Journal of Steroid Biochemistry, 1975, Vol. 6, pp. 755–760. Pergamon Press. Printed in Great Britain

MINERALOCORTICOID ACTIVITY IN PATIENTS IN THE EARLY BENIGN PHASE OF ESSENTIAL HYPERTENSION

JACQUES GENEST, WOJCIECH NOWACZYNSKI, OTTO KUCHEL, FRANZ MESSERLI, ROGER BOUCHER and MANUEL ROJO-ORTEGA

Multidisciplinary Research Group of Hypertension of the Medical Research Council of Canada, 110 Pine Avenue West, Montreal, Quebec H2W 1R7, Canada

SUMMARY

The major parameters of aldosterone metabolism (daily secretion and excretion rates, metabolic clearance rate and plasma levels) have been measured in carefully selected patients in the early benign phase of essential hypertension and in control subjects by double isotope dilution assay and, more recently, by radioimmunoassay. The results show: (1) a significant mean increase in plasma aldosterone concentration in two different groups of patients in the early benign phase of essential hypertension despite a daily secretion and excretion rate which was found normal in the great majority of patients; (2) a decreased metabolic clearance rate in 80% of all hypertensive patients studied; (3) a decrease in hepatic blood flow as measured by a constant infusion or indocyanine green. These observations are consistent with the absence or the blunting of aldosterone response to a variety of stimuli (severe sodium restriction, furosemide administration, severe hemorrhage) which increase plasma renin activity. Plasma progesterone concentrations are significantly increased in patients in the early phase of benign essential hypertension and their response to severe sodium restriction is significantly smaller than that of normal subjects. These studies indicate definite disturbances in aldosterone metabolism and regulations in the early benign phase of essential hypertension.

INTRODUCTION

The study of mineralocorticoid hormones in patients with essential hypertension is of great importance because of the many disturbances of sodium regulation observed in this disease. These disturbances include:

1. A greater and more rapid rejection of intravenously administered salt loads. Similar findings have been obtained in hypertensive animals.
2. A higher incidence of hypertension in populations receiving a high salt intake.
3. An increased arterial sodium content in various types of experimental hypertension.
4. The antihypertensive effectiveness of the rice–fruit diet or of severe sodium restriction to 10 m-equiv of sodium per day, of natriuretic agents and of spironolactone.
5. The natriuretic effects of intravenously administered angiotensin despite the aldosterone stimulation similar to that obtained in normotensive subjects.
6. The potentiation of the hypertensive effects of steroids by administration of salt.
7. The production of hypertension in salt-sensitive strains of rats or by high sodium intake in humans.
8. The pressor response to cross-transfusions of blood from an experimental renal hypertensive rabbit (one clipped kidney with contralateral nephrectomy) to a high salt-fed rabbit.

Our hypothesis was based on the assumption that some of these disturbances were caused by, or related to, abnormal regulation or metabolism of mineralocorticoid hormones.

Our first report in 1956 [1] of increased aldosterone excretion in patients with severe essential or malignant hypertension has been generally confirmed. On the other hand, our findings in 1958–1960 [2–4] of a significant mean increase in aldosterone excretion in patients with benign essential hypertension with the use of a physicochemical method of determination of aldosterone after two chromatographic purification of urinary extracts [5] could not be confirmed by several groups of workers using more precise methods based on double isotope dilution assays [6–10]. On the basis of these latter findings, Laragh has repeatedly emphasized that the "metabolism" of aldosterone was normal in the early phase of essential hypertension and that the disturbances in aldosterone secretion rate and excretion rate were manifestations of the latter stage of the disease and associated with advanced arteriolar disease in the kidneys [7, 11]. When Conn reported in 1967, in his Harvey Lecture [12], results of urinary aldosterone in patients with essential hypertension using a double isotope dilution assay and which were the mirror image of those we had previously reported, our group decided to restudy the whole problem in depth.

There were two major factors that could explain the source of the confusion and of differences in results by the various groups:

1. Besides the different methodologies, it was obvious that measurements of secretion and excretion

rates of aldosterone were wrongly assumed to represent "metabolism" or regulation. They involved only measurements of input and output but two most important points were forgotten: the level of hormone present in plasma for delivery at receptor sites and the response of aldosterone to various physiological stimuli.

2. It was evident in reviewing the various papers published on the subject that the criteria used for selection of patients were very loose and that populations studied were mixed ones and very nonhomogeneous.

It was therefore thought essential by our group: (a) to define with great precision the type of patients to be studied; (b) if aldosterone and/or other mineralocorticoid hormones were involved in the sodium disturbances seen in essential hypertension, and in the mechanism of essential hypertension, to concentrate our studies in the early benign phase of essential hypertension; and (c) to measure simultaneously the daily aldosterone secretion and excretion rates, metabolic clearance rate and plasma levels with precise and sensitive methods based on double isotope dilution assays or radioimmunoassay.

