Maluf, N. S. R.
1938. *Physiol. Rev.*, 18: 28.
Pearl, R. and S. L. Parkér
1924. Am. Nat., 58: 193.
Sanford, E. W.
1918. *Jour. Exp. Zool.*, 25: 355.
Singh-Pruthi, H.
1925. *Jour. Exp. Biol.*, 3: 1.
Slowtzoff, B.
1904. *Franz Hofmeister's Beitr. z. Chem. Physiol. Pathol.*, 4: 23.
1905. *Ibid.*, 6: 163.
1905a. *Ibid.*, 6: 170.
1909. *Biochem. Zeit.*, 19: 504.
Tangl, F.
1909. *Arch. ges. Physiol.*, 130: 1.
Teissier, G.
1928. *C. r. Soc. Biol.*, 99: 602.
Weber, H.
1931. *Zeit. Morph. Ökol. Tiere*, 23: 575.
Wodsedalek, J. E.
1917. *Science*, 46: 366.
1921. *Anat. Rec.*, 20: 222.

FURTHER STUDIES ON INCUBATION OF TURTLE (*MALACLEMYS CENTRATA* LAT.) EGGS[1]

In earlier reports (Cunningham and Hurwitz, 1936, and Cunningham and Huene, 1938) it was shown that the increase in weight of various reptile eggs during incubation could be accounted for, at least for the most part, by the absorption of water.

The studies did not positively exclude the possibility that mineral salts were also taken up by the egg. An examination of the data of Karashima (1929) indicates that both mineral salts and carbohydrates increase in the first fifteen-day period of incubation in the albumen and yolk of the sea turtle, *Thalassochelys corticata*. The source of this increase was not specified, although it may be assumed to be the shell. The present studies consider the whole egg as a unit and attempt to discover whether minerals from the environment enter the egg.

[1] These experiments were carried out at the U. S. Fisheries Laboratory at Beaufort, N. C., and the authors express appreciation to the bureau for the use of its facilities, and to Dr. H. F. Prytherch, director, and Mr. Charles Hatsel, foreman, for their cooperation.

It is unfortunate that the complete story can not be followed in each egg and that a somewhat statistical method has to be followed in order to ascertain whether or not elements other than water are added from the environment during incubation. Such procedure involves the use of a reasonable number of eggs at each of the selected ages. In these experiments the number was limited by the time available for the analyses. The eggs were studied individually, since by this method significant variations might become evident.

Newly laid eggs were taken from the nest before it was covered. These were weighed and immediately desiccated and reweighed to a constant figure. The dried mass was then ashed and weighed. Other eggs were incubated for 33 days under normal conditions and a similar procedure followed. Still others were incubated for 60 days and similar data collected.

The composite data for each group are shown in Table I. The data show a very slight increase in dry weight in the 33-day group over the fresh eggs. This would appear, however, to be well within experimental error. The increase in ash weight, however, is greater, but when taken into consideration with the slight rise in organic materials it is probably again within the range of experimental error.

TABLE I

WEIGHTS OF ASH AND ORGANIC MATERIAL AT VARIOUS STAGES OF DEVELOPMENT

No. eggs	Days incubated	Average wet wt.	Max. Min.	Average dry wt.	Max. Min.	Average org.* wt.	Max. Min.	Average ash wt.	Max. Min.
20	0	10.581	11.668 7.283	3.117	3.445 1.629	2.764	3.072 [illegible]	.3531	.4341 .2478
20	33	12.441	14.826 10.701	3.155	3.672 2.769	2.784	3.356 2.529	.3714	.4313 .3120
25	60	13.326	16.585 11.273	2.800	3.645 2.005	2.429	3.090 1.727	.3712	.4160 .2597

* Organic material.

The variations in dry weight, ash content and organic material between eggs in a single group are far greater than the differences in averages of the different groups. While there is much overlapping from one group to the other the maximum ash in each group shows a steady but comparatively slight regression during incubation.

Taken as a whole, it appears as if the mineral salts (ash) are reasonably constant during incubation. Further evidence for this conclusion, secured by a different procedure, will be presented later. The slight rise in organic material, when there should have been an appreciable loss at 33 days, can not be so easily explained, but the considerable drop before the 60th day indicates it may be an error in technique; it might be accounted for either by proportionately thicker shells or larger yolks in this particular group of eggs.

The problem of absorption of minerals was also approached from another angle. Eggs were placed upon a rack in a humidity chamber. A piece of cheese-cloth, one end of which dipped into distilled water, was placed under the eggs and folded back so as to cover the tops of the eggs. A glass plate, slightly raised to allow admission of air without too rapid evaporation of water, was placed over the top of the chamber. The distilled water was changed from time to time to prevent stagnation. A control, using tap

TABLE II

COMPARISON OF NEWLY HATCHED TURTLES FROM EGGS SUPPLIED WITH DISTILLED WATER ONLY WITH THOSE INCUBATED IN NATURE

	Number embryos	Average wet wt.	Max. Min.	Average dry wt.	Max. Min.	Average organ. wt.	Max. Min.	Average ash wt.	Max. Min.
Normal	10	7.860	9.8141	2.138	2.9056	1.915	2.636	.223	.2693
			7.2409		1.9710		1.754		.2054
Experimental	5	7.517	7.8594	2.055	2.2412	1.830	1.976	.225	.2485
			7.2613		1.9382		1.721		.2107

water (artesian well), was also set up. The jars were exposed to ordinary room temperature. In both cases the development proceeded to hatching, and gross examination revealed no anomalies of development, either in form or time required for hatching as compared to the normal in nature. The newly hatched embryos, supplied during incubation with distilled water, when analyzed and compared with embryos hatched under normal conditions, showed no significant differences. The data are given in Table II.

It is quite evident that all the mineral salts necessary for development are contained in the eggs; in fact, there is an excess, which may be partly accounted for by the minerals remaining in the shell at the time of hatching.

These experiments indicate that when the egg of the diamond back terrapin is taken as a whole it contains (with the exception of water) all the ingredients, in sufficient quantity, to insure the proper development of the embryo.

BERT CUNNINGHAM
M. W. WOODWARD
JANIS PRIDGEN

DUKE UNIVERSITY

LITERATURE CITED

Cunningham, B., and A. P. Hurwitz
1936. AM. NAT., 70: 590–595.
Cunningham, B., and E. Huene
1938. AM. NAT., 72: 380–385.
Karashima, J.
1929. *Jap. Jour. Biochem.*, 10 (2): 369–374.