animals were given 150 mg/kg of tetracycline hydrochloride by intraperitoneal injection and they were killed 1 h later.

Immediate frozen sections at 5 μ were examined by transmitted UV-light and also by ordinary histological techniques. The tetracycline in normal renal tubules appears as a very fine dust of yellow fluorescence in the epithelium (Figure 1). On the other hand, in necrotic epithelium it accumulates as coarse clumps of brightly fluorescent material (Figure 2). This is seen in the cells of a few tubules as early as 4 h after they have been killed (i.e. before the histological change is apparent) and in all the necrotic tubules at 24 h. This is the same whatever the manner of killing the cells, whether by nephrotoxins or ischaemia. The large take-up of tetracycline by the cells of renal tubules killed by mercuric chloride has been noted by previous workers[4] and a similar phenomenon has been observed in cultures of kidney cells which have been damaged by viruses[5].

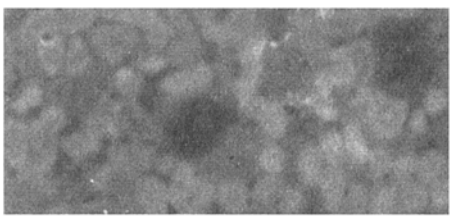

Fig. 1. Tetracycline in normal renal tubules (× 95).

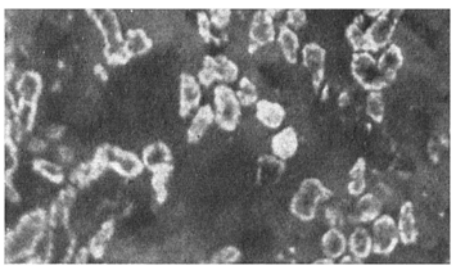

Fig. 2. Accumulation of tetracycline in the epithelium of convoluted tubules damaged by mercuric chloride (× 95).

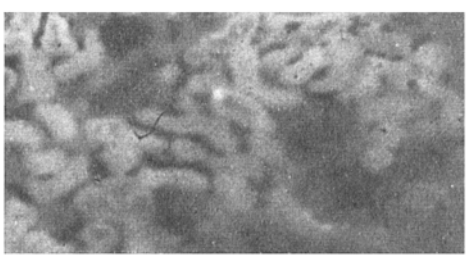

Fig. 3. Junction of normal renal cortex and area where the tubules fail to take up tetracycline. 1 h after injection of pituitrin to oestrogen-sensitized rat (× 95).

Variations in the uptake of tetracycline are also of value in assessing the renal blood flow under different experimental conditions. (1) If the renal pedicle is clamped permanently and tetracycline given 4 h later, it is not taken up by the kidney. This is what would be expected because there is no renal blood flow. (2) If the renal pedicle is clamped for 1 h and tetracycline administered 4 h after the removal of the clamp, the kidney takes up tetracycline in large amounts. This is indicative of a good re-establishment of the circulation through the kidney. (3) If the renal pedicle is clamped for 4 h and tetracycline is given 4 h after the removal of the clamp, none of the substance accumulates in the kidney. This is because after 4 h of ischaemia the circulation through the kidney cannot be re-established. These findings can be applied to the condition of the renal circulation in the oestrogen-sensitized rats which are given an injection of pituitrin. If the tetracycline is given 1 h after the subcutaneous injection of pituitrin, the renal cortex shows a very patchy take-up and there are several wedge-shaped areas in which no tetracycline accumulates (Figure 3). It appears reasonable to infer that there is no circulation at that time in these areas. It has been demonstrated by other methods that the pituitrin damage is due to spasm of branches of the renal artery[3]. However, if the tetracycline is not given until 4 h after the injection of pituitrin, the tetracycline is widely distributed throughout the cortex though some tubules show a greater accumulation of tetracycline than others. This may be taken to indicate that the renal blood flow has returned almost to normal within 4 h after the administration of pituitrin.

Zusammenfassung. Tetracyclin wird in mit vorübergehender Ischämie oder mit verschiedenen Nephrotoxinen geschädigten Tubulusepithelzellen intensiver gespeichert. Diese zunehmende Speicherung ist bereits nachweisbar, wenn histologische Veränderungen noch unausgeprägt sind.

