rat α_2-M inhibited trypsin, chymotrypsin, plasmine, thrombin and elastase. The effect on the tumour growth in serum-supplemented Dulbecco-Voigt medium was checked by measuring daily the mitotic frequency of the leukaemia cells in a cell counter. In the test series, the medium contained additionally 0.1 mg α_2-M (or other globulin fractions). The starting concentration of L 1210 was always between 8×10^4–2×10^5 cells. Following the inoculation of 2×10^7 minced cells of the DÄNA-435 tumour into R-rats, the tumour was either allowed to develop unhindered or, after 2 days incubation period, a dosage of 0.5 mg α_2-M suspended in normal rat serum was administered to the animal. The total tumour mass was then compared in the control animals and those rats given α_2-M after a total period of 12 days. As internal controls, the leukaemia cells were incubated, respectively enriched serum was administered to R-rats with the following other serum protein fractions: haptoglobin (0.8 mg), α_2-lipoprotein (0.15 mg) and α_1-globulin (0.06 mg). In this context it should be pointed out that coeruloplasmin, a normal constituent of the α_2-globulin fraction in human and rabbit serum, behaves as an α_1-globulin. To determine the turnover of the injected α_2-M in the blood in some experiments [^{125}J]-labelled α_2-M (prepared by the method of CESKA, SJÖDIN and GROSSMÜLLER[10]) was administered. The specific activity of the [^{125}J] was 9.4 mCi/m*M*.

Results. When leukaemia cells (L 1210) were incubated in presence of 0.1 mg α_2-M, they failed to divide any further. Of all the other serum protein fractions tested in concentrations related to the physiological range, only α_1-globulin (0.06 mg) proved to be effective, although the extent was considerably less, i.e. about 40%, than was observed in presence of 0.1 mg α_2-M. It is likely that this limited action of α_1-globulin was due to the α_1-antitrypsin activity in this serum fraction. That it is most probably a protease inhibition which is responsible for the α_2-M action on leukaemia cell division is supported by the dramatic reduction in tumour growth which occurred also on incubating L 1210 with protease inhibitors from potatoes (15 µg/10^5 L 1210 cells) or with Trasylol.

In the in vivo experiments on R-rats, raising the serum level of the α_2-M fraction proved equally effective as concerned the tumour growth. Whereas the tumour mass of the 24 control rats was between 4.4–5.6 g 12 days after DÄNA-435 inoculation, in 34 rats given 0.5 mg α_2-M the tumour weight was only 2.1–3.4 g after periods as long as 18–23 days. In 11 other rats, the tumour regressed altogether, while only in 4 rats the rate of tumour growth reached a level comparable to the untreated controls between 15–18 days after inoculation. That we are dealing with a true α_2-M effect is indicated by the absence of similar growth retardation when either α_2-lipoprotein or haptoglobin were given. Only with the haptoglobin fraction was in some cases a limited inhibitory effect noted, so that a 5 g tumour mass was only attained at a period of about 15 days. The dosage of α_1-globulin of 0.06 mg which proved to be effective in vitro on leukaemia cells could not be tested systematically as toxic side effects occurred with a sizeable number of the test animals.

The use of [^{125}J]-labelled α_2-M revealed that the mean half-time for the serum protein is close to 7 days, which is somewhat faster than the turnover time observed with human patients[11].

Discussion. Results both on leukaemia cells and the solid DÄNA-435 tumour of R-rats indicate that α_2-macroglobulin exerts a major inhibitory effect on tumour growth. At least for the leukaemia cells, the mechanism responsible seems to involve proteases necessary for cell division, which substantiates BURGER's hypothesis[5]. Comparative studies using α_1-lipoprotein, haptoglobin or α_1-globulin suggest that this effect on growth retardation seems to be fairly specific for α_2-M. The slow turnover time of [^{125}J] α_2-M makes it rather likely that a high serum concentration will persist around the model tumour for almost the length of the normal growth period.

