mines[15,16]; the signs of sympathetic activation in the heart[3,12] have already been mentioned. It has recently been shown[8] that the cardiac catecholamine stores are not reduced during hypothermia if the animals have undergone sympathectomy according to the present procedure immediately before cooling. It can therefore be assumed that the reduction of cardiac catecholamines during hypothermia requires an intact sympathetic neuron link to the heart. Hence, it seems that the decrease in cardiac noradrenaline registered in the present sympathectomized animals (Table) reflects a decrease caused mainly by the denervation rather than by the additional hypothermia. For this reason, the percental decrease in noradrenaline of the hearts in the Table has been calculated from the values obtained in the normothermic, methoxamine-treated group of animals.

Since the reduction in cardiac noradrenaline was generally more pronounced in the 3-day group (Table), it appears that cardiac noradrenaline continues to decrease also beyond the second day after sympathectomy. In view of these considerations it is obviously impossible to correlate the catecholamine level remaining in the heart 2–3 days after sympathectomy to the frequency of ventricular fibrillation during deep hypothermia. For example, it is probable that denervation in the 3 surviving animals from the 2-day group has been more complete than indicated by the noradrenaline figures (55, 68 and 73% reduction), and that the amount of cardiac noradrenaline representing *intact* sympathetic nerves was in fact lower.

The frequency of ventricular fibrillation during hypothermia is considerably lower in animals subjected to sympathectomy by the same operation procedure immediately before cooling, even though the level of cardiac noradrenaline remains unchanged[8]. Hence, there is strong reason to believe that the high rate of ventricular fibrillation in the present series – in spite of a pronounced reduction of the cardiac noradrenaline concentration – is caused by the progressive development of supersensitivity of the myocardial receptors after denervation[6]. The fact that the surviving 5 animals (Table) had the lowest noradrenaline concentration in the 2-day and 3-day groups, respectively, indicates that the denervation has been most complete in these animals. It is therefore conceivable that the progressive supersensitivity of the myocardial receptors to catecholamines released from the intact cardiac adrenergic nerves has been the most important factor in the development of ventricular fibrillation in animals subjected to hypothermia 2–3 days following cardiac sympathectomy. If circulating catecholamines from other parts of the sympatho-adrenal system had been of any importance in the supersensitivity mechanism it could have been expected that all animals should have developed ventricular fibrillation under the present conditions.

In conclusion, the present series of experiments show that hypothermia per se results in a reduction in cardiac noradrenaline, probably by a central activation mechanism. Attempts to reduce the cardiac noradrenaline stores through sympathectomy 2 or 3 days before cooling does not afford any significant protection against spontaneous ventricular fibrillation. This is probably due to the rapid development of supersensitivity of the myocardium to catecholamines, notably the noradrenaline released from the cardiac adrenergic nerves if the denervation has not been sufficiently complete[18].

Zusammenfassung. Versuche mit Katzen haben gezeigt, dass Hypothermie in einer Reduzierung des Noradrenalingehaltes im Herzen resultiert (zentrale Aktivierung). Der Versuch, den Noradrenalingehalt im Herzen durch Sympathektomie 2 oder 3 Tage vor Unterkühlung zu reduzieren, ergibt aber keinen signifikanten Schutz gegen spontanes Ventrikelflimmern. Dieses Verhalten beruht wahrscheinlich auf der schnellen Entwicklung der Empfindlichkeitssteigerung des Myokards gegenüber Katecholaminen und besonders Noradrenalin, das von adrenergen Herznerven freigesetzt wird (bei unvollständiger Denervierung).

K. C. Nielsen, Ch. Owman and E. Rosengren

Neurosurgical Clinic A, University Hospital of Lund, Institute of Anatomy and Histology, Department of Pharmacology, Lund University, 223 62 Lund (Sweden) and Department of Neurological Surgery, New York University Medical School, New York (N.Y., USA), 18 July 1968.

[15] T. G. Brown and M. V. Cotten, Fedn Proc. Fedn Am. Socs exp. Biol. *15*, 405 (1956).
[16] W. Holobut, Acta physiol. hung. *29*, 383 (1966).
[17] B. Falck and Ch. Owman, Acta Univ. Lund. II 7, 1 (1965).
[18] Supported by grants from the Association for the Aid of Crippled Children, New York; and from Anna-Lisa and Sven Eric Lundgren's Foundation.

Detection of Antibody and Antigen in the Milkweed Bug, *Oncopeltus fasciatus*

Most of the attempts to demonstrate antibody production in insects have been with microorganisms or particulate antigens and the tests have been the standard serological ones[1–3]. Kamon and Shulov[4] immunized locusts with scorpion venom but they could not demonstrate any antibody production.

We have used 2 complex soluble antigen systems, rabbit serum or goat globulin, and 2 very sensitive detection tools, the fluorescent antibody technique[5] and the Ouchterlony technique[6], in an attempt to detect antibody production and to trace the fate of the antigen in the large milkweed bug, *Oncopeltus fasciatus* (Dallas). The antigen injection was always given with a microinjector and a 30 gauge needle at the base of a metathoracic leg of an insect which was 24 h past the molt into the last nymphal stadium. The control insects were injected with

[1] J. D. Briggs, *The Physiology of Insecta* (Ed. M. Rockstein; Acad. Press, New York 1964), vol. 3.
[2] R. E. Gingrich, J. Insect Physiol. *10*, 179 (1964).
[3] J. M. Stephens, *Insect Pathology* (Ed. E. Steinhaus; Acad. Press, New York 1963).
[4] E. Kamon and A. Shulov, Invert. Path. *7*, 192 (1965).
[5] A. H. Coons, J. C. Snyder, F. S. Cheever and E. S. Murray, J. exp. Med. *91*, 31 (1950).
[6] A. J. Crowle, *Immunodiffusion* (Acad. Press, New York 1961).

