and 250 µl aliquots of these suspensions were pipetted into centrifuge tubes. Then, 250 µl of physiological medium were added and the samples were mixed and allowed to stand for 10 min at 0 °C. Lastly, 0.5 ml of a radioactive solution which provided final concentrations of 2.1×10^{-7} *M* of U-^{14}C-L-glutamate (New England Nuclear Corp.; 234 mCi/mmole) and 2,3-^{3}H-L-aspartate (New England Nuclear Corp.; 26 Ci/mmole) were added, and the samples were mixed and allowed to stand at 0 °C for 15 min before final centrifugation at $17{,}000 \times g$, 30 min. Pellet and supernatant fractions were prepared for determination of radioactivity[10,15]. The use of 0.5×10^{-6} *M* ^{14}C-sucrose (New England Nuclear Corp.; 505 mCi/mmole) in the media in identical experiments permitted correction for the amounts of ^{3}H and ^{14}C entrapped in supernatant fluid of the pellets. All data were corrected for the amounts of amino acids present as occluded supernatant fluid of the pellets[10,16].

'Binding' of glutamate and aspartate to synaptosomal fractions of 6 regions of the feline brain

Brain region	nmole/g P_2 in non-sucrose space	
	^{14}C-glutamate	^{3}H-aspartate
Cerebellar cortex	10.7 ± 0.5 [b]	7.1 ± 0.4
Cerebral cortex	3.2 ± 0.1	3.2 ± 0.2
Pons	2.7 ± 0.05 [b]	1.9 ± 0.04
Medulla oblongata	2.9 ± 0.06 [b]	2.0 ± 0.05
Caudate nucleus	3.9 ± 0.1 [a]	3.3 ± 0.15
Corona radiata	1.6 ± 0.1 [a]	1.2 ± 0.1

Final concentrations of ^{14}C-glutamate and ^{3}H-asparate in the medium were 2.1×10^{-7} *M* (see Methods); radioactivity was determined by a double-isotope method[10]. Means ± SEM; 17 or 18 tissue samples in all cases; [a] and [b] indicate, respectively, $p < 0.01$ and $p < 0.001$, when these values are compared with corresponding values for ^{3}H-aspartate; all values for the 'binding' of ^{14}C-glutamate and ^{3}H-aspartate to particles of cerebellar cortex were significantly greater ($p < 0.001$) than those for all other regions (Student's *t*-test; two tailed).

Results and discussion. Results shown in the Table indicated that both amino acids were bound to a considerable extent to particles prepared from all regions of the brain that were studied, and that pronounced regional differences existed in this 'binding'. The order of potency of 'binding' of glutamate and aspartate to the particles was; cerebellar cortex ⩾ caudate nucleus ≧ cerebral cortex > medulla oblongata ≅ pons > corona radiata. Glutamate was bound to a greater extent than aspartate to particles of all regions studied, except for cerebral cortex. This study has revealed that significant differences exist in the 'binding' of glutamate and aspartate to synaptosome-enriched fractions of some cerebral structures. These differences, like those observed in studies of the 'binding' of GABA and glycine to synaptosomal fractions of cerebral cortex and spinal cord[9,10], cannot be explained simply by differences which exist in the endogenous tissue contents of these amino acids, and consequently, they could be related to the physiological actions of these amino acids, e.g., to mechanisms of transmitter-inactivation ('re-uptake') and/or receptor-interaction. It is noteworthy that the results presented herein revealed regional differences that could not be shown clearly by kinetic studies of the uptake processes for these amino acids, e.g.,[5,6]. Such differences might even become more pronounced if binding studies are carried out in the absence of Na^{+} (see ref.[17]).

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Deficiency in Renomedullary Prostaglandin Synthesis Related to the Evolution of Essential Hypertension

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Summary. Continued PG synthesis in the early stages of essential hypertension might reflect an activation of the renal antihypertensive function in respect to neurogenic and/or hormonal pression stimuli, subsequently a deficiency of renal PG synthesis related to irreversible changes with the kidney would lead to the prepoderance of a pressure mechanism, resulting to a further increase of blood pressure.

