SERUM DIAGNOSIS AND RHOPALOCERA

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Serum reactions are one of the methods in the hands of the biochemists for detecting the subtle differences in the make-up of the protoplasm and proteins of animals. Numerous experimentors have investigated the reactions of vertebrate proteins and more recently proteins and protein allies derived from invertebrates have been used. Unfortunately, the method requires special training, a sizable laboratory, experimental animals, such as guinea pigs or rabbits, and time. Thus it is cumbersome for general routine in investigating the relationship of animal forms.

The method is based on the fact that a foreign substance injected into an animal causes a definite and specific reaction. This may be any one of several types. In this work we are interested in but one type, the production of *precipitins* or *agglutinins*, which, as their names indicate, cause a precipitation or agglutination of the irritating medium upon a future injection. They are derived by injecting into a guinea pig (or any suitable animal) a suspension of the protoplasm or protein; in our case we used that of *Eurymus philodice*. This irritating material is termed the "antigen."

As the antigen is absorbed into the system of the experimental animal, the blood builds up the combative elements, in our case precipitins or agglutinins. These are called the "antibodies." When the blood serum has built up considerable of these it is said to be "sensitized" to the antigen used (i.e. protoplasm of *Eurymus philodice* in this experiment). An injection of the antigen at this time will cause the blood serum to precipitate it and bring about an anaphylactic shock or even death. So instead of causing the reaction to take place in the guinea pig and thereby needing a great number of sensitized animals, we

draw from its heart 8–10 milliliters of blood, allow this to coagulate, and recover the serum. This serum contains the antibodies and the precipitating reactions between them and the antigen can be carried on in a test tube in a constant temperature bath at blood heat, 37.5° C.

The intensity of serologic reactions are recorded as follows:—

4 plus—extremely strong, entirely precipitated or agglutinated.
3 plus—strong, almost but definitely not a complete reaction.
2 plus—a fair reaction, probably 50% perfect.
1 plus—a good, recognizable reaction, not very intense.
plus-minus—a slight or doubtful reaction.
negative—no reaction.

The more intense is the reaction the closer relation it indicates between the sensitizing and reacting antigens. The less intense reactions will be differentiated more strongly in the higher dilutions than the lower dilutions of the antigen.

The authors are interested in insects and wished to determine if closely related forms give specific reactions when the usual methods applied to serum work are used; and, if these did not, just how closely forms might be related and still react specifically. In each of the three recorded experiments the sensitized serum was obtained from guinea pigs inoculated with "philodice antigens" described in Experiment One.

EXPERIMENT I

Ten male specimens of *Eurymus philodice* were macerated entire with 20 mls of physiologic saline solution. The mash was then held at 50° C. for one hour, filtered hot through coarse filter paper and sterilized at 58°–60° C. for one hour. The resultant antigen was yellow and clear. It contained 0.004 grams of extracted material per milliliter.

A 700 gram male guinea pig was selected and the fluid injected subcutaneously daily in doses beginning with 0.5 ml and progressing geometrically until a total of 7.5 mls had been injected amounting to 0.029 g. of "philodice antigens." On the tenth day five milliliters of blood were drawn from the heart and the serum separated. This was reacted undiluted with vary-

ing dilutions of the standardized antigens at 37.5° C. These standardized antigens were made from males of *Papilio Troilus, Pieris rapæ, Eurymus philodice, Eurymus eurytheme* and *Argynnis cybele.* Our standard was arbitrary, one milligram of suspended material in each milliliter of physiologic saline solution, and was prepared in a manner identical to the suspension used for the injections.

After 48 hours at 37.5° C. the following readings were noted:

	ANTIGEN DILUTION			
	1:1	1:10	1:100	1:1000
Eurymus philodice	4 plus	4 plus	t.c.	1 plus
Pieris rapæ	2 plus		4 plus	1 plus
Papilio troilus	neg.	3 plus	1 plus	neg.
Argynnis cybele	neg.	neg.	neg.	neg.

(t.c.—tube cracked during incubation)

The insects used in this experiment were papered specimens 17 to 18 months old. The results showed us that it would be profitable to repeat the experiment in the summer when fresh materials were available. Experiment I may be interpreted to show a very strong inter-family reaction (*Papillionidae* vs. *Nymphalidæ*) with a distinct differentiation between sub-families (*Papillioninæ* vs. *Pierininæ*) related to the insect used as sensitizer. (Note reaction of antigen dilutions *1:100* and *1:1000.*)

EXPERIMENT II

The first experiment was repeated as planned with freshly killed specimens and the results verify Experiment I. As will be seen by the following readings there is a distinct inter-generic differentiation (*Eurymus* vs. *Pieris*) in dilutions 1:10 and possibly an Order reaction in *1:1 dilution,* indicated by the "plus-minus" of *Argynnis,* both of which were lost by the use of dried specimens.

	ANTIGEN DILUTION			
	1:1	1:10	1:100	1:1000
Eurymus	4 plus	4 plus	4 plus	2 plus
Pieris	4 plus	2 plus	3 plus	plus-minus
Papilio	3 plus	1 plus	2 plus	neg.
Argynnis	plus minus	neg.	neg.	neg.
control	neg.	neg.	neg.	neg.

EXPERIMENT III

The third series of tests were carried on with dried insects to determine if two species as closely related as *E. philodice* and *E. eurytheme* would show any differentiation. No evidence that would tend to prove it was found in dilutions up to 1:100,000 and with incubation up to 72 hours.

CONCLUSIONS

1. The serum of guinea pigs sensitized by the injection of suitably prepared insect antigens, as of male *Eurymus philodice*, showed a specific reaction when the family was used as a unit, and

2. There was a distinct inter-subfamily reaction when dried insects were used, and

3. There was a distinct inter-generic reaction when freshly killed insects were used, and

4. There was no inter-specific reaction when dried, very closely allied species of insects were used, as *E. philodice* and *E. eurytheme*.

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