In this presentation, I will discuss: (1) the rigid criteria used for the selection of patients with early benign essential hypertension; (2) the results of measurements of plasma concentration and metabolic clearance rate of aldosterone in such patients; (3) the cardiac index and splanchnic blood flow index; and finally (4) the plasma progesterone concentrations. My collaborator, Dr. Wojciech Nowaczynski, will cover in this Symposium other aspects of aldosterone metabolism (urinary excretion of the two major metabolites, protein binding of aldosterone by the transcortin-like globulin), the disturbances of aldosterone response to posture and the daily secretion rate of 18-hydroxy-deoxycorticosterone in the same patients.

PATIENTS

The criteria for selection of patients in the early benign phase of essential hypertension were: (a) absent or minimal symptoms; (b) age between 25 and 50; (c) no abnormality found on physical examination, absence of retinopathy and of any definite signs of arterio-atherosclerosis of the large vessels; (d) normal plasma sodium, potassium and bicarbonate concentrations; (e) normal renal function as measured by blood urea, serum creatinine, creatinine clearance, rapid sequence intravenous pyelography and renal arteriography, and (f) normal electrocardiogram and chest X-ray without evidence of left ventricular hypertrophy.

All patients had been followed in the Hypertensive Clinic for several weeks to several months and had consistent blood pressure readings above 140/90 mm Hg. Following admission in the Clinical Investigation Unit, the patients were divided into two subgroups depending on their blood pressure response to rest and reassurance. Blood pressure was taken every hour from 8 am to 8 pm both in recumbent and upright postures every day. One subgroup of patients had blood pressure levels always remaining over 140/90 mm Hg and was called the stable benign essential hypertensives; the other group had a decrease of blood pressure to levels below 140/90 mm Hg after the first day or two in the Unit and was called labile. All patients were placed on a standard diet containing 135 m-equiv sodium and 90 m-equiv of potassium per day and steroid analyses were made between the 4th and the 7th day. Many were afterwards placed on a severe sodium restriction diet to 10 m-equiv/day or received a salt load of 300 m-equiv/day with the same amount of potassium (90 m-equiv/day). Blood was always taken between 8:30 and 9 am with the patients in recumbent position.

METHODS

Aldosterone measurements were based on a double isotope dilution assay previously described [13] and more recently on a specific and sensitive radioimmunoassay [14]. The metabolic clearance rate of aldosterone was measured by the constant infusion technique of Tait[15, 16]. The procedures used for plasma progesterone levels were based on competitive protein binding assay of Murphy[17], that of plasma renin activity on the procedure of Boucher[18]. Cardiac index and splanchnic blood flow index were measured using a constant infusion of indocyanine green by the dye dilution technique described by Stewart[19] and Hamilton *et al.*[20] and the splanchnic blood flow calculated according to the formula of Bradley[21].

RESULTS

1. *Plasma aldosterone*

Two different groups of patients in the early benign phase of essential hypertension had measurements of plasma aldosterone under controlled conditions of dietary sodium intake (at 135 m-equiv/day) and of recumbency. The first group was studied with the double isotope dilution assay which included 40 patients and 20 normal control subjects. The results shown in Fig. 1 indicate that the mean concentration was significantly ($P < 0{\cdot}01$) higher in hypertensive patients (17·2 ng%) than in control subjects (7·5 ng%). The significance of the difference remains whether the group is subdivided according to normal or low plasma renin activity [22–24].

A second group was studied by a specific and sensitive radioimmunoassay for aldosterone (Fig. 2). Again, the mean plasma aldosterone concentration in patients with early and stable essential hypertension ($n = 42$) was significantly increased ($P < 0{\cdot}01$) when compared to the group of control subjects ($n = 42$). The increase was greater ($P < 0{\cdot}001$) in those