Die Untersuchung der tubulären Tetracyclinaufnahme ist auch für die Beurteilung renaler Blutverteilungsänderungen wertvoll.

E. TAPP, R. CARROLL, and K. KOVÁCS

Department of Pathology, University of Liverpool (England), April 10, 1964.

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The Chemoreflex Induced by Intraarterial Injection of Aza-azepinophenothiazine, Veratridine and Bradykinin into Femoral and Brachial Arteries of Innervated and Divided Limbs of a Dog

Since HUNT[1] observed reflex changes of the systemic blood pressure induced by the electrical stimulation of the central end of cut sciatic nerve, many authors have confirmed the general sympathetic excitation as the due cause of this reflex. However, what kind of afferent fibres are brought to excitation is still the question. The authors were wholly unprepared for the evident result of pressor reflex induced by an intraarterial injection of an aza-azepinophenothiazine derivative into the femoral artery of divided limb of a dog, which, up to this point, had

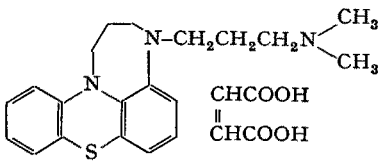
[1] R. HUNT, J. Physiol. *18*, 381 (1895).

appeared unfruitful after the use of more than 50 miscellaneous compounds in which not only effective vasoconstrictors but also vasodilators were included. In the course of the study, bradykinin[2] and veratridine[3] of the compounds supposed to cause pain sensation were found also to elicit the reflex changes of blood pressure. The picture of these reflexes was very similar to the sequence when the afferent fibre in the sciatic nerve was electrically stimulated.

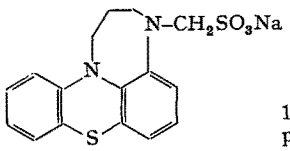
One divided hindlimb of dog A with intact nerves was perfused by the cross-circulation technique using a donor dog B. A sigmamotor pump was placed in the arterial side of the cross circulation to maintain the constant volume perfusion. The initial perfusion pressure was adjusted to the carotid arterial pressure of dog A. 0.1 ml of drug solution was given *via* a rubber tube close to the arterial cannula over a period of 10 sec. The carotid pressure of dog A and the perfusion pressure to the divided limb were registered on the smoked drum. 10 mg of heparin sodium were given initially to dog B, with an additional dose of 2 mg after a 1 h interval.

(1) *Dimethylaminopropyl-tetrahydro-aza-azepinophenothiazine malate (RPP 201)*[4]*. A reflex rise of the systemic blood pressure accompanied by the respiratory stimulation was observed following the injection of this compound into the femoral artery of the divided hindlimb, without exception, in 21 experiments. This phenomenon could be observed repeatedly at 10 min intervals. The smallest dose necessary was different in each case, varying from 100 to 500 μg. At these dose levels, this compound dilated the peripheral circulation of the hindlimb, but in smaller doses it resulted in an initial dilation followed by a more

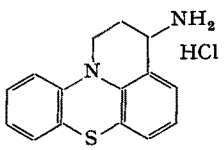
RPP 201



4 (3-dimethylamino-propyl)-1, 2, 3, 4-tetrahydro-4 aza-azepino-phenothiazine malate



1, 2, 3, 4-tetrahydro (1, 4) diazepino-phenothiazine methanesulfonate



3-amino-2, 3-dihydro-1 H-pyrido-phenothiazine hydrochloride

[2] L. F. CHAPMAN and H. G. WOLFF, Science *128*, 1208 (1958).
[3] W. FLACKE, Arch. exp. Path. Pharmak. *241*, 170 (1960).
[4] This study was supported by grants of the Pharmacological Research Foundation Inc., the Takamine Institute of Sankyo and the Squibb Institute for Medical Research. These compounds were kindly given to us by Dr. G. SUNAGAWA of the Takamine Institute*, Professor O. KRAYER of Harvard Medical School** and Dr. M. BODANSKY of the Squibb Institute and Professor M. CATTELL of Cornell University Medical College***.

