artery so that the sharp medial edge of neocortical necrosis represents the boundary zone or 'watershed' between the territories of the anterior and middle cerebral arteries (ACA and MCA). A typical 'watershed' lesion as well as a marked loss of neurones in the outer layers was seen in the contralateral neocortex of 1 of 6 animals killed at 24 h.

As neuronal damage was unequivocal after a survival of 4 h, intervals between 24 and 4 h were omitted and animals surviving 3, 2, 1 and $^1/_2$ h were studied, as well as 6 killed at the end of the 40 min exposure to nitrogen ('0' survival). A narrow band of finely spongy tissue along the line of the ipsilateral ACA/MCA 'watershed' was recognizable in animals surviving from 4 to $^1/_2$ h and contained neurones in stages of ischaemic change. Encrustations of the Golgi network were visible here and also in scattered neurones in the neocortex and pyriform cortex after a survival of $^1/_2$ h (Figure 2b). The earliest evidence of ischaemic damage, visible in 2 animals after-'0' survival, took the form of microvacuolation of neuronal cytoplasm in scattered cells in the outer layers of the neocortex, in the pyriform cortex and in many cells of the hippocampus (H.l. and endfolium Figure 2c). Ischaemic cell change with encrustations was visible in the hippocampus after 1 h. Ischaemic damage in the thalamus, striatum and amygdaloid nucleus was less frequent and severe than in the neocortex.

In the contralateral neocortex and pyriform cortex of animals surviving from '0' h upwards, ischaemic cell change had reached the same stage as in the ipsilateral hemisphere but was less extensive. Typical lesions in the contralateral ACA/MCA 'watershed' were seen in three animals killed after 1, 3 and 4 h.

Of 28 animals exposed to nitrogen alone by the method already described, 11 (39%) died during the exposure. Of the 17 survivors the brains were histologically abnormal in 14. Ischaemic cell change occurred bilaterally in the neocortex, always sparing the ACA territories. Sharply-defined lesions in the ACA/MCA 'watersheds' were seen in two animals after 4 and 24 h. Ischaemic cell change was also seen in the pyriform cortex and hippocampus, but only occasionally in the basal ganglia. In the neocortex microvacuolation of neurones was visible after '0' survival and encrustations of nerve cells in animals surviving $^1/_2$ h and more.

The occurrence of lesions in neocortical 'watersheds' suggests that a major reduction in overall cerebral blood-flow had occurred[7,8], most probably due to myocardial hypoxia. A similar reduction in cerebral blood flow is the most probable explanation for the damage in the contralateral neocortex observed when exposure to nitrogen followed unilateral carotid ligation.

In the 'LEVINE preparations', swelling of the ipsilateral hemisphere was recognizable from $^1/_2$ h onwards, but was not detected before 3 h by MACDONALD and SPECTOR[9]. SPECTOR[2,3,10] has employed the contralateral hemisphere as a control in biochemical and enzymatic studies. In the present series, ipsilateral lesions, no more severe than those illustrated by SPECTOR[10], were often associated with variable contralateral alterations.

In contrast to the demonstration of ischaemic alterations in neurones from '0' survival onwards in the present series, the earliest evidence of similar changes has been minimal shrinkage of neurones and sponginess of tissue after 1 h obtained by ZEMAN[11], ischaemic cell change in the caudate nucleus after 3 h and in the neocortex and hippocampus after 4–6 h obtained by BECKER and BARRON[4], and in the hippocampus after 10–18 h obtained by SPECTOR[3].

Zusammenfassung. Es wird gezeigt, dass Schädigungen von Nervenzellen nach dem Aussetzen in eine Stickstoffatmosphäre, mit oder ohne Abklemmung der Carotis, histologisch erkennbare Veränderungen ergeben.

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Neuropsychiatric Research Unit, Medical Research Council Laboratories, Carshalton (Surrey, England), March 17, 1966.