The identification of the potent antihypertensive prostaglandins (PGs) in the renal medulla[2,3] has provided further support for the concept that states of animal and human hypertension may result from a deficiency of renal depressor systems[4]. Since natural PGs occurring in the urine have been thought to reflect renal PGs synthesis[5], the detection of urinary PGs in patients with arterial hypertension could serve as a useful tool for testing this hypothesis. We therefore decided to invesigate the presence of substances with chromatographic behaviour and the bioassay properties of PGs in the urine of a group of patients with essential hypertension. The results are suggestive of a deficiency in renomedullary PG synthesis related to the evolution of the hypertensive disease.

Patients and methods. 21 patients with essential hypertension, 15 males and 6 females, aged 19 to 53

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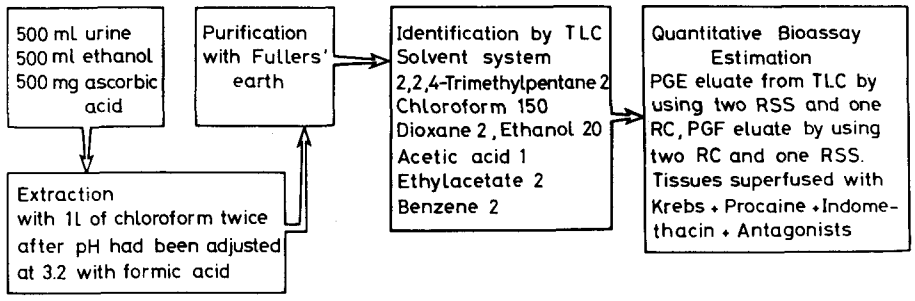

Fig. 1. A schematic portrayal of the procedure used for extraction, purification and quantitative bioassay estimation of prostaglandins.

years (mean age 36.2) were studied. In all patients, diastolic pressure was consistently in excess of 100 mm Hg on outpatient blood pressure recordings during the previous year, and no cause of secondary hypertension could be identified by extensive investigation. Patients were untreated or had discontinued therapy at least 1 week before study and were maintained on a 120 mEq sodium diet. Bioassays were performed on extracts of 500 ml from 24 h urine collections, after chromatographic separation into PGE and PGF groups. A modification of the quantitative bioassay technique presented in detail elsewhere[3] was used (Figure 1). Eluates from PGE corresponding areas were assayed as ng of PGE_2 equivalent and eluates from PGF corresponding areas as ng of $PGF_{2\alpha}$ equivalent. Since PG metabolites appearing in the urine generally have less biological activity than the parent compound[6] it might be assumed that most of the biologically active substances detected in this study were natural PGs.

Analytical methods. The procedure used for the extraction, purification, chromatographic identification and quantitative bioassay estimation of prostaglandins is shown in Figure 1.

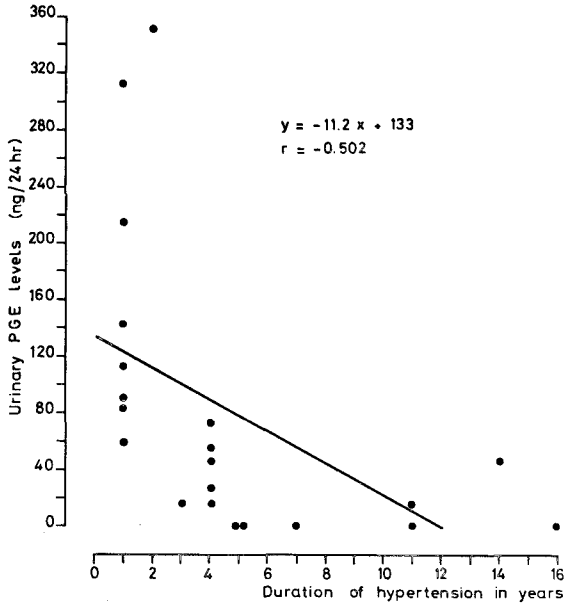

Fig. 2. Significant inverse correlation between the renal PGE secreted per 24 h and the duration of the hypertensive disease. This deficiency of the renal medulla in prostaglandin synthesis and release could rather be the consequence than the cause of the hypertensive disease.