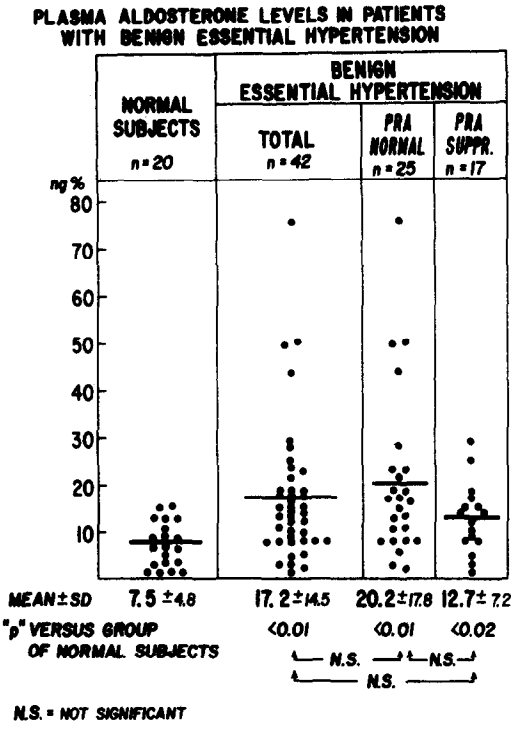

Fig. 1. Plasma aldosterone concentration, measured by a double isotope dilution assay, in control subjects and patients in the early benign phase of essential hypertension.

patients in whom plasma renin activity measured simultaneously was below 0·1 ng/ml/h.

Since the daily excretion rate of aldosterone was normal in the great majority of patients and since

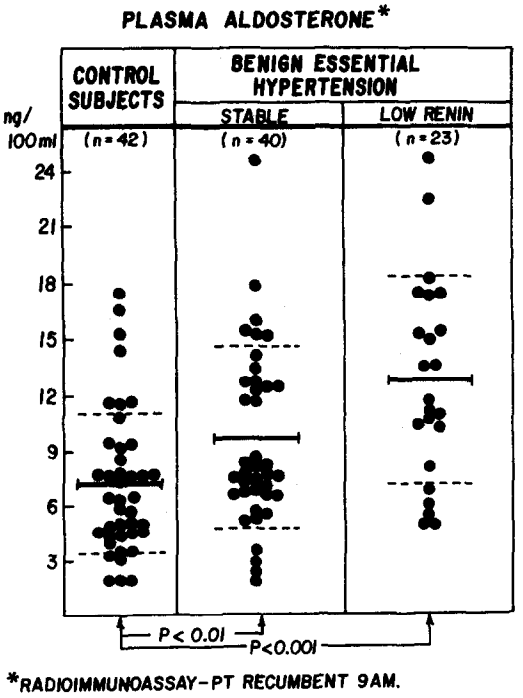

Fig. 2. Plasma aldosterone concentration measured by a specific and sensitive radioimmunoassay in control subjects and patients in the benign stable phase of essential hypertension.

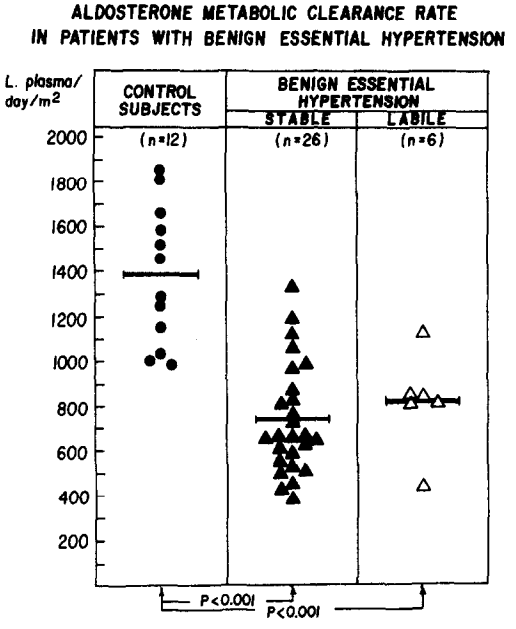

Fig. 3. Metabolic clearance rate of aldosterone determined by a constant infusion technique in patients in the early benign phase of essential hypertension and in control subjects.

the daily secretion rate was slightly lower (87 vs 107 μg/day in the control group) [25], these findings could not be explained otherwise than by a decrease in metabolic clearance rate.

2. *Metabolic clearance rate of aldosterone*

The metabolic clearance rate of aldosterone was measured in 32 patients with benign essential hypertension and in 12 normal control subjects (Fig. 3). The results show a significant decrease ($P < 0.001$) with more than 80% of hypertensive patients having metabolic clearance rate below the lower range of normal. The mean metabolic clearance rate was 867 ± 270 liters of plasma/day/m^2 as compared to 1480 ± 285 in normal control subjects. A significant correlation could be demonstrated in hypertensive patients between the metabolic clearance

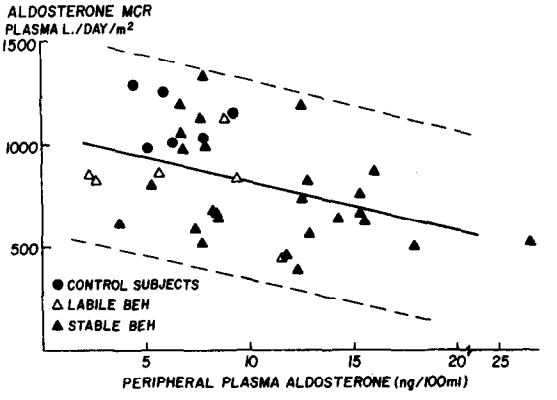

Fig. 4. Positive correlation between the metabolic clearance rate of aldosterone and its plasma concentration measured simultaneously in the same subjects and patients.

rate of aldosterone and its peripheral plasma levels measured simultaneously (Fig. 4).