[7] K. J. ZÜLCH, Proc. 2nd Internat. Congr. Neuropathol., London (Excerpta Medica Foundation, Amsterdam 1955), p. 251.
[8] J. H. ADAMS, J. B. BRIERLEY, R. C. R. CONNOR, and C. S. TREIP, Brain, in press.
[9] M. MACDONALD and R. G. SPECTOR, Br. J. exp. Path. *44*, 11 (1963).
[10] R. G. SPECTOR, Br. J. exp. Path. *42*, 623 (1961).
[11] W. ZEMAN, in *Selective Vulnerability of the Brain in Hypoxaemia* (Ed. J. P. SCHADÉ and W. A. MCMENEMEY; Blackwell, Oxford 1963).

Effects of Angiotensin II and Epinephrine Infusion on Juxtaglomerular Cell Granularity and Arterioles of the Rat Kidney[1]

In general, when a kidney is exposed to hypertensive blood pressure levels either in vivo or in vitro the granularity of the juxtaglomerular cells decreases[2–5], whereas kidneys protected from excessive elevation of blood pressure show an increased number of granules in juxtaglomerular cells. Angiotensin both increases aldosterone secretion by the zona glomerulosa of the adrenal cortex[6,7] and directly promotes vasopressor activity on intravenous infusion[8–13]. Indeed, ASSALI and WESTERSTEN[12] have suggested that angiotensin II is the most potent known constrictor of peripheral arteries, particularly the renal arteries.

The present study was performed to observe the changes in granularity of the juxtaglomerular apparatus in a kidney exposed in vivo to high blood pressure that was maintained by administration of angiotensin II or epinephrine.

16 female Sprague-Dawley rats weighing 100–150 g were divided into 2 groups of 8 rats each. The left kidney of each rat was removed under ether anesthesia and 2 h later infusion was begun with the rats now anesthetized with Nembutal (0.06–0.08 ml/100 g body weight).

In group 1 synthetic angiotensin II was infused for 1 h through a polyethylene tube (PE 50) inserted in the jugular vein. The dosage and rate were controlled manually in order to maintain systolic blood pressure above 180 mm Hg. The blood pressure was measured directly from the carotid artery through PE 60 poly-

ethylene tubing, using a Statham transducer and an Offner RS Dynograph.

In group 2 epinephrine (1-epinephrine bitartrate) was infused for 1 h subcutaneously at a rate sufficient to maintain the systolic blood pressure above 180 mm Hg.

Both kidneys were fixed in Helly's solution, sectioned at 5 μ, stained with hematoxylin and eosin, periodic acid Schiff, Verhoeff, Van Gieson and Bowie stains for microscopic study. Juxtaglomerular granularity was assessed by HARTROFT's method[14] and is expressed in the following presentation as the juxtaglomerular index (JGI).

Blood pressure response. When a single dose of angiotensin II was given intravenously, blood pressure increased to 200–220 mm Hg systolic and 160–180 mm Hg diastolic within 10–30 sec and returned to the preinjection level after 3–5 min. However, when angiotensin II was given repeatedly and the dosage continuously increased, blood pressure levels above 180 mm Hg systolic and 150 mm Hg diastolic could be readily maintained. Angiotensin II did not show an acute vasopressor effect on subcutaneous injection. Attempts to maintain continuously high blood pressure levels by intravenous infusion of epinephrine were unsuccessful, because toxic effects appeared very early. However, blood pressure levels of 200 mm Hg systolic and 160 mm Hg diastolic could be maintained by repeated subcutaneous injection.

Juxtaglomerular index. As shown in the Table, the JGI of group 1 increased from 22.6 $\pm$ 1.06 to 28.4 $\pm$ 1.68. This difference is significant ($p < 0.02$). In contrast, the JGI of epinephrine-infused rats (group 2) decreased from 22.3 $\pm$ 1.72 to 17.3 $\pm$ 1.49, but this was not significant ($p < 0.1$).

In group 1 the kidneys showed moderate dilatation and cast formation in distal convoluted and collecting tubules. Small arcuate and interlobular arteries were contracted as judged from the marked wrinkling of the internal elastica and thickened media (Figure A). Slight vacuolation of the media was noted but the severity of this change was not significantly different from controls.