Successive steps of the procedure were as follows.

a) Extraction. In 500 ml of urine equal volume of ethanol and 500 mg of ascorbic acid were added. After adjustment of the pH to 3.2 with formic acid the solution was extracted twice with equal volumes of chloroform. The extract was evaporated to dryness and formic acid was removed by oxygen-free nitrogen.

b) Purification. The residue was dissolved into 20 ml of ethanol 20% in saline and 1 g of Fullers' earth was added to the solution. The mixture was shaken for 10 min, allowed to stand at −20 °C for 10 min and centrifuged at 3,000 rpm for 15 min. After readjustment of the pH to 3.2 with formic acid the supernatant was extracted twice with equal volumes of chloroform. The extract was evaporated to dryness and formic acid was removed by oxygen-free nitrogen.

c) Identification. The residue was dissolved into 1.0 ml of chloroform and 200–300 µl were applied to thin-layer chromatographic plates (TLC ready platic sheets F 1500, silica gel 20 × 20, acid resistant; Carl Schleicher & Schüll D-3354 Dassel W-Germany). Standard PGE_2 and $PGF_{2\alpha}$ and the extracted material were developed in the following solvent system: 2,2,4-trimethylpentane 2, chloroform 150, dioxane 2, ethanol 20, acetic acid 1, benezene 2, ethylacetate 2. The standards were visualized with phosphomolybdic acid and heat (110 °C). Corresponding areas of the extract chromatogram opposite the PGE_2 and $PGF_{2\alpha}$ standard spots were scraped off and the biologically active substances were eluted from silica gel with 30 ml of ethanol. The eluates were evaporated to dryness and the residues were stored at −20 °C. They were dissolved in 2 ml of Krebs solution just before bioassays.

d) Quantitative bioassay estimation. 3 assay organs, 2 rat stomach strip (RSS) and 1 rat colon (RC) were used for the assay of the PGE compounds, and 1 RSS and 2 RC for the assay of the PGF compounds. The tissues were superfused with Krebs solution at the rate of 10 ml min^{-1} by a roller pump. The composition of the Krebs solution in $g \cdot l^{-1}$ of distilled water was as follows; NaCl 6.56, KCl 0.33, $CaCl_2$ 0.40, KH_2PO_4 0.16, $MgSO_4$ $7H_2O$ 0.29, $NaHCO_2$ 0.86, Na_2HPO_4 0.41, NaH_2PO_4 0.13, glucose 1.00. Like UNGER, STAMFORD and BENNETT[7] we found that substances like acetylcholine, histamine, noradrenaline, 5-hydroxytryptimine, angiotensin II, vasopressin and bradykinin, which affect the assay organs, were not extracted by the method used. However, in order to increase sensitivity and specificity and to diminish spontaneous contractions of the tissues, the following substances in mg/l were added to the Krebs

[6] P. PIPER, in *The Prostaglandins* (Ed. M. GUTHBERT; Heinemann, London 1973), p. 126.

solution: procaine hydrochloride 50, indomethacin 2, hyoscine methylbromide 0.1, phenoxybenzamide hydrochloride 0.1, propranolol hydrochloride 2 and methysergide bimalate 0.2 (final concentration of active bases). The solution was gased with a mixture of 5% carbon dioxide in oxygen. Contractions of the assay tissues were transduced through microdisplacement myograph transducers and were recorded at a physiograph (Narco biosystem Inc., Huston, Texas). After equilibration of the whole system for 2 h to acquire more sensitivity and baseline level stability, 1.0 ml of various concentrations of solutions of PGE_2 was perfused and the contractions recorded. Then, 1.0 ml of eluate from the TLC area corresponding to the PGE_2 standard spot (dissolved into 2.0 ml of Krebs) was similarly perfused and the contractions recorded. A second measurement was made with the other half of the eluate after calibration with PGE_2. A similar procedure was adopted for the eluate of the areas corresponding to the $PGF_{2\alpha}$ standard spots. When the response to the first half of the eluate was more than that to 5.0 ng of standard PGs the second half was further diluted with Krebs solution.

