage.

Materials and methods. Embryonating eggs of the White Leghorn were selected at random from several large and representative batches. All of the eggs used contained actively motile embryos with pronounced peripheral blood vessels visible upon candling. The eggs were incubated at 38°C and a relative humidity of 40 to 60%. Age of the embryo was designated as the number of days of incubator life. The embryos subsequently dying in the course of the experimental work were assumed to be dead when examination by candling disclosed that spontaneous motion of the embryo had ceased and the peripheral blood vessels had retracted. Infection with Japanese encephalitis virus (Nakayama strain, egg-adapted) was accomplished by inoculation of the chorio-allantoic membranes of 10-day-old embryos with 0.1 ml doses of a 1% suspension of infected chick-embryo tissue in phosphate buffered saline solution, pH 7.8. Twenty to 100 inoculated eggs were used in each experiment, with an equal number of control eggs from the same group. The eggs were maintained at 35°C and examined by candling at 4-hour intervals. All embryos which died less than 48 hours after inoculation were discarded. When the mortality ratio exceeded 20% (generally 60 to 72 hours after inoculation) both the infected and the normal

TABLE I. Pentose and Desoxyribonucleic Acid Concentrations in Infected and Normal Chick Embryo Tissues. 7 experiments.

	Pentose (mg % wet tissue)				Desoxyribonucleic acid (mg % wet tissue)			
	Infected embryos			Normal embryos	Infected embryos			Normal embryos
	I Dead	II Living, hemorrhagic	III Living, non-hemorrhagic	IV	I Dead	II Living, hemorrhagic	III Living, non-hemorrhagic	IV
	71	92	105	131	217	241	229	237
	37	92	96	99	166	210	212	208
	50	93	94	89	200	214	216	212
	60	70	100	104	233	202	196	220
	47	60	109	112	170	181	220	195
	49	80	101	106	176	219	201	204
	48	58	71	92	198	201	211	195
Mean	51.7	77.9	96.6	104.7	194.3	209.7	212.1	210.1

control eggs were harvested. The infected embryos were divided into three groups at the time of harvest: I) embryos dead 4 hours or less, II) embryos living but profusely hemorrhagic, and III) embryos living and not hemorrhagic. Group IV consisted of the normal embryos from the control group. The embryos were removed from the shells and allowed to drain free of egg fluids. After weights were taken, the embryos were macerated in a Waring Blendor in a solution of sodium chloride (0.1 M), sodium citrate (0.1 M), and sodium desoxycholate (0.2%) to prepare homogenates containing 20% (wet weight) of tissue. This procedure was based on the technic devised by McCarty and Avery (6) and modified by Schneider(7). After standing for 2 hours at 4°C, the homogenate was deproteinized by shaking for 10 minutes with ½ volume of a mixture (after Sevag(8)) of chloroform (4 volumes) and isoamyl alcohol (1 volume). Separation of the phases was accomplished by centrifugation for 1 hour at 2°C at a mean acceleration of 2000 g. The supernatant aqueous phase was removed by siphonation. The chloroform phase was discarded. The mass of insoluble material at the interface was resuspended in a small volume of the disrupting fluid and re-extracted. Aliquots of the pooled aqueous phase were analyzed without further purification. Duplicate or triplicate determinations were made in all cases. It was demonstrated that the aqueous extracts as prepared contained more than 99% of the total extractable pentose and DNA.

Total pentose (excluding desoxypentose) was estimated by the orcinol reaction of Kerr and Seraidarian(9) with reference to a standard solution of d-ribose. The specificity of the orcinol reaction for pentose in the presence of other sugars has been verified by Brown(11) and Fernell and King(12). Seventy to 85% of the pentose measured is accountable as ribonucleic acid (RNA) on the basis of its contribution to the optical density of the solution at 258 mμ. The results of the analyses were reported in terms of pentose, however, to avoid the introduction of errors in conversion. Desoxyribonucleic acid (DNA) was estimated by a modification (10) of the Dische diphenylamine reaction. The reference standard was a solution of sodium desoxyribonucleate.*

Results. The pentose and DNA concentrations found in the infected and normal embryos in 7 experiments are shown in Table I. A variance analysis of the data by a modification of the method of Snedecor(13) is summarized in Table II. Pentose concentration in the infected embryos was significantly lower than that of the normal embryos. The diminution in pentose concentration is greatest in the dead embryos, and the living hemorrhagic embryos were lower in pentose content than the non-hemorrhagic infected embryos. DNA concentration was not significantly different among any of the groups.