In group 2 the kidney changes consisted of hyperemia, capillary dilatation and congestion. Some of the arcuate and interlobular arteries were markedly dilated, thin-walled and contained numerous erythrocytes (Figure B). In the more severe cases, interstitial bleeding was observed. Although a few casts were present in the tubules, their incidence and severity were much less than in group 1.

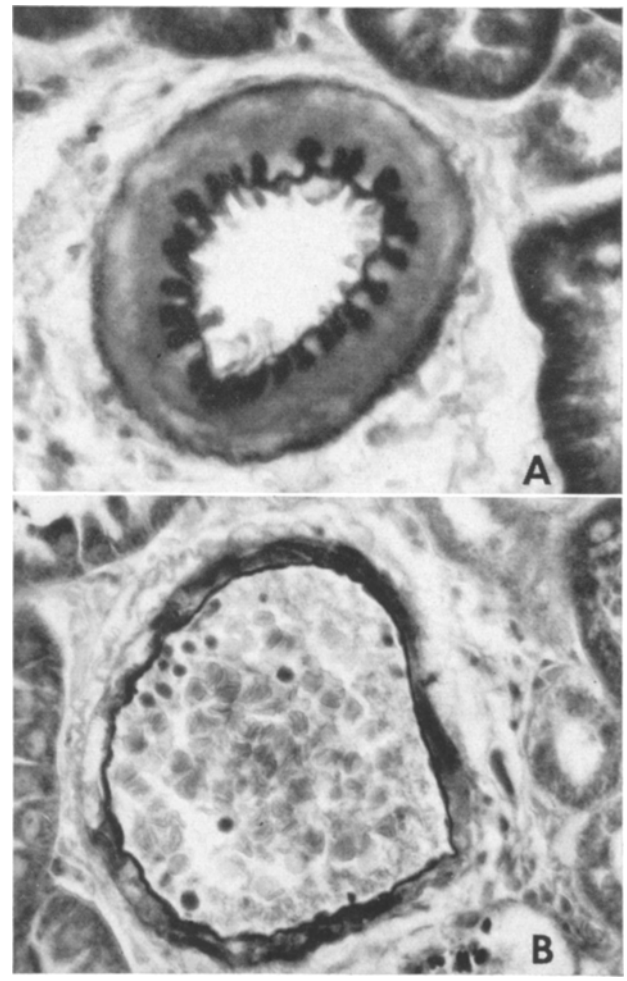

Fig. A. Section through an interlobular artery of a rat infused with angiotensin II. The wall is thickened and shows a smudgy cellular appearance of the smooth muscle and a greatly contracted internal elastic lamina.

Fig. B. Section through a small arcuate artery, showing the great dilatation of the vessel which is reflected by the thin wall and stretched internal elastic lamina. (Both Verhoeff. × 800.)

Juxtaglomerular index (JGI) of rats infused with angiotensin II and with epinephrine

Group 1			Group 2		
Angiotensin II infusion			Epinephrine infusion		
Rat	Before	After	Rat	Before	After
3	22.1	32.8	1	17.6	17.7
4	24.3	27.6	2	25.5	16.9
5	23.6	34.8	3	16.9	15.3
6	27.8	26.8	5	24.3	14.0
7	23.3	29.4	6	16.8	18.0
8	22.7	30.4	9	26.2	16.8
9	20.0	26.0	10	29.7	26.8
10	16.7	19.6	11	21.4	12.7
Mean	22.6	28.4	Mean	22.3	17.3
	$\pm$ 1.06	$\pm$ 1.68		$\pm$ 1.72	$\pm$ 1.49
$p < 0.02$			$p < 0.1$		

[1] This investigation was supported by U.S.P.H.S. Grant No. HE 06975 of the National Heart Institute.