Results. Our results are illustrated in Figures 2 and 3. Mean values of 79.3 (SE = 21.4) ng/24 h, assayed as ng of PGE_2 equivalent, and of 47.6 (SE = 8.6) ng/24 h, assayed as ng of $PGF_{2\alpha}$ equivalent, were respectively obtained for PGE and PGF group. In 5 patients no detectable values of either PGE or PGF could be obtained. The duration of arterial hypertension in these patients was 5 years or more. Furthermore, in the overall group of patients, there was a significant inverse relationship between the known duration of hypertension in years and both urinary PGE (r = 0.502, Figure 2) and urinary PGF (r = −0.732) levels. These findings might be interpreted as indicative of a depression in renal PG synthesis related to the evolution of essential hypertension.

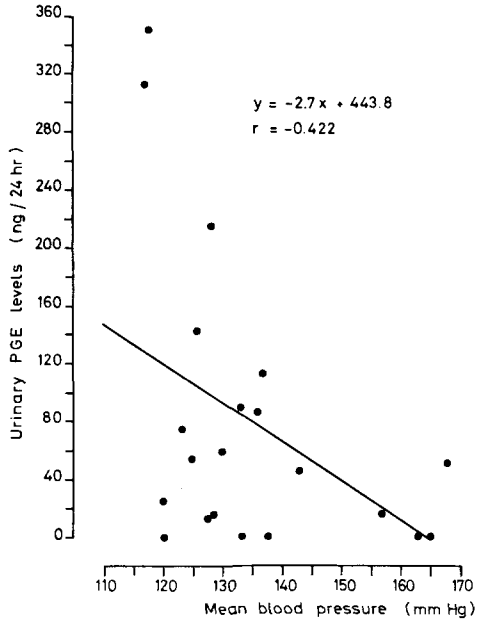

Fig. 3. Significant inverse correlation between the renal PGE secreted per 24 h and the mean blood pressure in the same hypertensive patients as in Figure 2. The progressive rise of the blood pressure during the hypertensive disease could be due in part to the deficiency of the renal medulla in synthesis and release of the potent hypothensive, antiadrenergic, diuretic and natriuretic PGE. The release of PGE following expansion of the extracellular space[8,9,12] prevents the rise of the blood pressure by a complicated mechanism described elsewhere[9]. In the case of hypertensive patients the deficiency of renal medulla in prostaglandin synthesis and release could deprive these patients of the protective homeostatic role of PGE on blood pressure, blood volume and sodium and water balance regulation.

Discussion. The wide variety of factors which are known to influence PG synthesis and/or release[6-9] makes it very difficult to evaluate the exact mechanism by which arterial hypertension could lead to deficient PG synthesis. Low plasma-renin concentrations have been supposed to reflect a long-term effect of the raised blood-pressure on the kidney[10,11]. Possibly, irreversible changes within the kidney, related to the duration of the hypertensive state, could also account for the deficiency in PG synthesis by the renal medulla. On the other hand, patients with low plasma-renin have been reported to be significantly older than patients with normal plasma-renin concentration[11]. Although we did not find any significant correlation between age and either PGE (r = −0.213) or PGF (r = −0.359) levels in the urine, it must be noted that the age range in our group of patients was different than that reported in the study in which plasma-renin was measured. Furthermore, it has been suggested that the duration of hypertension rather than age per se is also the important factor in the relatinship between plasma-renin and age[10]. Nevertheless, no significant correlation between plasma-renin activity and urinary PGE (r = −0.381) or PGF (r = −0.131) levels was found in our study.

It is of interest to note that a significant inverse relationship was also found in the hypertensive patients between mean blood-pressure and urinary levels of both PGE (r = −0.422, Figure 3) and PGF (r = −0.527) group. In view of the known antihypertensive properties of renomedullary PGs, and especially of the PGE group, these findings might suggest a causal relationship between raised blood-pressure and the decreased levels of PG in the urine. Since a significant positive correlation was present in our group of patients between mean blood pressure and the duration of arterial hypertension (r = 0,487), a tentative conclusion could be that while continued prostaglandin synthesis in the early stages of essential hypotension might reflect an activation of the renal anti-hypertensive function in respect to neurogenic and/or hormonal pression stimuli, subsequently a deficiency in renal PG synthesis related to irreversible changes within the kidney would lead to the preponderance of a pressor mechanism, resulting to a further increase of blood-pressure. It is evident that this conclusion is only speculative and that much more work will have to be done before the exact role of renal PGs in essential hypertension can be clarified.

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