It seems likely that the α_2-M effect accounts also for the action of α_2-globulins against leukaemia induction as the result of X-ray irradiation in mice[12]. The α_2-M fraction, by means of its protease inhibitory action, may further slow down the accumulation of toxic cell debris as the result of cell lysis following tumour therapy[1] or major injuries, explaning in this way the neutralization by α_2-globulin of otherwise lethal γ-ray damage[13]. To what extent temporary increases in the α_2-M level may prove useful in the control of tumour growth in human patients remains to be seen in view of the known interactions with the blood clotting system[14].

Zusammenfassung. Versuche an Leukämiezellen L 1210 und in vivo-Versuche an DÄNA 435-tragenden R-Ratten haben gezeigt, dass α_2-Makroglobulin selektiv das Tumorwachstum hemmt.

M. VON ARDENNE and R. A. CHAPLAIN[15]

Research Institute Manfred von Ardenne, DDR–8051 Dresden-Weisser Hirsch, Zeppelinstrasse 7, 11 May 1973.

[10] M. CESKA, A. V. SJÖDIN, F. GROSSMÜLLER, Biochem. J. *121*, 139 (1971).
[11] R. KLUTHE, N. KLEINE and U. HAGEMANN, Experientia *22*, 385 (1966).
[12] I. BERENBLUM, M. BURGER and A. KNYSZYNSKI, Nature, Lond. *217*, 857 (1968).
[13] M. G. HANNA, P. NETTESHEIM, W. D. FISCHER, L. C. PETERS and M. W. FRANCIS, Science *157*, 1458 (1967).
[14] E. WERLE Ärztl. Forsch. *22*, 41 (1968).
[15] Present address: Kurklinik Pitzer, Genthstr. 9, D–6208 Bad Schwalbach (Germany).

Myonecrosis Induced by Scorpion Venom

Arthropods, mainly insects, are the natural prey and food of scorpions. However, up to now there is no report on the effect of scorpion venom on insect tissue. Therefore we thought it interesting to study the pathological changes induced by scorpion venom in an insect. This report describes our findings in the striated muscle of cockroaches that had been injected with Brazilian scorpion venom.

Materials and methods. The experiment was carried out on adult cockroaches (*Periplaneta americana*). The insects were injected in the posterior part of the abdomen with 12 µg of crude venom of the scorpion *Tityus serrulatus* dissolved in the proper physiological solution. Physiological solution was used as a control. All insects were injected with a fluid volume of 0.03 ml. The muscular tissue was removed from the anterior part of the thorax

24 h after the venom administration. The excised tissue was fixed in a 1.4% glutaraldehyde solution in cacodylate buffer (pH 7.3) for 2 h, post-fixed in 1% osmium tetroxide for 1 h, dehydrated in graded acetones solutions and embedded in Epon. Ultrathin sections were double stained with uranyl acetate and lead citrate, and examined in a Zeiss EM 9S-2 electron microscope.

Results. The control insects injected with physiological solution exibited no lesions of myonecrosis. Electron microscopic observations disclosed various levels of degeneration among muscle fibres in cockroaches injected with scorpion venom. Apparently normal fibres were intermixed with necrotic fibres (Figure 1). Vacuoles of varying sizes lying under the plasma membrane or between myofibrils were observed (Figure 1). Many vacuoles possessed myelin figures (Figure 2). As the necrosis progressed, the vacuoles became smaller. Myofibrillar organization was lost until the myofilaments became reduced to a disoriented meshwork of fine filaments (Figure 1, upper right). Remarkable swellings of mitochondria, including irregular cristae, many vesicles and ruptured cristae, were present (Figure 2). In completely degenerated muscle fibres, the plasma and basement membranes disappeared (Figure 1, at the arrows). The intermuscular nerves showed some axonal degenerative changes (Figure 1).

Discussion. Myonecrosis of striated muscle has been studied by means of the light and electron microscopes. A variety of toxins produces myonecrosis[1-8]. The types of injuries that develop as consequence of toxic agents vary from cloudy swelling of the sarcoplasm to total necrosis of the muscle fibre.