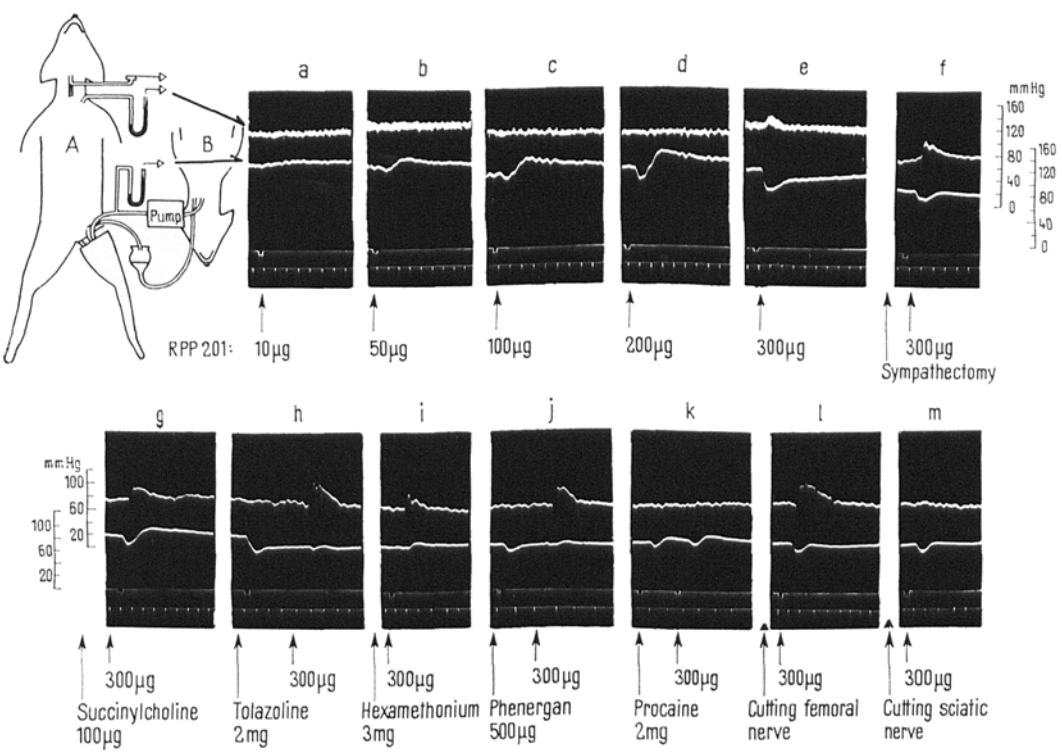


Fig. 1. The curves show the carotid pressure, the perfusion pressure of divided hindlimb of dog A, the mark of injection and the time mark at every 30 sec. All drugs were given *via* the rubber tube connected to the perfused femoral artery. a, b, c, d and e, 10, 50, 100, 200 and 300 μg of RPP 201; f, 300 μg of RPP 201 after sympathectomy; g, the same after 100 μg of succinylcholine; h, the same after 2 mg of tolazoline; i, the same after 3 mg of hexamethonium; j, the same after 500 μg of phenergan; k, the same after 2 mg of procaine; l, the same after cutting femoral nerve; m, the same after cutting sciatic nerve.