0.7% saline. All insects were anesthetized with CO_2 and bled or sacrificed 24 h after the injection.

The smears and tissue sections were examined for fluorescence with an AO Fluorostar microscope and a Fluorolume illuminator with a 200 watt mercury arc lamp. An AO exciter filter No. 693, which transmitted light with a wave-length of 325 nm, and a barrier filter No. 706 were used.

The methods used for the detection of antibody are summarized in Table I. We prepared the fluorescein-rabbit serum[7] and in some cases we adsorbed it with tissue powder[8] prepared from milkweed bugs in order to decrease non-specific staining. The Ouchterlony tests were conducted with hemolymph from the injected bugs and rabbit serum. No specific fluorescence was seen on any of the preparations and all the Ouchterlony tests were negative.

Table I. Methods used to detect antibody production in the milkweed bug

Antigen injected	Hemolymph smears	Frozen sections[11]	Paraffin sections[12]	Detection method
Rabbit serum				
2 µl	×		×	Fluorescein-rabbit serum
0.1 µl	×	×		Adsorbed fluorescein-rabbit serum
5 µl				Ouchterlony at 24 h
1 µl				Ouchterlony at 24 h
0.1 µl				Ouchterlony at 24 h

Table II. Methods used to detect injected antigens in the milkweed bug

Antigen injected	Hemolymph smears	Frozen sections	Paraffin sections	Detection method
Fluorescein-goat globulin				
2 µl	×		×	Direct microscopic examination
Rhodamine-rabbit serum				
5 µl		×		Direct microscopic examination
6 µl[a]		×		Direct microscopic examination
7 µl[a]	×		×	Direct microscopic examination
10 µl	×			Direct microscopic examination
Rabbit serum				
2 µl			×	Stained with fluorescein-antirabbitglobulin
0.1 µl				Ouchterlony, 197 h
5 µl				Ouchterlony, 197 h
Fluorescein-rabbit serum				
5 µl				Excreta
10 µl				Excreta

[a] Concentrated 3 times.

The fate of the injected antigen was followed with tissue sections, blood smears, excreta studies and Ouchterlony tests (Table II). The fluorescein-goat globulin was purchased (Nut. Biochem. Corp., Cleveland); the fluorescein-rabbit serum and the rhodamine-rabbit serum were conjugated in our laboratory[7]. The rhodamine-rabbit serum was concentrated with a Schleicher and Schuell Collodion Bag Suction Apparatus. For the excreta studies, each bug was injected, anesthetized with CO_2 and attached by its dorsal thorax to an applicator stick with beeswax. The bugs were then fixed in place over pieces of filter paper which absorbed all their excretions. The bugs had access to water but no food during the collection period. After 24 h the filter papers were examined for fluorescence with a portable UV-light (2537 Å).

No fluorescence was detected in the tissues sections, blood smears or excreta.

For determining how long the antigen remained in the hemolymph, 8 bugs were bled (and sacrificed) at 24 h intervals after injection for the duration of the fifth stadium. Ouchterlony plates were prepared with hemolymph from each bug and goat antirabbit globulin (Nut. Biochem. Corp.). The plates showed that the rabbit serum was present in the hemolymph throughout the fifth stadium (197 h). In some cases the injected bugs were allowed to molt to the adult but the rabbit serum could not be detected after the molt.

In summary, we have extended the work on immunological responses of an insect to include the use of soluble antigens. We have used the sensitive fluorescent methods which have not been used previously to detect antibodies in insects (although KRYWIENCZYK[9] and SINHA and REDDY[10] used them to detect viruses in insects), and we have shown that the antigen remains in the hemolymph for an extended period of time. Our negative results corroborate the results of others in suggesting that conventional antibodies probably do not exist in insects[13].

Zusammenfassung. Mit Hilfe der Fluoreszenzmarkierung von Proteinen und Ouchterlonys Methode wurde festgestellt, dass Insekten keine Immunität besitzen; Blutserum des Kaninchens blieb während 197 h im Hämolymph der Insekten.

DOROTHY FEIR and G. T. SCHMIDT

Biology Department, St. Louis University, St. Louis (Missouri 63104, USA), 19 July 1968.

[7] H. RINDERKNECHT, Nature *193*, 167 (1962).
[8] A. H. COONS, E. H. LEDUC and J. M. CONNOLLY, J. exp. Med. *102*, 49 (1955).
[9] J. KRYWIENCZYK, J. Insect Path. *5*, 309 (1963).
[10] R. C. SINHA and D. V. R. REDDY, Virology *24*, 626 (1964).
[11] J. E. TOBIE, W. BURGDORFER and C. L. LARSON, Expl Parasit. *11*, 50 (1961).
[12] G. SAINTE-MARIE, J. Histochem. Cytochem. *10*, 250 (1962).
[13] This research was supported in part by St. Louis University CRIC grants Nos. 64-03 and 65-03.