acid-soluble collagen in the skin of old hamsters.

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Antigenic Relationships in Insect Extracts.* (23725)

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Recent findings have indicated that dust from disintegrated insects may constitute a common type of antigen responsible for asthma and hay fever(1). One of the problems raised in this connection is whether the observed group reactivity to insects in allergic subjects is due to identical allergens in the extracts of various insects. An unequivocal answer may be obtained only by isolation and comparison of purified allergens from different insects. Useful information about the relationship of extract constituents which may also serve as a guide to fractionation of insect extracts may, however, be obtained by immunological study.

Antigens of several grass pollen extracts were studied by Gosselin *et al.*(2) using hemagglutination. They found many cross reactions among the grasses but also a high degree of specificity by inhibition technics. Gel diffusion has been applied extensively to allergen extracts by Wodehouse(3). Although our plan of study of antigenic relationships of insect extracts included such technics as anaphylactic shock, the production of asthma in the guinea pig from antigen aerosols, Schultz-Dale reaction and passive transfer reactions with reagins, the present study is confined to hemagglutination and specific precipitation in agar.

Materials and methods. The insects used for this study were chosen to satisfy the following requisites: (1) previously demonstrated wheeling skin reactions and clinical allergy in man; (2) close taxonomic relationship between some forms; (3) fairly distant biologic relationship between others; (4) availability of adequate quantities of a pure breed of insect. *Insects.* Mayfly (Ephemoptera), fly (Phormia regina), cricket (Acheta domestica), cockroach (Blatella germanicum) and silk pupa (Bombyx mori) were dried, ground, the ether soluble fraction discarded and dried again. *Extracts.* One gram of dried powder was extracted overnight in 10 ml saline with shaking. Extracts were clarified by centrifugation, merthiolate was added and the solution was frozen. This extract was designated 1:10, and subsequent dilutions were expressed on this basis. *Antisera.* Rabbits were immunized with 4 ml of 1:10 extract in Freund's adjuvant. After 4 weeks test bleedings were made and the gel precipitin pattern observed. Where necessary a second injection was made at this time. Sera were prepared from periodic bleedings and pools made in accordance with gel precipitation data. *Hemagglutination.* The tannic acid technic of Boyden(4) as modified by Stavitsky(5) was employed both for hemagglutination and hemagglutination inhibition titers. Hemagglutination inhibition was performed by preincubating 4 agglutinating doses of serum in all tubes with graded dilutions of antigen followed by addition of coated cells. Extracts were usually diluted to 1:100 for the purpose of coating sheep cells since more concentrated extracts often caused spontaneous agglutination or lysis. *Agar Diffusion.* The triangle plate method of Jennings

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TABLE I. Hemagglutination Titers of Antisera to Insect Extracts.

Antigen*	Sera				
	Mayfly	Fly	Roach	Cricket	Silk
Mayfly	1600	6400	0	800	0
Fly	0	10240	1600	800	20
Roach	6400	0	10240	800	0
Cricket	0	25600	800	10240	20
Silk	0	0	0	20	25600

* Tannic acid treated sheep cells coated with insect extract.

and Malone(6) was used. Plates were placed in moistened Petri dishes and covered with another dish of the same size and sealed with scotch tape to prevent desiccation. Patterns were generally recorded after development for 1 week at room temperature.

Results. Hemagglutination. Extensive cross reactions shown in Table I were obtained when the agglutination titer of each serum was determined against sheep cells coated with each extract. Inhibition of agglutination in the 5 homologous systems of extract coated cells and corresponding serum was appreciable only with the homologous extract as indicated in Table II. Inhibition of the above cross reactions (Table I) was often exhibited by extracts other than the cross reacting pair as is shown in Table III.

Agar precipitation. When an extract was placed in one depot of a triangle plate and its homologous serum in another, a number of bands were seen emanating as straight lines from the apex of the clear agar triangle formed between the two wells. The number of zones of precipitation obtained were as follows: Mayfly 3, fly 2, roach 6, cricket 4 and silk 3. When the third unused depot of the above experiment was filled with a heterologous extract, the pattern was virtually the same as before. In most cases, however, an additional diffuse and faint zone was seen emanating from the adjacent apex and forming a continuous arch into the apex from which the homologous zones originated. This band indicated a cross reaction between the heterologous extract and the serum and that this antigen was similar to or identical with some component in the homologous extract.

