obtained against the purified neurotoxin. This partial identity possibly reflects an additional antigenic determinant of the neurotoxin in its native state in the whole venom, in which it appears to be bound to a lipoprotein[5]. In immunoelectrophoresis using a 5% concentration of whole venom as antigen, one precipitation line was obtained. When using a 10% venom concentration, however, 2 additional weak lines were noticed. Presumably, these represented a very low concentration of antibodies elicited by minute quantities of contaminating proteins present in the purified antigen used for immunization.

SUMYK and KASHIN[7] obtained an antibody specific for the main neurotoxic component of cobra venom by immunizing rabbits with a purified neurotoxic preparation. Our results demonstrate that pure viper neurotoxin can be rendered immunogenic by binding to ion exchange resin. The single antibody obtained provides further evidence for the homogenicity of the purified neurotoxin preparation[8].

Résumé. L'anticorps contre la neurotoxine du venin de *Vipera palestinae* est obtenu chez des lapins par immunisation de la neurotoxine purifiée liée à la cellulose carbométhylique.

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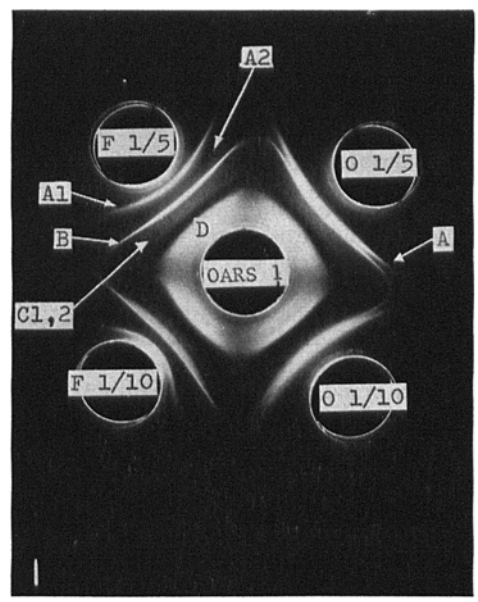
An Immunologically Detectable Change in the Egg White of Chicken Eggs Following Pre-Incubation Storage

Results of studies by WAITE[1] on the deleterious effects of prolonged pre-incubation storage on the hatchability of chicken eggs have been adequately corroborated (e.g. [2]) and extended to other species of the domestic fowl, i.e. turkeys[3], ducks[4], and geese[5]. Nearly $^2/_3$ of the food material available to the avian embryo is contained in the egg white. In the domestic chicken it is ingested mainly in the second half of the incubation period, although considerable evidence exists[6] pointing to its physiological importance during early embryogenesis as well.

In this connection, it is conceivable that the well documented physicochemical changes in the egg white of eggs held for extended periods of time (cf. ROMANOFF and ROMANOFF[7]) may be directly related to reduced viability of embryos developing within such eggs. The fact that changes do take place, on molecular level, in the egg white during the in situ storage was demonstrated by BAKER and MANWELL[8], who reported that the resolution of egg white proteins by starch electrophoresis became progressively more difficult as the age of the egg increased. This suggested that a further exploration of biologically significant changes in the extra-nuclear components of the egg during pre-incubation storage was needed. Accordingly, a study was undertaken with this in mind, the results of which are reported below.

Eggs laid by a White Leghorn pullet provided the necessary experimental material. Specimens of the 'fresh' egg white were collected from eggs 9 h after they were laid and of the 'old' egg white from eggs stored for 21–25 days at 13.5 °C and 80% relative humidity. Each specimen was identified as to its source.

The anti-egg white sera came from seven 1-year-old rabbits, each rabbit having received a series of 10 injections of the egg white from a specific specimen. This resulted in 7 different antisera: 4 from rabbits injected with 'fresh' egg white, and 3 from rabbits injected with 'old' egg white. All injections were via the ear vein route. Prior to injection, each egg white specimen was homogenized, filtered and then diluted 1:7.5 with a 0.9% saline. The first injection contained 1 cm³ of the above solution; each of the subsequent 9 injections contained 2 cm³. The injections were carried out every other day. 10 days after the last injection, a test blood sample was taken via the heart puncture. The definitive sample was collected on the following day and immediately stored at 5 °C for 24 h. The antiserum was obtained by centrifuging the blood coagulum at 2,500 rpm for 30 min and drawing off the clear serum. Complement inactivation was achieved by heating the antiserum for 30 min at 56 °C. Following



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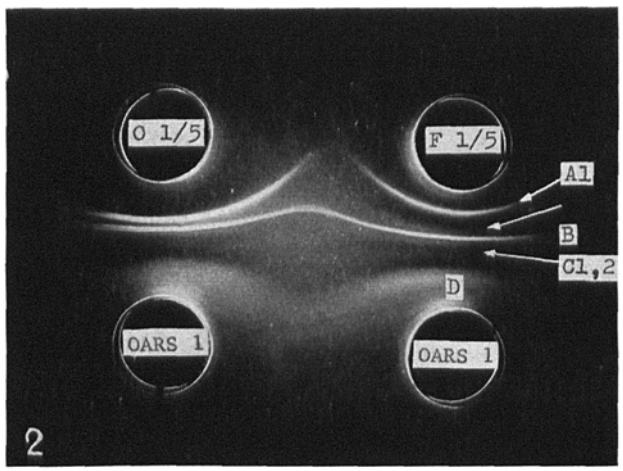

