Occurrence of Lysozyme in Bird Egg Albumins. (18520)

A. N. SMOLELIS AND S. E. HARTSELL. (Introduced by H. Koffler)

From the Department of Biological Sciences, Purdue University, Lafayette, Ind.

It has been reported by Fleming(1), Florey(2), Prickett, Miller and McDonald (3,4), Bradford and Roberts(5) and others, that lysozyme is widely distributed in animal tissues and fluids. Although none of these authors attempted to assign structural function to this enzyme, it is interesting to note the frequent occurrence of lysozyme. Quantitative data relative to the enzyme is not available, although a number of qualitative tests, some of which approximated quantitative procedures, have been made. Since Smolelis and Hartsell(6) described an accurate method for the determination of lysozyme from natural materials, it has been possible to accumulate more complete data.

Fleming(7) reported that lysozyme was present in the white of eggs from the thrush, the wagtail and the moorhen. Is it probable that the concentration of lysozyme in tissues or fluids is determined, in part, by the heredity of the species? A study of the lysozyme content of a number of egg albumins was carried out as a first step toward an answer to this question. Samples were obtained from chicken, bantam hen, guinea hen, goose, duck, turkey, quail, pheasant, chuckar partridge, horned lark, herring gull, and although not avian in origin, a number of turtle eggs.

Methods. The fresh eggs were broken and the whites separated from the yolks. These whites were then pooled and blended in a Waring blendor for 5 seconds. After mixing, the material was distributed into small test tubes, quick frozen and stored at −9°C. By handling in this manner it was possible to carry out the assays at convenient times. Earlier work had shown that no loss in the titer of egg albumin occurred after storage for several weeks. Lysozyme determinations were made in accordance with the method described by Smolelis and Hartsell(6). The results are presented in Table I. No calculated values are shown for the lysozyme contained in the goose or turkey egg albumins. Although these materials were soluble in phosphate buffer at pH 6.2, it was not possible to arrange a series of dilutions which resulted in turbidity values applicable to the standard curve. It may be that the lysozyme in the albumin of these birds was not free, therefore could not act as it would in pure form.

TABLE I. Lysozyme Titers of Frozen Bird Egg Albumins.

Source	No. of tests	Lysozyme, mg per ml* (avg)	Range
Bantam hen	5	6.9	6.4 -7.5
Quail	5	6.7	6.6 -6.7
Chicken	6	6.5	6.3 -6.9
Chuckar partridge	5	5.3	4.0 -6.0
Pheasant	7	3.4	2.9 -4.1
Guinea hen	5	3.1	2.8 -3.4
Duck	4	1.8	1.7 -2.0
Horned lark	4	1.0	0.9 -1.1
Herring gull	8	0.89	0.81-1.0
Goose	6	—	
Turkey	6	—	

* Expressed as mg of crystalline lysozyme per ml of albumin.

From these results it can be seen that an appreciable difference in lysozyme concentration exists between different bird egg albumins. However, a more complete survey is necessary before generalizations regarding species characteristic can be made. In attempting to determine lysozyme concentrations of turtle eggs a solubility problem was presented. In phosphate buffer, pH 6.2, a precipitate was formed. As an alternative method of assay for these albumins, agar cups were used. Previous experience had shown

1. Fleming, A., *Proc. Roy. Soc.*, (London), B, 1922, v93, 306.

2. Florey, H., *Brit. J. Exp. Path.*, 1930, v11, 251.

3. Prickett, P. S., Miller, N. J., and McDonald, F. G., *J. Bact.*, 1937, v33, 39.

4. Prickett, P. S., Miller, N. J., and McDonald, F. G., *J. Bact.*, 1933, v25, 61.

5. Bradford, W. L., and Roberts, J. B., *J. Pediat.*, 1936, v8, 24.

6. Smolelis, A. N., and Hartsell, S. E., *J. Bact.*, 1949, v58, 731.

7. Fleming, A., *Lancet*, 1929, pt. 1, 217.

that the agar cup method for determining lysis was neither very accurate nor very consistent for the quantitation of lysozyme, nevertheless, it was applied to the albumins which did not lend themselves to turbidimetric assays.

Petri dishes containing 15 ml of nutrient agar were seeded with 4 ml of agar containing *Micrococcus lysodeikticus* cells. Nine-millimeter cups were formed in the agar and filled with the turtle egg albumin. For comparison, lysozyme dilutions were also prepared in the same manner. After 14 hours of incubation, the zones of inhibition surrounding the cups were measured. These were indicative of the bacteriostatic nature of the turtle egg albumin. Diffusion of some component, presumably lysozyme, in the turtle egg albumin prevented the growth of *M. lysodeikticus*. To further determine the bacteriolytic effect of this material upon the test species, another series of plates was prepared. These were seeded with ultraviolet, light-killed *M. lysodeikticus* cells and cups made as before. The amount of cells added was sufficient to impart a definite turbidity to the agar. After incubation, the zones surrounding the cups were measured. The clearing of the cells adjacent to the cup indicated that the material in the turtle egg albumin was lysozyme because the lysis of the dead cells is currently considered as a specific enzymatic reaction between lysozyme and its aminopolysaccharide substrate. Using this method, it was possible to demonstrate that undiluted turtle egg albumin caused a 20 mm zone of inhibition and a 17 mm zone of lysis. A 1:10,000 dilution of crystalline lysozyme caused a 20 mm zone of inhibition and a 14 mm zone of lysis. In this manner lysozyme was demonstrated in turtle egg albumin.

These results indicate the distribution and quantitative variation of this enzyme in egg albumins. There is variation between species of birds in the lysozyme content of their eggs.

Received January 30, 1951. P.S.E.B.M., 1951, v76.

Relation of Clearance and Distribution of Injected Glutathione to Protection Against Radiation Injury.* (18521)

E. P. CRONKITE,† W. H. CHAPMAN,† AND GEORGE BRECHER.

From the Naval Medical Research Institute and the National Institutes of Health, Bethesda.

In a series of studies on the effect of glutathione (reduced) on radiation injury it has been shown that this sulfhydryl tripeptide will protect significantly when administered before irradiation(1,2), that the radiation dosage mortality curve is significantly shifted to the right above an LD_{20}(3), and that there is histologic evidence of more rapid regeneration of the hematopoietic organs in the glutathione treated mice(4). With the publication of evidence by Forrsberg(5) that bacteria, skin, and hair follicles could be protected by the local action of cysteine, the question naturally arose as to whether glutathione protection of the irradiated animal arose from selective concentration of reduced glutathione in radiosensitive tissues essential to survival. Accordingly studies on the clear-

* Opinions and assertions are those of the authors and are not to be construed as reflecting the policies or opinions of the Navy Department at large.

† Naval Medical Research Institute, Bethesda, Md., From Report No. 2, Project NM 006 012.05, dated June 27, 1950.

1. Cronkite, E. P., and Chapman, W. H., *Fed. Proc.*, 1950, v9, 329.

2. Chapman, W. H., Sipe, C. R., Eltzholtz, D. C., Chambers, F. W., Jr., and Cronkite, E. P., *Radiology*, December, 1950.

3. Chapman, W. H., and Cronkite, E. P., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 318.

4. Cronkite, E. P., Brecher, G., and Chapman, W. H., PROC. SOC. EXP. BIOL. AND MED., in press.

5. Forssberg, A., *Acta Radiol.*, 1950, v33, 296.