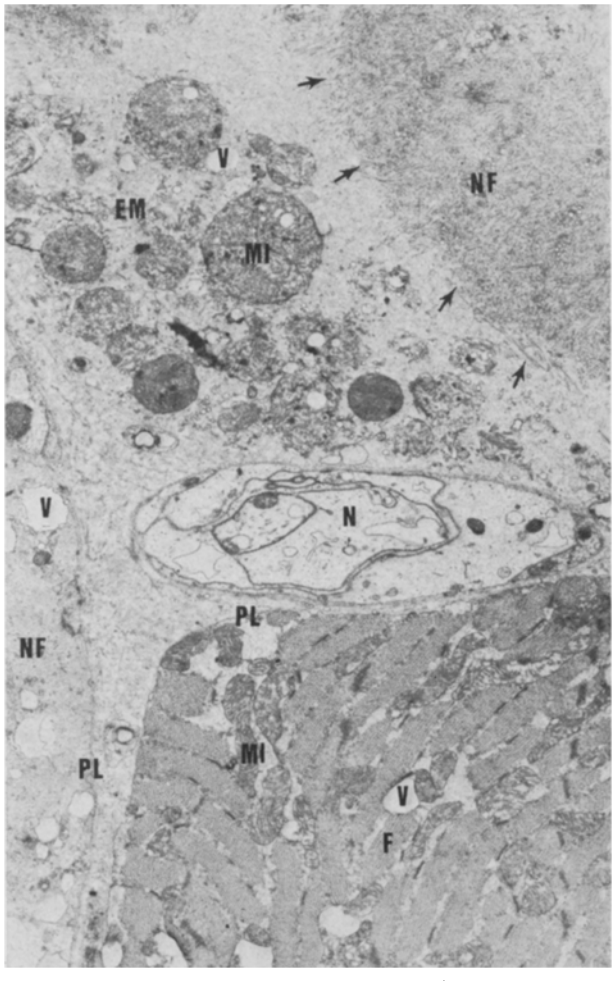

Fig. 1. Myonecrosis induced by scorpion venom. ×6,760.

[1] R. J. Stenger, D. Spiro, R. E. Scully and J. M. Shannon, Am. J. Path, *40*, 1 (1962).
[2] H. M. Price, D. C. Pease and J. M. Blumberg, Lab. Invest. *11*, 549 (1962).
[3] P. J. Anderson and S. K. Song, Proc. 5th int. Congr. Neuropath. *100*, 687 (1966).
[4] P. J. Anderson and S. K. Song, J. Neuropath, exp. Neurol. *25*, 149 (1966).
[5] R. D. Adams, D. Denny-Brown and C. M. Pearson, *Diseases of Muscle. A Study in Pathology* (Harper and Row, Publishers, New York 1967).
[6] S. W. Strunk, C. W. Smith and J. M. Blumberg, Am. J. Path. *50*, 89 (1967).
[7] J. M. Stringer, R. A. Kainer and A. T. Tu, Toxicol. Appl. Pharmac. *18*, 442 (1971).
[8] J. M. Stringer, R. A. Kainer and A. T. Tu, Am. J. Path. *67*, 127 (1972).