Discussion. Infection of 10-day-old chick embryos with Japanese encephalitis appears to

* Obtained from Nutritional Biochemicals Corp.

TABLE II. Variance Analysis of Data.

Source of variation	DF	SS	MS	F
Pentose				
I II III vs IV	1	4,517	4,517	39.3*
I vs II III	1	5,881	5,881	51.1*
II vs III	1	1,226	1,226	10.6†
Between exps.	6	2,146	358	3.1‡
Interactions:				
(I II III vs IV) × exps.	6	514	86	
(I vs II III) × exps.	6	734	122	
(II vs III) × exps.	6	823	137	
Error	(18)	(2,071)	115	
Total	27	15,842		
Desoxyribonucleic acid				
Between groups	3	1,432	477	2.2
" exps.	6	4,025	671	3.1‡
Error	18	3,874	215	
Total	27	9,331		

Level of significance:
* 0.1% † 1% ‡ 5%

effect a reduction in pentose content of the tissues prior to death of the embryo. This alteration in tissue composition is demonstrable by analysis of the entire body of the embryo, indicating that most or all of the cells of the body are affected. The mechanism of the alteration has not been elucidated. Since the virus of Japanese encephalitis is pantropic in the chick embryo(14), it is possible to infer that the invasion of virus particles has impeded aerobic glycolysis in the individual cells. The phenomenon would then be parallel to what happens in bacteria invaded by phage(4,5). However, the organized structure of the embryo could sustain functional defects on a higher physiological level which would elicit the same result. A respiratory insufficiency in the dying embryo, for example, might force the individual cells to abandon aerobic glycolysis. A comparative study of embryos dying from other causes will be required for the elucidation of the observations reported. It will also be of interest to determine the correlation between the metabolic state of the embryo and its virus content.

Summary. The tissues of chick embryos dead or dying following infection with the virus of Japanese encephalitis are significantly lower than normal in pentose concentration but no significant differences in desoxyribonucleic acid concentration were demonstrated. The factors responsible for the decrease in pentose have not been elucidated.

The authors are indebted to Dr. H. C. Batson for his supervision of the statistical procedures and to Major Morris D. Schneider for his valuable suggestions during the course of the work.

1. Caspersson, T. O., *Cell Growth and Cell Function,* 1950, W. W. Morton and Co., Inc., New York.

2. Novikoff, A. B., and Potter, V. R., *J. Biol. Chem*, 1948, v173, 233.

3. Rothstein, N., *Variation in Nucleic Acid Content of the Deveolping Chick Embryo,* M. S., Thesis, The George Washington Univ., Washington, D. C., May 27, 1953.

4. Cohen, S. S., *Bact. Rev.,* 1951, v15, 131.

5. Cohen, S. S., and Roth, L., *J. Bact.,* 1953, v65, 490.

6. McCarty, M., and Avery, O. T., *J. Exp. Med*, 1946, v83, 97.

7. Schneider, M.D., *J. Bact.,* 1952, v63, 495.

8. Sevag, M. G., Lackman, D. B., and Smolens, J., *J. Biol. Chem.,* 1938, v124, 425.

9. Kerr, S. E., and Seraidarian, K., *ibid.,* 1945, v159, 211.

10. Sevag, M. G., Smolens, J., and Lackman, D. B., *ibid.,* 1940, v134, 523.

11. Brown, A. H., *Arch. Biochem.,* 1946, v11, 269.

12. Fernell, W. R., and King, H. K., *The Analyst,* 1953, v78, 80.

13. Snedecor, G. W., *Statistical Methods, 4th ed.,* 1950, The Iowa State College Press, Ames, Iowa.

14. Olitsky, P. K., and Casals, J., *Viral Encephalitides,* Ch. 8, in Rivers, T. M., *Viral and Rickettsial Infections of Man,* 1948, J. P. Lippincott Co., Philadelphia.

Received March 8, 1954. P.S.E.B.M., 1954, v86.