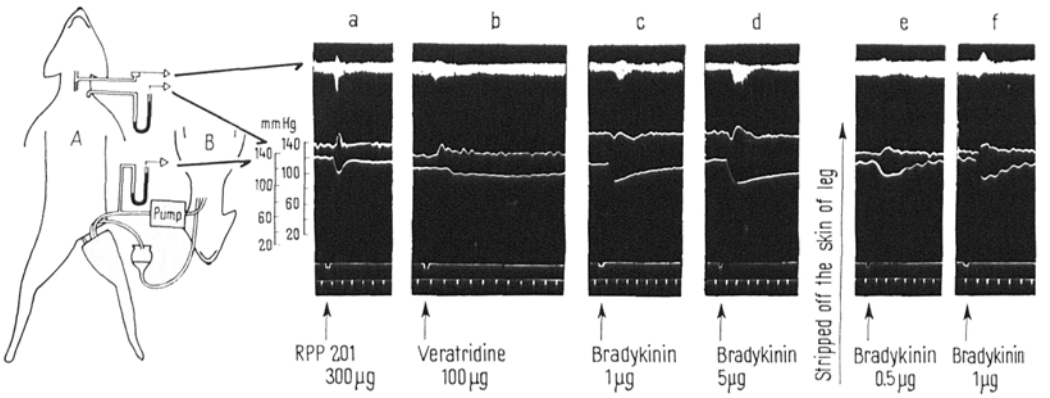


Fig. 2. The curves show the respiratory movement by Marey's tambour, the carotid pressure, the perfusion pressure of divided hindlimb of dog A, the mark of injection and the time mark at every 30 sec. a, 300 µg of RPP 201; b, 100 µg of veratridine; c and d, 1 and 5 µg of bradykinin; e and f, 0.5 and 1 µg of bradykinin after stripping off the skin of a divided hindlimb.

eminent constriction without any sign of reflex, either in the blood pressure or in the respiratory movement. The reflex rise of systemic pressure was not blocked by pretreatment with atropine, tolazoline or phenergan, by total extirpation of lumbal and sacular paraganglia or by cutting the femoral nerve, but was temporarily blocked by the use of procaine and completely blocked by cutting the sciatic nerve (Figure 1). The reflex response was reduced but still existed in a pithed animal at the first cervical level. In the divided forelimb cross circulation, this reflex response was induced in the same way as in the hindlimb preparation. Of phenothiazine compounds tested, 3-amino-2,3-dihydro-pyridophenothiazine was active but by far the weakest. Tetrahydro-diazepino-phenothiazine methane sulfonate, chlorpromazine, and phenothiazine itself were not effective.

(2) *Veratridine*[4**]. Veratridine, like aza-azepinophenothiazine, induced only a pressor reflex, but reflex by veratridine lasted longer than that by aza-azepinophenothiazine. Veratridine dilated the femoral artery but with a delay of more than 30 sec after the onset of reflex (Figure 2, b).

(3) *Bradykinin (synthetic)*[4***]. Synthetic bradykinin induced a depressor reflex with a small dose (1 µg), but an initial depressor was followed by pressor reflex after a larger dose (5 µg). Respiratory stimulation was observed after both doses. After stripping off the skin of the limb, the depressor reflex disappeared and the pressor reflex appeared (Figure 2, c, d, e and f).

The authors would like to advance the working hypothesis that aza-azepinophenothiazine derivatives and veratridine may act as releasers of the bradykinin[4].

Zusammenfassung. Reflektorische Blutdrucksteigerung wurde durch intraarterielle Injektion von Tetrahydro-aza-azepinophenothiazin und Veratridin am Präparat des innervierten Hinterbeins vom Hund hervorgerufen. Während kleine Dosen von Bradykinin nur leichte Blutdrucksenkung verursacht, führen grössere Dosen zu nachhaltigem Blutdruckanstieg. Bei Abhäuten des Hinterbeinpräparates unterblieb der Primäreffekt.

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Department of Pharmacology, Tohoku University School of Medicine, Sendai (Japan), December 3, 1963.

Evidence for the Renal Origin of Urinary Kinin

The presence of a biologically active polypeptide in urine was first demonstrated by BERALDO[1] in 1952. From that time, several pharmacologists[2–6] have investigated the substance and found that the urinary kinin is identical with or closely related to the nonapeptide bradykinin. The excretion of urinary kinin in man was studied in our laboratory[7]. In the course of this work, we were interested in the problem of whether the urinary kinin originates from circulating blood or not, because the origin of kinin in urine has been unknown to date. It is the purpose of this paper to report the results of the experiments on this problem.

Firstly, a kinin-forming enzyme kallikrein (Padutin, Bayer) was diluted with 5% glucose and infused intravenously in a patient in a dose of 3 units/min for 30 min. The blood pressure dropped by 30/20 mmHg and the patient felt itching in the face and chest, but no serious side effects were observed during the infusion. The urines before and during the infusion were separately collected,

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