Antibody Against Purified Viper Neurotoxin

Snake venoms are complex mixtures of toxins and enzymes with varying immunogenic activity. In general the hemorrhagic venom toxins are strong antigens, while the neurotoxins have low immunogenic potency. Thus, antisera produced against *Vipera palestinae* venom, which is both hemorrhagic and neurotoxic, are rich in anti-hemorrhagic antibody but have low antineurotoxic titer[1]. In previous studies[2-4] it was demonstrated that the immunogenicity of the *Vipera palestinae* neurotoxin could be raised by binding it to carboxymethyl cellulose. Since the neurotoxin preparation used still contained other proteins, the antiserum comprised numerous antibodies in addition to the antineurotoxic one. The purification of the *Vipera palestinae* neurotoxin, which proved to be a basic protein with a molecular-weight of 12,000[5], prompted a trial to obtain the single antineurotoxic antibody. Two principles were applied: isolation of antibody from antigen-antibody complex, and immunization with purified antigen.

Vipera palestinae neurotoxin was purified as described previously[5]. Whole venom was chromatographed on a DEAE-cellulose column providing a neurotoxic fraction devoid of hemorrhagic toxin. The 'DEAE-cellulose neurotoxic fraction' was further fractionated by differential salt precipitation and methanol-chloroform extraction. Anti-*Vipera palestinae* horse serum, prepared with whole venom and crude neurotoxic fraction bound to carboxymethyl cellulose[4], was incubated with the purified neurotoxin and the precipitate formed was separated by centrifugation. Upon treatment with 0.02*M* HCl – 0.15*M* NaCl (pH 2.0), according to the method of GIVOL et al.[6], 17% of the precipitate dissolved. Chromatography on a Sephadex G 75 column separated between the antibody which eluted first and the low molecular weight antigen. The antibody yield, however, was poor. Application of a similar method by SUMYK and KASHIN[7] for the purification of anti-cobra neurotoxin antibody has been also unsuccessful.

Treatment of the antigen-antibody precipitate with 8*M* urea in 0.15*M* NaCl – 0.01*M* sodium phosphate buffer pH 7.4 according to FRIEDMAN et al.[8] resulted in 25–36% dissolution. Chromatography of the dissolved precipitate on a carboxymethyl cellulose column furnished a fraction containing antibody capable of forming a precipitate with the purified neurotoxin as demonstrated by immunodiffusion. This fraction, however, contained also antigen, as indicated by immunodiffusion against anti-vipera venom serum.

Since by these methods either too small amounts of antibody or impure antibody preparations were obtained, immunization with purified antigen was tried. The purified neurotoxin was bound to soluble carboxymethyl cellulose, 2.5% solution in 0.005*M* phosphate buffer pH 6.8[2], and the complex used for immunization in rabbits. The primary dose was a total of 1 mg resin-bound neurotoxin mixed with Freund's adjuvant, injected intradermally into the foot pads and into various sites of the back skin. 10 days later a booster injection containing 2 mg resin-bound neurotoxin was given i.m. and bleeding was performed after 1 more week. The precipitin titer of the anti-neurotoxin serum, using a 1% venom solution in saline as antigen, was 128. 1 ml of the antiserum was capable of neutralizing up to 70 LD_{50} of the 'DEAE-cellulose neurotoxic fraction'. After a second booster injection 135 LD_{50} were neutralized.

The antiserum produced against the purified neurotoxin exhibited in immunodiffusion upon reaction with the purified neurotoxin a single line, identical with the precipitin band obtained upon reaction with the whole venom (Figure 1). This antiserum exhibited in immunodiffusion also a single line upon reaction with the crude 'DEAE-cellulose fraction' (Figure 2). In contrast, antiserum produced against whole venom gave multiple precipitin bands with the crude neurotoxic fraction, one of which indicating partial identity with the antibody

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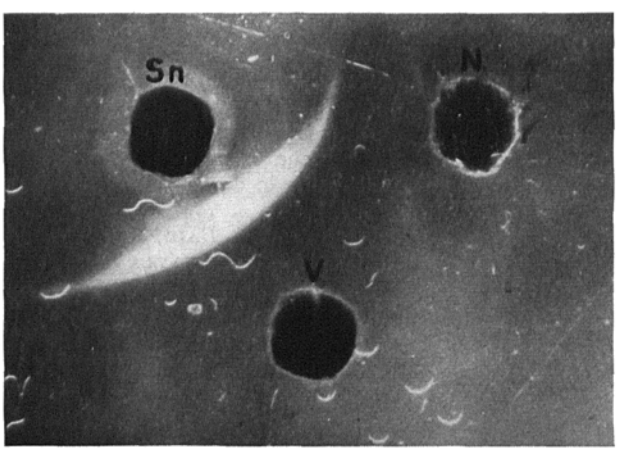

Fig. 1. Serum (Sn) produced against resin-bound pure neurotoxin. Antigens: whole venom (V) and pure neurotoxin (N).

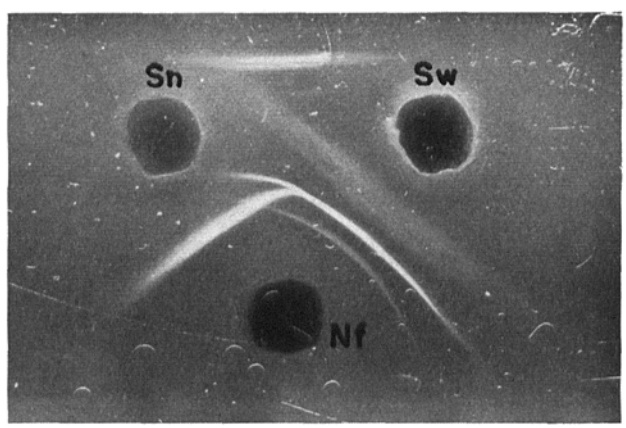

Fig. 2. Serum (Sw) produced against whole venom and serum (Sn) produced against resin-bound pure neurotoxin. Antigen: 'DEAE-cellulose neurotoxic fraction' (Nf).

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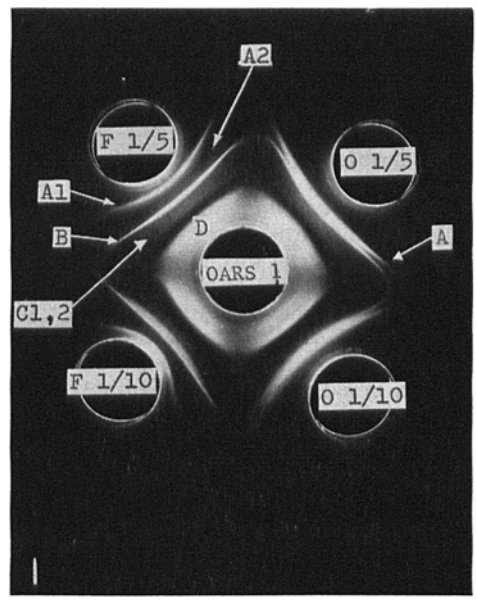
An Immunologically Detectable Change in the Egg White of Chicken Eggs Following Pre-Incubation Storage

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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