[2] L. TOBIAN, Physiol. Rev. *40*, 280 (1960).
[3] L. TOBIAN, J. THOMPSON, R. TWEDT, and J. JANECEK, J. clin. Invest. *37*, 660 (1958).
[4] P. M. HARTROFT, J. exp. Med. *105*, 501 (1957).
[5] L. TOBIAN, A. TOMBOULIAN, and J. JANECEK, J. clin. Invest. *38*, 605 (1959).
[6] A. J. MARX, H. W. DEANE, T. F. MOWLES, and H. SHEPPARD, Endocrinology *73*, 329 (1963).
[7] P. J. MULROW and W. F. GANONG, Circulation *25*, 213 (1962).
[8] J. F. HERRICK, A. C. CORCORAN, and H. E. ESSEX, Am. J. Physiol. *135*, 88 (1941).
[9] R. G. ABELL and I. H. PAGE, J. exp. Med. *75*, 305 (1942).
[10] E. G. MCQUEEN and R. B. I. MORRISON, Br. Heart J. *23*, 1 (1961).
[11] Y. J. KATZ, P. PATEK, and S. BERNICK, Circulation Res. *11*, 955 (1962).
[12] N. S. ASSALI and A. WESTERSTEN, Circulation Res. *9*, 189 (1961).
[13] F. B. BYROM, Br. J. exp. Path. *45*, 7 (1964).
[14] P. M. HARTROFT and W. S. HARTROFT, J. exp. Med. *97*, 415 (1953).

The observations reported herein show a discrepancy between the acute effects of elevated blood pressure as produced by angiotensin II and epinephrine on the granularity of the juxtaglomerular apparatus and confirms the previously reported observations of KATZ et al.[11]. Although both compounds are vasoconstrictive agents[8,10,16–18] their effects on various vascular beds appear to differ inasmuch as angiotensin II has a preferential constrictor action on renal vessels which is not possessed equally by epinephrine. MCQUEEN and MORRISON[10] have shown that angiotensin produces marked depression of glomerular filtration rate, whereas epinephrine causes no significant change in this parameter despite considerable reduction in renal plasma flow. On the other hand, ASSALI and WESTERSTEN[12], HERRICK et al.[8] and DEL GRECO and PAGE[15] have reported that angiotensin also produces reduced renal blood flow in the dog. In view of TOBIAN's proposal that juxtaglomerular cell granularity is inversely proportional to the renal perfusion pressure it is possible that the changes observed in the JGI in the present study may be causally related to different hemodynamic responses of the renal vasculature to angiotensin II and epinephrine.

The contraction of interlobular and small arcuate arteries in rats infused with angiotensin correlates well with the findings of KATZ et al.[11], but evidence of degenerative changes in the vessel walls was not observed. The changes which this substance produces in renal hemodynamics suggests that the pre-glomerular vascular contraction may reduce the pressure impinging on the juxtaglomerular cells, thereby providing the stimulus for granule accumulation. The hyperemia and congestion of glomerular capillaries, the dilatation of post-glomerular veins and pre-glomerular arteries and arterioles seen in the rats infused with epinephrine suggest that vasoresistance may be post-glomerular, thereby elevating pre-glomerular arterial pressure and volume, this producing stretch which would then act as the stimulus for the slight fall in the JGI.

The rapidity of the change in juxtaglomerular cell granularity under the condition of angiotensin II infusion in this experiment suggests also that a direct feedback mechanism may be involved, quite independent of hemodynamic effects. Elucidation of this possibility, however, must await further experimentation in which hemodynamic changes are controlled.

These findings show that, although both angiotensin II and epinephrine are highly active direct vasoconstrictors capable of maintaining elevated systolic blood pressure, they differ in their effects on individual vascular beds and juxtaglomerular cell granularity.

