This intimal thickening was found in nearly all the preparations of older spontaneously hypertensive rats. This area was frequently inhabited by a variable number of cells of varying size.

On the other hand, the vessel walls of the normotensive control animals never presented intimal thickening up to the age of 21 weeks, whereas some degree of intimal swelling was present in 30-week-old rats. This subendothelial proliferation was either concentric or restricted to one or more focal areas. These observations are illustrated in the Figure. Furthermore, the distance between the elastic laminae of the media was generally larger and relative hypertrophy of the smooth muscle occurred in the blood vessels of SHR. These differences were found in the abdominal aorta as well as in the other vascular preparations.

Discussion. Our observations concerning the blood pressure level in SHR are in agreement with the finding that the recent generations of this strain develop hypertension within the first 7 weeks of life[8]. This high blood pressure level is not accompanied by relative bradycardia, which provides an additional indication that the baroreceptor regulation has been shifted towards a set-point of higher blood pressure values. The histological findings demonstrate an intimal thickening in vessel walls of spontaneously hypertensive rats, more particularly in the region of the carotid sinus and of the aortic arch, where stretch receptors are located at the margin between the media and the adventitia of the vessel walls[9]. Medial hypertrophy was generally found in SHR irrespective of the topographical origin of the blood vessel. Similar modifications have been described in rats after induction of DOCA-hypertension[10].

According to REMINGTON[11] the arterial wall may be considered as an inner, distensible coat (the media-intima layer) covered by an outer restricting jacket (the adventitia).

The thickening of the inner coat may constitute a damping factor for the transmission of the intravascular pressure pulse through the vessel wall towards the stretch receptor endings. The mechanical properties of the intimal and medial structures apparently differ widely. Since techniques are not yet available for measuring the distribution of mechanical strains across the vascular wall thickness[12], the extent of baroreceptor resetting due to intimal swelling and/or medial hypertrophy will be difficult to evaluate.

In any case, a damping of the pressure pulse signal resulting from this vascular thickening could precisely lead to the type of baroreceptor resetting which has been described[4,5]. Whether these histological modifications of the vascular wall in the stretch receptor areas of SHR play a causative role or are merely a consequence of the high blood pressure, cannot be settled at present. They certainly constitute another loop in the vicious circle of spontaneous hypertension[13].

Zusammenfassung. Nachweis, dass in Ratten mit genetischem Hochdruck und normalem Herzrhythmus Verdickungen des Endotheliums im Karotissinus und im Aortabogen beobachtet werden. Eine Hypertrophie der Gefässmuskulatur war nachweisbar, was zu einer Abschwächung des Drucksignals zur Stelle der Pressorezeptoren führte. Diese dürften teilweise verantwortlich sein für die Verschiebung der Pressorezeptor-Steuerung bei genetisch hypertensiven Ratten.

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[8] K. OKAMOTO, Y. YAMORI, A. OOSHIMA, C. PARK, M. HAEBARA, M. MATSUMOTO, T. TANAKA, T. OKUDA, F. HAZAMA and M. KYOGOKU, *Spontaneous hypertension* (Ed. K. OKAMOTO; Igaka Shoin Ltd., Tokyo 1972), p. 1.

[9] A. ABRAHAM, *Microscopic Innervation of the Heart and Blood Vessels in Vertebrates Including Man* (Pergamon Press; London 1969).

[10] H. WOLINSKY, Atherosclerosis *11*, 251 (1970).

[11] J. W. REMINGTON, in *Handbook of Physiology* (Ed. W. F. HAMILTON and P. Dow; Am. Physiol. Soc., Washington 1963), sect. 2, vol. 2, Circulation.

[12] P. M. REES and P. JEPSON, Circulation Res. *26*, 461 (1970).

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The Inhibitory Effect of α-2-Macroglobulin on Tumour Growth

In a previous description of the detoxification effect exerted by α_2-globulin during massive tumour lysis[1], an interesting side effect was noted in a few of the tumour-bearing rats. Remnants of tumour cell aggregates, which normally supported a restoration of the original tumour mass within a 10-day-period, exhibited a much reduced mitotic activity, so that an interval of about 18–23 days elapsed before the tumour became palpable again. Testing various identifiable subfractions of the α_2-globulins revealed that only the α_2-macroglobulin exhibited a major delaying action on tumour growth. As yet the only identifiable biochemical property of α_2-macroglobulin is its binding to, and its potent inhibitory effect on various proteolytic enzymes[2–4].

Material and methods. Leukaemia cells (L 1210) and the DÄNA-435 tumour of the R-rat (Berlin-Buch) have been used. The reason behind the choice for these model systems was that leukaemia cells produce proteases essential for cell division[5], while the DÄNA-435 tumour is an immunologically well-controlled tumour. The α_2-macroglobulin (α_2-M) was prepared from rat serum as described by previous authors[6–8]. The serum was initially fractionated on a DEAE column. The α_2-M fraction was concentrated by dialysis through PM-30 Diaflo membranes followed by equilibration on Sephadex G-100. The purity of the α_2-M fraction was checked by immunoelectrophoresis with the aid of specific anti-serum[9]. Using standard enzyme essays[3,4] it could be shown that

[1] M. VON ARDENNE and R. A. CHAPLAIN Z. ges. inn. Med. *26*, 175 (1971).

[2] M. STEINBUCH and CH. BLATRIX Rev. fr. Etud. clin. biol. *13*, 142 (1968).

[3] J. BIETH M. PICHOIR and P. METAIS FEBS Lett. *8*, 319 (1970).

[4] K. JAMES, F. B. TAYLOR and H. H. FUDENBERG, Biochim. biophys. Acta *133*, 374 (1966).

[5] M. M. BURGER, Nature Lond. *227*, 170 (1970).

[6] J. W. MEHL, M. Y. PARK and W. O'CONNELL, Proc. Soc. exp. Biol. Med. *122* 203 (1966).

[7] D. S. PEPPER, Biochim. biophys. Acta *156*, 327 (1968).

[8] A. F. PARISI and B. L. VALLEE Biochemistry *9*, 2421 (1970).

[9] J. J. SCHEIDEGGER Int. Arch. Allergy appl. Immun. *7*, 103 (1956).

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.

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[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).
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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