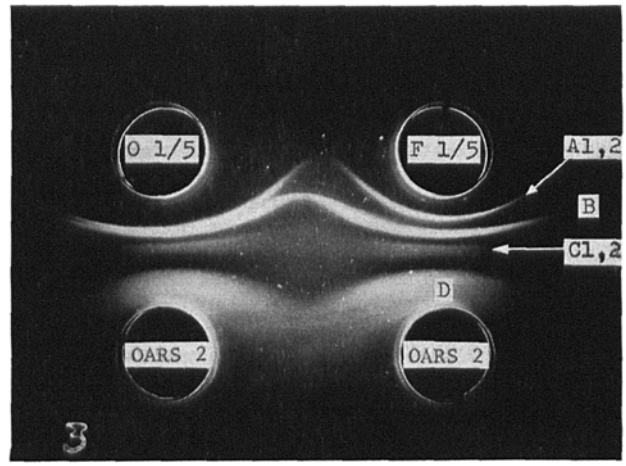

Precipitin reactions, showing a positional difference for line A between 'fresh' and 'old' egg white antigens and the anti-'old' egg white rabbit serum. F and O designate 'fresh' and 'old' egg white specimens, respectively. Fractions indicate dilution. Figs. 1 and 2. Line A_2 facing the 'fresh' antigen well ($F\ ^1/_5$) is jointly visible between lines A_1 and B; it is absent on the side of the 'old' antigen ($0\ ^1/_5$). Line A_1 has moved farther from the antigen well when the antigen was 'old' egg white. In both figures, the antiserum was provided by the same rabbit (No. 1). Fig. 3. Line A (A_1 and A_2 combined), which has again moved farther from the antigen well when the latter contained 'old' egg white, overlaps the area occupied by line B. The corresponding lines facing the antigen well containing 'fresh' egg white have maintained their identity. The antiserum was provided by rabbit No. 2.

the addition of 0.01% merthiolate, the antisera were stored at – 20 °C until needed.

The 7 antisera were tested against the egg white obtained from 5 freshly laid eggs (i.e. eggs used within 9 h of oviposition) and 5 'stored' eggs (stored for 25 days at 13.5 °C and 80% relative humidity), all laid by the original hen. Before the test each specimen of the egg white used as antigen was diluted with a 0.9% saline in a dilution series, ranging from 1:5 to 1:640.

The testing procedure was OUCHTERLONY's double diffusion agar plate method[9]. The plate was made up of 1.25% agar dissolved in a 0.9% saline solution, to which was added 0.01% merthiolate. The mixture was buffered at pH 7.0 with a phosphate buffer. Each of the wells measured 3 mm in depth and 8 mm in diameter. The distance between reactant wells was 8–10 mm. Before they were 'read', the testplates were kept 2 weeks at 37 °C and 80% relative humidity.

Regardless of the antiserum-antigen combination, the general precipitin pattern was found to be similar to that recently reported from this laboratory[10], both as to the principal precipitin lines and reaction to the antigen dilution gradient. Typically, the pattern consisted of line A (or of 2 sub-lines A_1 and A_2), line B, 2 fainter lines C_1 and C_2, and a broad D complex (Figure 1). As noted in the above cited report[10], line A, depending on the balance of concentration between the antigen and the antiserum, can separate into a strong line A_1 and a faint line A_2. This separation was also observed in the present study, but *only with the fresh egg white antigen.*

Furthermore, line A consistently was found to be farther removed from the antigen well when the latter contained 'old' egg white than was the case when 'fresh' egg white was the antigen (Figure 2). In fact, at times such a shift led to a partial 'fusion' of lines A and B (Figure 3). This characteristic advance of line A, when the antigen well contained egg white of eggs stored for 25 days before the test, was in evidence regardless of the 2 types of antisera used in the present study.

According to the immunological analysis of the chicken egg white[10], line A represents the reaction between the gamma globulin molecules of the antigen and those of the antiserum against the whole egg white. All other things being the same, the shifting of the front toward the antiserum well, when the antigen was 'old' egg white, points to a reduction in the molecular size of the gamma globulin fraction of the egg white proteins in eggs kept for extended periods of time under conditions which today are considered optimal or near-optimal for the pre-incubation storage of chicken eggs, i.e. 13.5 °C and 80% relative humidity. SATO and NAKAMURA[11] observed marked changes in the electrophoretic mobility of several components of the globulin complex in the egg white of chicken eggs stored for prolonged periods of time. Extending their results to the present study, one can postulate that the antigen, represented by line A, may exist in a highly polymerized form in the fresh egg and in a relatively depolymerized form in the stored egg. In the latter case, a higher rate of mobility would be expected from the antigen. This could result not only from the smaller size of the depolymerized antigen molecules per se, but also from an increase in the number of determinant groups capable of reacting with the available antibody molecules.

Such depolymerized gamma globulin could in part, at least, be responsible for decrease in the biological quality of avian hatching eggs during extended pre-incubation storage[12].

Zusammenfassung. Veränderungen des Hühnereiweisses durch Lagerung können in der Immunpräzipitation (Ouchterlony-Test) erfasst werden.

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