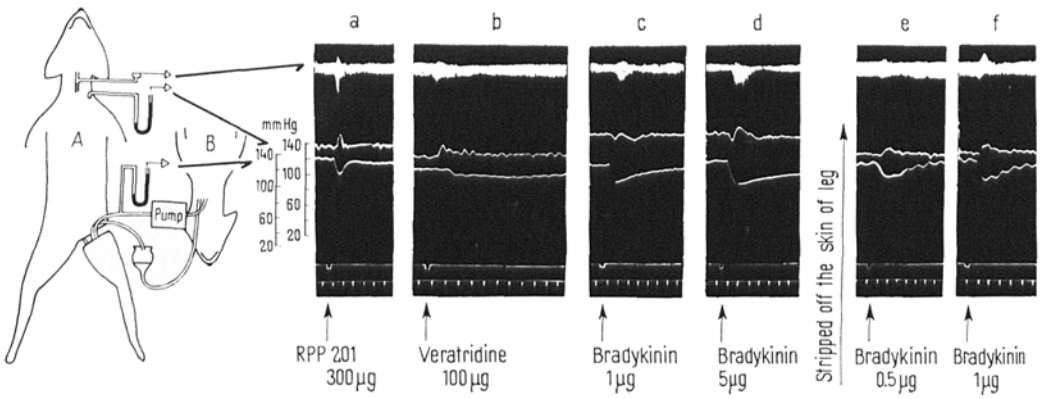

Fig. 2. The curves show the respiratory movement by Marey's tambour, the carotid pressure, the perfusion pressure of divided hindlimb of dog A, the mark of injection and the time mark at every 30 sec. a, 300 µg of RPP 201; b, 100 µg of veratridine; c and d, 1 and 5 µg of bradykinin; e and f, 0.5 and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

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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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extracted and assayed for their kinin contents according to the method previously reported[8]. The excretions of kinin in pre-infusion and infusion periods were 5.3 and 11 ng/min respectively. This degree of increase is quite insignificant, because normal excretions of urinary kinin are 3.8 ~ 28 ng/min (mean 13.2, standard deviation ± 6.3).

Secondly, the nonapeptide bradykinin, synthesized and kindly supplied by Sandoz Pharmaceutical Co., was dissolved in 5% glucose and infused into another patient in a total dose of 450 μg for 27 min. Tachycardia, hypotension (140/70 → 100/40) and flushing in the face were observed during infusion, but no other side effects were encountered. Assaying the urines, again no significant change was proved in the kinin excretions, i.e. 9.8 and 19 ng/min respectively. Taking the extra kinin (output during infusion minus preinfusion control) as originated from the external source, the rate of excreted kinin to the total infused dose was only 0.056%.

Thirdly, bradykinin was infused into the renal artery in dogs. Under pentobarbital anaesthesia, thin polyethylene tube was inserted into one renal artery *via* the femoral route. Urines were obtained from ipsilateral ureter which was also cannulated by polyethylene tubing. Three different doses of bradykinin, 0.75, 1.5, and 3.0 μg/kg, were dissolved separately in 15 ml 5% glucose, and infused for 15 min each. Control urine was obtained during the infusion of glucose only. Remarkable diuresis was elicited by the kinin infusions, but the arterial blood pressure remained unchanged. As listed in the Table, the kinin excretions were not significantly affected by the infusion of bradykinin.

Changes in kinin output during bradykinin infusion into renal artery in a dog[a]

Infusion rate (μg/kg/min)	Total dose infused (μg)	Control excretion (μg/15 min)	Excretion during infusion (μg/15 min)	Increase in kinin excretion (μg/15 min)	Rate of excretion to infused dose[b] (%)
0.05	9.7	0.24	0.19	−0.05	−0.27
0.10	18.4		0.40	+0.16	+0.86
0.20	37.5		0.39	+0.15	+0.40

[a] In two other dogs similar results were obtained.
[b] Increased kinin was assumed to originate from infused bradykinin.

It has previously been reported[9] from this laboratory that circulating angiotensin is not excreted in urine in any significant amount. Since the molecular weights of biologically active polypeptides are around 1000, they may easily be filtered through the glomeruli, but they do not appear in urine. This fact seems to suggest that the polypeptides are re-absorbed by tubular cells perhaps in the proximal convolution.

If the urinary kinin does not originate from circulating blood, where does it come from? From the results described above, it seems very probable that the kinin is produced and secreted by renal tubular cells perhaps in the lower part of the nephrons. Urinary kallikrein also differs from blood or pancreas kallikrein, and is thought to be secreted by the kidney itself. As is the case with kallikreins in urine, saliva or pancreatic juice, biological significance of urinary kinin remains to be elucidated in future[10].

Zusammenfassung. Nach intravenöser Kallikrein- oder Bradykinininfusion wurde an den Patienten keine signifikante Zunahme der Kininausscheidung beobachtet. Bradykinininfusion in die Nierenarterien des Hundes führte zu keiner Vermehrung der Kininexkretion im Harn. Die Ergebnisse bestätigen, dass Kinin im Harn nicht aus dem zirkulierenden Blut stammt, sondern von den Tubulusepithelien gebildet und sezerniert wird.

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Evaluation of the Method of Rivanol Precipitation of [^{35}S] Sulphated Mucopolysaccharides Present in the Material Obtained from a Small Group of Cells

The formation of rivanol and sulphated mucopolysaccharide (SM) complexes, practically insoluble in water, has been described by Whitehouse and Boström[1]. This phenomenon was employed to obtain protein-free SM complex from small groups of cartilage cells. Presence of SM in chondrocytes has been previously described[2].

3 ml of the medium[2], containing 15 μc [^{35}S]Na_2SO_4 (The Radiochemical Centre, Amersham), were equilibrated for 15 min at 37°C. Then, halved epiphyseal cartilages (femur) isolated from 14-day-old rats were added and incubation conducted for 30 min at 37°C. Following incubation the tissue was fixed in 95% ethanol overnight and then embedded in paraffin. 10 μ sections were passed through benzene, absolute and 95% ethanol, then 80% ethanol saturated with non-radioactive Na_2SO_4. Following inorganic sulphate exchange, the chondrocytes were separated from intercellular substance[3] and then collected in groups of 20 cells. The cells were taken at random from the same region of the section, using de Fonbrune micromanipulator. After evaporation of ethanol, 0.32

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