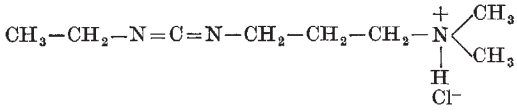
Biologically Active Antibodies to Histamine

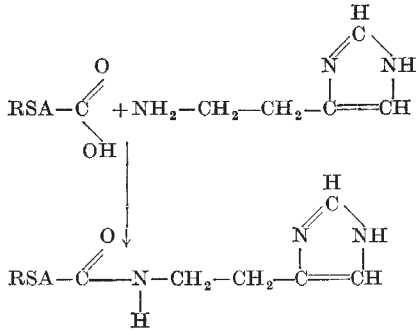
It has been known for some time that peptides, amino-acids and proteins containing the substituents COOH and NH_2 can be linked together by the use of water soluble basic carbodiimides[1]. Goodfriend *et al.*[2] showed that linking the polypeptides of bradykinin and angiotensin with rabbit serum albumin (RSA) by the use of carbodiimides produced a complex which was highly antigenic. Subsequently Davis *et al.*[3] showed that the antibodies induced by the bradykinin–RSA complex were protective against the biological effects of bradykinin, and Oken *et al.*[4] obtained similar results with angiotensin. So far the use of carbodiimides has been confined to protein and peptide syntheses.

We have made a preliminary study to determine whether or not linkages between RSA and non-peptide compounds containing the NH_2 but not the COOH substituent could be made, and whether or not the resulting complex was antigenic. The compound chosen for the study was histamine, which was linked to RSA using the carbodiimide

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl



The most likely reaction is between the primary amine of histamine and a carboxyl group from the protein. The chemical reaction is shown as:



The resulting complex was used to immunize guinea-pigs using the immunizing regimen described for bradykinin[2,3] whereby a first immunizing dose is given in the pads of the paws with second and third immunizing doses given intradermally in the back at intervals of 21 days.

Dilutions of the serum which produced a reaction on Ouchterlony plates against the histamine–RSA complex varied between 1 : 16 and 1 : > 256, with 80 per cent occurring at dilutions of 1 : 64 or greater. The ability of the antibodies in the serum to neutralize the biological effects of histamine was tested by making a LD_{50} mixture of histamine (0·19 mg/kg body weight for the guinea-pig) either with non-immune serum (control) or with immune serum (test) and injecting these intravenously into guinea-pigs.

Table 1. MODIFICATION OF LD_{50} DOSE OF HISTAMINE BY ANTIBODIES INDUCED BY AN ANTIGEN PRODUCED BY A COMPLEX OF HISTAMINE AND RABBIT SERUM ALBUMIN

	Reaction					
	None	Mild	Moderate	Severe	Death	Total
LD_{50} in non-immune serum						
No. of animals	0	6	4	3	11	24
Percentage	0	25	16	13	46	100
LD_{50} in immune serum						
No. of animals	2	22	6	0	3	33
Percentage	6	67	18	0	9	100

Table 1 shows that the LD_{50} intravenous dose of histamine made up with non-immune serum produced close to the expected 50 per cent death rate with 59 per cent of the animals in the severe reaction and death categories. On the other hand, the LD_{50} intravenous dose of histamine made up with immune serum produced only 9 per cent of animals in the severe reaction and death categories with 73 per cent of animals in the no reaction and mild reaction categories. This shift in distribution was statistically highly significant ($P < 0{\cdot}005$) using the χ^2 test for significance.

The presence of antibodies in actively immunized animals produced no obvious adverse effects in terms of changes in growth rate, overt behaviour or physical appearance. These results indicate that antibodies which neutralize histamine can be induced by an antigen resulting from covalently bonding histamine to a large protein molecule such as RSA.

Thomas R. A. Davis
Kathleen M. Meade

Life Sciences Division,
Arthur D. Little, Inc.,
Cambridge, Massachusetts.

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[1] Sheehan, J. C., and Hlavka, J. J., *J. Org. Chem.*, 21, 439 (1956).
[2] Goodfriend, T. L., Levine, L., and Fasman, G., *Science*, **144**, 1344 (1964).
[3] Davis, T. R. A., and Goodfriend, T. L., *Amer. J. Physiol.*, **217**, 73 (1969).
[4] Oken, D. E., and Biber, T. U. L., *Amer. J. Physiol.*, **214**, 791 (1968).

Examination of "Species Specificity" of Avian Interferons

Interferon preparations usually show some degree of species specificity in their antiviral effects, exerting their greatest effect on growth of viruses in cells of the species which produced the interferon, and being less active or totally inactive in cells of other species[1–3]. Recent studies have shown that species specificity is far from absolute: certain human and primate interferons have proved active in rabbit cells[4]; the activity of monkey interferon in human cells was first noted by Isaacs *et al.*[5]; and cross-reactivity of human and monkey interferon systems at the level of the taxonomic family has been demonstrated[6]. Mouse serum interferon expresses one-twentieth of its mouse cell activity on other rodent cells[7].

These findings suggest that the closeness of the genetic relationship between two species may determine the ability of their interferons to exhibit heterologous activity. To examine this possibility, we have studied the reactivity of avian interferons from three orders of the class *Aves* on cells of closely and distantly related avian species.

Embryo fibroblasts from one-half term embryonated eggs were used, and interferons were produced either in eggs or in the cell cultures. Eggs were inoculated with influenza A–1 virus, strain WS–157. Allantoic fluid was harvested after 48 h incubation. Interferon was produced in cell culture by application of ultraviolet-inactivated Newcastle disease virus at a multiplicity of ten virus plaque forming units (pfu) per cell estimated before irradiation. Culture fluids were prepared after 25 h incubation at 37° C. All preparations were titrated to *p*H 2 with HCl and stored for 48 h at 4° C. After neutralization with NaOH solution, samples were centrifuged at 100,000*g* for 1·5 h. Supernatants were checked for the complete removal of virus by reinoculation into embryonated hens' eggs.

Interferon assays were done on monolayers of embryo fibroblasts in 60 mm Petri dishes. Two-fold dilutions of the samples were applied and left in contact with the cells for 20 h. The cells were then washed with balanced salts solution, and 50 to 80 pfu of vesicular stomatitis virus