When each of 2 troughs was filled with a different extract and the third with a serum heterologous to both extracts one diffuse band of identity was usually observed and in some cases a second similar zone appeared. Without exception all heterologous combinations resulted in at least one band of identity between both extracts. This band of identity is evidence for a common antigen in all of the extracts studied. Substitution of normal rabbit serum for the specific heterologous serum above also resulted in the appearance of zones of identity. Normal serum did not reproduce any of the specific homologous zones but only the heterologous bands of identity. Normal serum of 2-week-old rabbits gave parallel results to serum from older animals. If the observed reaction with non-immune serum is due to antibody it is probably due to natural rather than acquired antibody.

TABLE II. Homologous Inhibition Titers of Insect Extracts.

Homologous reaction*	Extracts				
	Mayfly	Fly	Roach	Cricket	Silk
Mayfly	25600	400	0	0	0
Fly	0	3200	0	0	0
Roach	0	0	4000	0	0
Cricket	0	0	0	1600	0
Silk	0	400	200	200	25600

* Reaction between extract coated cells and homologous antiserum, in fixed agglutinating concentration, in presence of dilutions of extract.

TABLE III. Heterologous Inhibition Titers of Insect Extracts.

System		Extracts				
		Mayfly	Fly	Roach	Cricket	Silk
Mayfly	Anti-fly	800	800	200	1600	0
"	Anti-cricket	1600	200	200	800	0
Fly	"	3200	6400	1600	6400	0
"	Anti-roach	0	6400	1600	0	0
Roach	Anti-mayfly	1600	200	6400	200	0
"	Anti-fly	0	800	3200	1600	0
Cricket	"	0	6400	200	6400	0
"	Anti-roach	0	0	800	400	0

Discussion. The results obtained with hemagglutination inhibition indicate that the antigens of major quantitative significance are specific for each extract since only the homologous extract could effectively inhibit a homologous reaction. The cross reactions obtained between extract coated cells and heterologous serum show that at least some

of the extracts contain similar or identical antigens in addition to the major specific antigens. Each cross reaction might represent a unique antigen common to the two interacting insect extracts or there might be an antigen common to all of the extracts. For each cross reacting pair one expects inhibition by extracts of each member of the pair involved. When extracts other than those of the cross reacting pair also inhibit this is evidence for a common antigen in all the inhibitory extracts. By this criterion there appears to be an antigen common to mayfly, fly, roach and cricket; one common to fly, roach and cricket; one common to fly and roach, and another to cricket and roach. It cannot be determined from these data whether these are single or multiple antigens. There is evidence then for cross-reacting antigens of wide and of limited distribution among the insects studied which cannot be correlated with insect taxonomy. The original source of these antigens is probably the insects themselves but could also be due to fortuitous factors such as common bacterial contaminants.

Gel diffusion studies were initiated in part to confirm and amplify the findings in hemagglutination. Although apparent confirmation of extensive cross reactions was obtained, normal rabbit serum produced the same patterns as anti-insect rabbit serum. Gel diffusion, therefore, can neither confirm nor negate the results obtained by hemagglutination.

This study introduces at least two interesting possibilities. One is that the observed bands of identity with normal serum are artefacts and therefore due warning should be given those using the reported methods. The second possibility is that the bands are specific reactions between heterogenetic components of the insect extracts with some natural antibody present in rabbit serum. One type of natural antibody that might be involved is the isoagglutinin. If this is the responsible agent then one would have to conclude that the extracts contained materials related to mammalian blood group substances. Investigation of certain aspects of this problem is being made.

Summary. 1. Extracts of mayfly, fly, cockroach, cricket and silk have been investigated by hemagglutination and agar precipitation technics. 2. Evidence has been obtained for the presence of specific antigens in each extract as well as antigens common to one or more of the other extracts. 3. The minimum number of antigens in each extract has been determined by agar diffusion. 4. The agar diffusion and hemagglutination data provide information helpful in purification studies.

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Cartesian Diver Technics for Embryonic Chick Hearts.* (23726)

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The embryonic chick heart is ideal for certain metabolic studies because it is readily available, inexpensive, small enough to permit ready diffusion of dissolved gases to and from all parts, yet large enough to be handled easily.

Such hearts, however, are too small for successful use in the conventional Warburg apparatus and too large for use in the cartesian diver as previously described(1,2,3). But, by adopting a feature of the Warburg, namely

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