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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mμl of 0.5 *N* NaOH was added to each group of cells. Constriction micropipettes, measuring volumes of the order of 0.1 mμl with 2–3% accuracy, were used (Figure). The micropipettes were made in the de Fonbrune microforge, siliconized and then calibrated by measuring the diameter of the distilled water drops let into the paraffin oil. The cells were left for 20 h at 4°C in the NaOH solution. Then alkaline extract was removed, dried on another slide and rinsed with 80% acetic acid to neutralize NaOH. Surplus acetic acid was evaporated.

The extract was treated overnight at 37°C with 0.20 mμl of 0.5% papain solution in 0.1 *M* phosphate buffer, pH 6.8, containing 5 m*M* cysteine and 5 m*M* EDTA. The solution obtained was then evaporated on another slide and the residual dissolved in 0.13 mμl of HCl solution, pH 1.5. No precipitate appeared. The solution was transferred onto another place and the same volume of 1% rivanol (2:5-diamino-7-ethoxyacridine lactate) solution in HCl, pH 1.5, freshly prepared just before use, was added. After the addition of rivanol a complex of rivanol-SM precipitates. The concentration of sodium acetate in the reacting mixture was about 0.6 *M*. Four samples of precipitates, placed on the same slide, were washed twice with water (0.2mμl each), then covered with a collodion membrane and subjected to autoradiography (stripping film AR-10, Kodak). Radioactivity was found in preparations after 4 weeks of exposure. Counts of silver grains were performed in the autoradiographs of precipitates on the same slide, and the results were expressed in terms of grains per preparation.

Silver grain counts in autoradiographs of rivanol-treated preparations, obtained from different groups of cells, gave relatively uniform results (Table). The coefficient of variation of 12% is comparable with that for the individual cell's nucleic acid determination[4]. The evaluation of the method on a scale of small groups of cells is possible when there is no significant variation in: (1) the degree of $^{35}S$ incorporation into SM contained in individual alkaline extracts (this is assumed by the authors), and (2) the relation of $^{35}S$–SM to the other $^{35}S$ components of the extract. From the results obtained, this seems to be the case. Thus, the rivanol precipitation of SM present in the extracts occurs quantitatively. However, as yet there is no way of estimating the amount of SM in the preparations obtained.

Number of silver grains in autoradiographs of total extracts and rivanol treated preparations. Each preparation obtained from 20 cells. Number of silver grains in total extract is the sum of grain number in autoradiographs of washings and precipitates of the respective alkaline extract. All washings and precipitates were placed and autoradiographed on the same slide. Background is already subtracted (0.5 silver grains per 29.2 $\mu^2$).

| Total extract, grain number $\cdot 10^3$ | Rivanol treated preparation grain number per 29.2 $\mu^2$ | total $\cdot 10^3$ | % of total extract |
|---|---|---|---|
| 38.0 | 21.4 | 33.6 | 88.4 |
| 35.1 | 14.6 | 31.6 | 90.0 |
| 46.4 | 18.3 | 41.1 | 88.5 |
| 40.9 | 16.0 | 33.9 | 82.8 |

*Résumé.* Les petits groupes des chondrocytes étaient isolés du cartilage après incubation avec [$^{35}S$]$Na_2SO_4$. Les mucopolysaccharides étaient extractés de ces cellules par la technique de micromanipulation. Le rivanol permet la précipitation quantitative des S-mucopolysaccharides extractés de ces groupes des cellules.

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## Localization of Monoamines in the Lower Brain Stem

With the help of a new fluorescence method for the histochemical localization of monoamines, it has been shown that noradrenaline and 5-hydroxytryptamine, in, for example, the hypothalamus and spinal cord, are accumulated in very high concentrations in synaptic terminals[1,2]. The terminals originate from two special types of neurons, which contain in their cell bodies low concentrations of the respective amines and which may be characterized as adrenergic and 5-hydroxytryptaminergic respectively[3]. Evidence has now been obtained that also the dopamine, which is to be found in significant amounts in the lower brain stem[4], is localized to special neuron systems.

Mesencephalon, pons and medulla oblongata were examined in some 250 adult male albino rats, with the method of FALCK and HILLARP[5] as described in detail in another paper[3]. Histochemical and pharmacological experiments (reserpine, reserpine-nialamide, *m*-tyrosine, α-methyl-*m*-tyrosine) were used to check the specificity of the fluorescence reactions[1–3].

Following formaldehyde treatment, a specific fluorescence due to the presence of monoamines developed in the pericarya of several groups of nerve cells, and in typical nerve terminals present, in varying abundance, almost everywhere in the lower brain stem. The terminals were

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