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Antigenic and Anaphylactoid Studies with Seromucoid and Seroglycoid.* (27206)

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Many agents are known to produce an anaphylactoid reaction in rats characterized by hyperemia, cyanosis and edema. Included among these are such diversified substances as dextrans(1), 48/80(2), hyaluronidase(3), polymyxin B(4), egg white(5), etc. The latter has been studied extensively and it has been established that the ovomucoid fraction is the causative factor(6). Since ovomucoid is classified as neutroglucoprotein, the study of neutroglucoproteins obtained from serologically different species might be of value in identifying a common moiety capable of engendering the hypersensitivity reaction. Equine seromucoid and bovine seroglycoid were used.

Materials and methods. Equine seromucoid (ESM) was prepared from serum according to the method of Rimington and Van den Ende(7). The following modification facilitates the subsequent removal of pigment: solid sodium bromide was added to one liter of serum until a specific gravity of 1.21 was reached. The resulting solution was centrifuged at 20,000 rpm for 12 hours and the supernatant layer, essentially containing the lipoproteins, was discarded. The residue was dialyzed against running water until free of bromide and further diluted to make a volume of 4 liters. After adjusting the pH of the solution to 4.7 with acetic acid, the mixture was heated until coagulation was complete following which the pH was readjusted. The coagulum was filtered off, the water-clear filtrate concentrated to a small volume and precipitated by addition of 2 volumes of 95% alcohol. The precipitate was collected by centrifugation, washed several times with absolute alcohol and then with ether. Further purification is not necessary for this technic results in a product which is white and not contaminated with yellow pigment.

Bovine seroglycoid (BSG), which is carried down with the albumin precipitate, was generously supplied by Mann Research Laboratories, New York City.

Two sets of experiments with rats were carried out: the first consisted of intact, white females of about 150 g in weight, and the second of comparable white females which were bilaterally adrenalectomized 24 hours prior to challenge. The rats of each group of 4 received 3 mg ESM or BSG in 1 ml saline given intravenously.

Results and discussion. None of the rats in either the intact or adrenalectomized groups exhibited any evidence of an anaphylactoid response to either ESM or BSG.

The anaphylactoid reaction in the rat is believed to be a response to histamine and/or serotonin released from degranulating mast cells(8). Since there are diverse histamine-releasing agents, the identification of a common prosthetic group among them would help clarify one of the mechanisms involved. Ovomucoid, OVO, a potent histamine releaser in the rat, is chemically related to both ESM and BSG by a similar carbohydrate complex. According to Stacey(9) these substances, neutroglucoproteins or mucoproteins, are protein-carbohydrate compounds composed of a relatively high protein content and a carbohydrate complex. The carbohydrate moiety of both OVO and ESM consists of D-mannose, D-galactose, and N-acetyl-d-glucosamine while BSG differs only by containing un-

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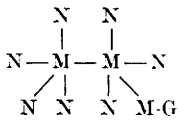
acetylacted D-glucosamine.

We failed to observe any serological cross reaction by the gel diffusion technic among the 3 mucoproteins and undoubtedly each exhibits its own species specificity. OVO has been said to be a poor antigen in the rabbit (10) and this has been demonstrated in the mouse(11), and rat(12), also. Similarly, ESM was found to be a poor antigen in the mouse, failing to engender anaphylactic sensitivity even by recourse to the pertussis-technic(13). In addition, ESM failed to induce a reaction in the mouse sensitized to equine serum and failed to cross react with antiequine serum-serum by the cellulose acetate immunoelectrophoresis technic. On the other hand, BSG was seen to be a strong antigen in that it engendered a hypersensitive state in the mouse by the pertussis-technic and also induced anaphylactic shock in the bovine serum albumin-sensitized mouse. These reactions may be a reflection of incomplete purification of BSG for the latter is prepared from BSA and, indeed, Rimington and Van den Ende(7) have stated that their preparations were certainly immunologically impure.

The prosthetic group of OVO is a hendecasaccharide containing 7 moles of N-acetyl-d-glucosamine (N), one mole of galactose (G) and 3 moles of mannose (M) represented as follows:



The polysaccharide is linked to the peptide chain of the protein by one of its glucosamine molecules(14). Although the carbohydrate complex of ESM has the same components, the latter are present in equimolecular proportions(9). As stated previously, the complex of BSG contains the unacetylated d-glucosamine. Neuberger and Yuill(15) have stated that there is no evidence that the carbohydrate complexes of ovalbumin and bovine ovomucoid enter into or influence their immunological specificity. In addition, Coghill and Creighton(16) could not demonstrate that the polysaccharide present in serum proteins behaved like a hapten. The serological specificity of the mucoproteins studied in this report is distinct and is quite likely dependent upon the protein carrier. The capacity to induce the anaphylactoid reaction is inferred to be related primarily to the protein moiety and appears to be a peculiarity of OVO, based on the carrier or the mode of its combination to the carbohydrate complex.

Summary. Neither seromucoid nor seroglycoid was capable of inducing an anaphylactoid response in the intact or adrenalectomized rat although they are classified, chemically, with ovomucoid. Seromucoid was not antigenic for the mouse. The antigenicity of seroglycoid may be attributed to traces of serum albumin.

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