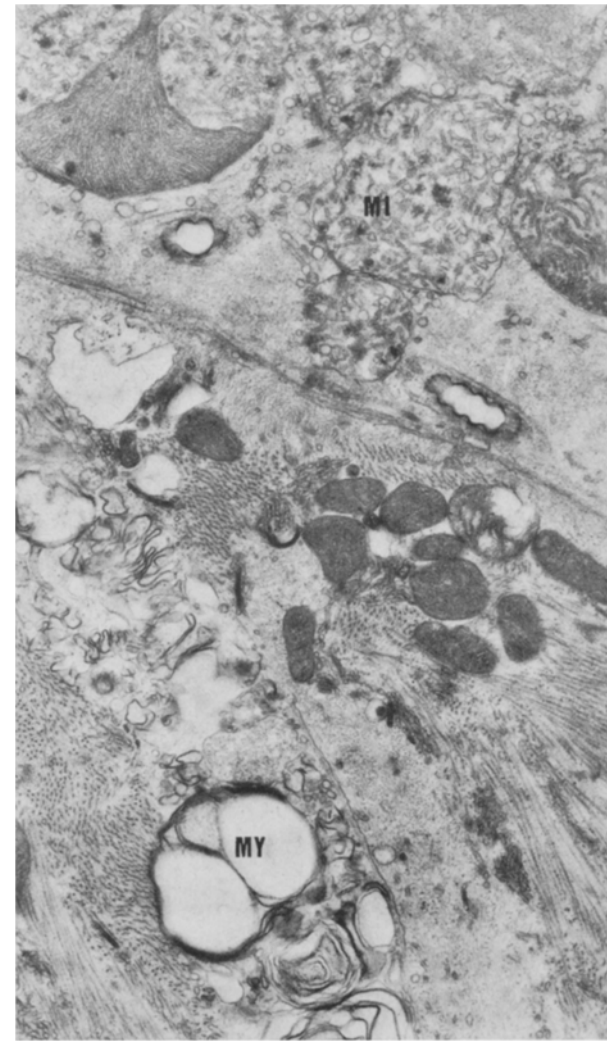

Fig. 2. Myonecrosis induced by scorpion venom. ×16,450. Abreviations used in the Figures: EM, endomysial space; F, myofilaments; MI, mitochondria; N, intermuscular nerve; V, vacuoles and vesicles; NF, necrotic muscle fibers; PL, plasma membrane; MY, myelin figures.

Myocardial pathological changes induced by scorpion venom consisted of edema and focal destruction of myofibrils and sarcoplasmic reticulum[9]. No lytic or disruptive lesions were demonstrated in the ultrastructural study of the frog sartorius muscle exposed to scorpion venom[10]. The pathological changes observed in the present investigation involved all components of the cockroach striated muscle, which suggests that crude venom of the scorpion *Tityus serrulatus* contains a potent toxin active in insect striated muscle.

JUNQUA and VACHON[11] cited numerous examples of differences in susceptibility of both vertebrates and invertebrates to scorpion venom. ZLOTKIN et al.[12,13] showed that scorpion venoms contain a variety of toxic proteins specifically active in mammals, insects or crustaceans. They suggested that this specificity might represent an adaptive process related to the survival of the species.

Scorpion venom produces spontaneous twitches or tetanic contraction in skeletal muscle[14-16]. This effect is independent of nerve stimulation, resulting from a direct stimulatory action on muscle cell membranes[14,15]. YAROM and MEIRI[10] studied the morphological alterations of the frog sartorius muscle exposed to scorpion venom with the aid of a technique used to detect intracellular calcium and magnesium. The results suggested that the venom acts directly on the muscle membrane, altering the calcium flux.

The pathological changes induced by scorpion venom in striated muscle of cockroach may be a nonspecific reaction to various kinds of injury, and it would be difficult to determine the nature of the venom damage from the histological changes alone. Cytochemical and biochemical studies need to be performed before many of these observations can be fully understood.

Zusammenfassung. Elektronenoptischer Nachweis, dass Skorpiongift (*Tityus serrulatus*) nach i.p. Verabreichung an der quergestreiften Muskulatur der Küchenschabe eine starke nekrosierende Wirkung ausübt.

M. A. ROSSI[17], A. L. FERREIRA, S. M. PAIVA and J. C. M. SANTOS

Laboratory for Electron Microscopy, Institute of Biology, State University of Campinas, Campinas (S.P., Brazil) and Dept. of Morphology, Medical School of Ribeirão Preto, Ribeirão Preto (S.P., Brazil), 16 April 1973.

[9] R. YAROM and K. BRAUN, Lab. Invest. *24*, 21 (1971).
[10] R. YAROM and U. MEIRI, Toxicon *10*, 291 (1972).
[11] C. JUNQUA and M. VACHON, *Les Arachnides Venimeux et leur venin* (Acad. Roy. des Sciences d'Outre Mer, Brussels 1968).
[12] E. ZLOTKIN, F. MIRANDA and S. LISSITZKY, Toxicon *10*, 207 (1972).
[13] E. ZLOTKIN, F. MIRANDA and S. LISSITZKY, Toxicon *10*, 211 (1972).
[14] K. R. ADAM and C. WEISS, Br. J. Pharmac. *14*, 334 (1959).
[15] K. R. ADAM, H. SCHMIDT, R. STAMPLI and C. WEISS, Br. J. Pharmac. *26*, 666 (1966).
[16] I. PARNAS, D. AVGAR and A. SHULOV, Toxicon *8*, 67 (1970).
[17] Present adress: Dept. of Pathology, Medical School of Ribeirão Preto, Caixa Postal 301, 14.100–Ribeirão Preto, S. P., Brazil.