Zusammenfassung. In Ratten wurde durch Injektion von Angiotensin II und Epinephrin akuter systemischer Hochdruck erzeugt. Angiotensin II verursachte Kontraktion der kleinen Arterien mit Zylindern in distalen Abschnitten und Sammelröhrchen. Der Juxtaglomeruläre Index (JGI) war leicht erhöht ($p < 0.02$). Epinephrin verursachte erhebliche Hyperämie und Dilatation der kleinen Arterien; der JGI war etwas erniedrigt ($p < 0{,}1$). Die Ergebnisse zeigen, dass, obwohl Angiotensin II und Epinephrin hochaktive Vasokonstriktoren sind und auch erhöhten Blutdruck aufrechtzuerhalten vermögen, sie verschiedene Effekte auf individuelle Gefässbereiche haben.

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Department of Pathology, State University of New York at Buffalo, Buffalo (New York, USA),
March 28, 1966.

[15] F. DEL GRECO and I. H. PAGE, Circulation *24*, 917 (1961).
[16] M. J. MANDEL and L. A. SAPIRSTEIN, Circulation Res. *10*, 807 (1962).
[17] B. G. ZIMMERMAN, F. M. ABBOUD, and J. W. ECKSTEIN, Am. J. Physiol. *206*, 701 (1964).
[18] J. C. ROSE, P. A. KOT, J. N. COHN, E. D. FREIS, and G. E. ECKERT, Circulation *25*, 247 (1962).

Über den zeitlichen Ablauf der Aktivierung von Cyclophosphamid im Menschen und die Testung der zytostatisch aktiven Form an Ehrlich-Mäuse-Aszites-Tumorzellen

Die Aktivierung von Cyclophosphamid im menschlichen Organismus ist unter den verschiedensten Gesichtspunkten erforscht worden. Untersuchungen der therapeutischen Eigenschaften des Cyclophosphamids unter Anwendung des Transportform-Wirkform-Prinzips stammen insbesondere von DRUCKREY et al.[1].

Über die Biochemie der Cyclophosphamidaktivierung gibt es zahlreiche Publikationen mit zum Teil noch unterschiedlichen Meinungen. Nach Ansicht von ARNOLD[2] und BROCK et al.[3] ist die Aktivierung von Cyclophosphamid ein Oxydationsprozess, der an mikrosomale Enzymsysteme, vor allem der Leber, gebunden ist. Nach RAUEN et al.[4,5] sind N-β-Chloräthyloxazolidon und N-β-Chloräthylaziridin die Endprodukte der Aktivierung von Cyclophosphamid.

Mit dem Mechanismus der zytostatischen Wirkung von aktivem Cyclophosphamid beschäftigte sich HOLZER[6], der die Wirkung alkylierender Substanzen am Beispiel Trenimon an der Hemmung der Milchsäuregärung von Ehrlich-Mäuse-Aszites-Tumorzellen untersuchte. Nach LISS et al.[7,8] ist die Hemmung der Milchsäuregärung durch alkylierende Substanzen die Folge einer primären Schädigung der Nukleinsäuresynthese der Zellen.

Für die in vitro Testung der Sensibilität menschlicher Tumoren gegenüber Cyclophosphamid benötigen wir

[1] H. DRUCKREY, D. STEINHOFF, M. NAKAYAMA, R. PREUSSMANN und K. ANGER, *Chemotherapie maligner Tumoren* (Schattauer Verlag, Stuttgart 1964), p. 11.
[2] H. ARNOLD, *Chemotherapie maligner Tumoren* (Schattauer Verlag, Stuttgart 1964), p. 37.
[3] N. BROCK und H. J. HOHORST, Arzneimittelforsch. *13*, 1021 (1963).
[4] H. M. RAUEN, A. REISCH und H. SCHRIEWER, Arzneimittelforsch. *14*, 161 (1964).
[5] H. M. RAUEN und K. NORPOTH, Naturwissenschaften *52*, 477 (1965).
[6] H. HOLZER, in *Chemotherapy of Cancer* (Ed. PL. H. PLATTNER; Elsevier IX Amsterdam 1964), p. 44.
[7] E. LISS und G. PALME, Naturwissenschaften *50*, 672 (1963).
[8] E. LISS und G. PALME, Z. f. Krebsforsch. *66*, 196 (1964).