Distribution du système glio-interstitiel chez les Mollusques: Les parties tonique et phasique du muscle adducteur postérieur chez *Anodonta*

L'étude de la répartition du système glio-interstitiel chez les Doridiens (Gastéropodes marins)[1,2] nous a permis d'établir que certaines parois musculaires comme celle de l'œsophage, contenaient un tissu glio-interstitiel abondant alors que celle du bulbe buccal en était complètement dépourvue. Les deux principales différences que nous avons notées entre les muscles de l'œsophage et ceux du bulbe buccal résident d'une part dans la nature de la contraction, de type tonique pour l'œsophage, phasique pour le bulbe et l'autre part dans le développement des espaces extracellulaires, beaucoup plus important dans l'œsophage.

Afin de déterminer s'il existait une corrélation entre les caractéristiques morphologiques et physiologiques des muscles et le développement du tissu glio-interstitiel, nous avons entrepris une étude comparative sur différents mollusques. Le muscle adducteur de l'Anodonte apparaît comme un matériel favorable puisqu'il comporte deux parties, une partie «phasique», à contraction rapide et brève, responsable de la fermeture des valves, et une partie «tonique», à contraction lente et prolongée, responsable du maintien de cette fermeture. Ces parties présentent l'avantage de pouvoir être distinguées à l'œil nu d'après leur couleur[3].

Partie tonique. Les fibres musculaires toniques sont de grande taille, avec un diamètre moyen d'environ 20 µm. Elles laissent entre elles de larges espaces extracellulaires conjonctifs (Figures 1 et 3). Nous ne reviendrons pas sur l'appareil contractile formé par les myofilaments, qui a déjà fait l'objet d'une étude détaillée[4]. Le réticulum est réduit à quelques citernes généralement associées à la membrane sarcoplasmique en dyades[5]. Les desmosomes entre myocytes sont fréquents, de même que les hémi-desmosomes accolés à la face interne de la membrane sarcoplasmique. Presque tous les prolongements nerveux sont étroitement associés à des prolongements de type glial contenant de gros granules de 5000 à 25000 Å, plus ou moins denses aux électrons. Cette téloglie[1,2] établit aussi de fréquents contacts avec les myocytes (Figures 1 et 3) mais n'émet pas de prolongement de type interstitiel comme l'on en a décrit dans plusieurs muscles toniques de mollusques[2]: elle reste toujours associée aux neurites[6]. Il faut noter également l'aspect particulier des contacts neuro-musculaires: de fins prolongements issus des myocytes vont à la rencontre des neurites qu'ils enveloppent parfois très étroitement. A ce niveau, on remarque des jonctions neuro-musculaires de type synaptique (Figure 1) présentant les différentiations caractéristiques des synapses formées entre éléments nerveux chez les mollusques[2,7].

[1] G. NICAISE, Thèse Doct. Etat Sci. Nat., Lyon, No 138 (1972).
[2] G. NICAISE, Int. Rev. Cytol. *34*, 251 (1973).
[3] I. Zs NAGY et J. SALANKI, Annls Biol., Tihany *37*, 131 (1970).
[4] I. Zs. NAGY, J. SALANKI et N. GARAMVOLGYI, J. Ultrastruct. Res. *37*, 1 (1971).
[5] R. H. NISBET and J. M. PLUMMER, Symp. zool. Soc. Lond. *22*, 193 (1968).
[6] I. Zs. NAGY et J. SALANKI ont décrit cette téologlie sous le nom de «special cells» sans préciser sa distribution dans l'adducteur de l'Anodonte.
[7] Des jonctions probablement similaires quoique moins différenciées ont été décrites dans le muscle adducteur antérieur d'*Anodonta* par I. Zs. NAGY et J. SALANKI[3].