Three major factors could explain such an important decrease in aldosterone metabolic clearance rate:

1. A decrease in hepatic blood flow. This could be very important, since Tait has demonstrated that about 97% of plasma aldosterone is completely cleared from blood during one passage through the liver [15].

2. A relative deficiency in the hepatic uptake of aldosterone or in the 4-ene-reductase activity responsible for the degradation of aldosterone into the tetrahydro derivative. Such a deficiency of this hepatic enzyme has been strongly suggested by Kornel and his group for cortisol in patients with essential hypertension [26, 27].

3. An increased protein binding of aldosterone in plasma resulting in a decreased hepatic uptake of aldosterone. The latter two possibilities will be discussed by Dr. Nowaczynski.

In 1952, Wilkins has reported that the hepatic blood flow was essentially normal in a poorly defined population of hypertensive patients, using the bromesulphalein method [28]. Later in 1964, Wolheim, using a colloidal chromophosphate preparation labelled with P^{32} found a significant decrease of 20% in hepatic blood flow in patients with essential hypertension [29]. Again the hypertensive population was a mixed one and poorly defined.

This aspect has been re-examined by Messerli in our laboratories [30]. The cardiac index, splanchnic blood flow index and hepatic resistance were measured simultaneously in 13 patients in the early benign phase of essential hypertension and in six normal subjects. As shown in Fig. 5, the findings indicate a significant ($P < 0{\cdot}05$) decrease in cardiac index with a more important ($P < 0{\cdot}01$) 12% decrease in splanchnic blood flow index and a significant ($P < 0{\cdot}01$) increase in splanchnic resistance. A significant correlation was found between mean arterial pressure and splanchnic blood flow index (Fig. 6).

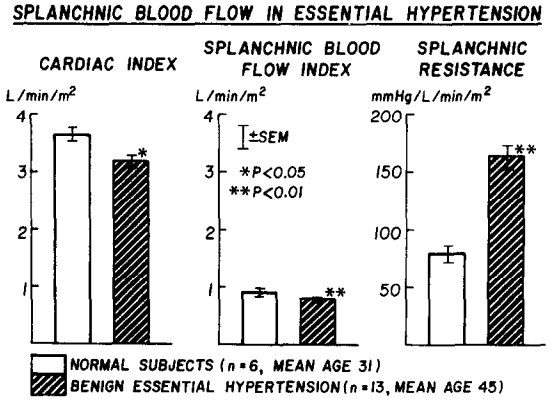

Fig. 5. Results of the measurements of the cardiac index, the splanchnic blood flow index and the splanchnic resistance, using the constant infusion of indocyanine green in normal subjects and in patients in the early benign phase of essential hypertension.

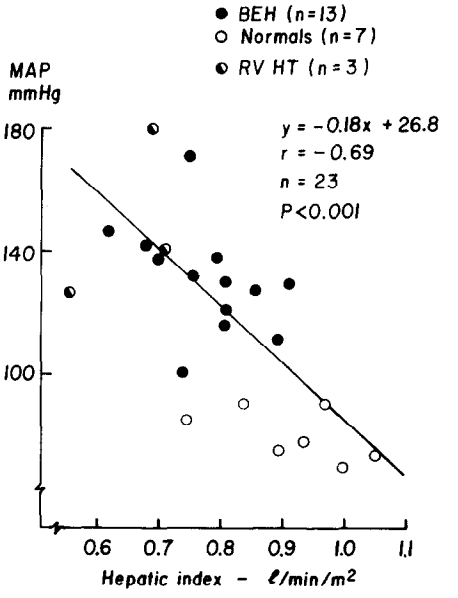

Fig. 6. Positive correlation between the mean arterial pressure and the splanchnic blood flow index measured simultaneously in seven control subjects, 13 patients in the early benign phase of essential hypertension and three patients with renovascular hypertension.

As important as these disturbances in aldosterone metabolism are, others appear just as significant, if not more, in the regulation of aldosterone under conditions of severe sodium restriction, depletion by furosemide administration, rapid reduction in blood volume by severe hemorrhage, posture and especially administration of salt loads. Many workers have confirmed the early findings of Helmer and Judson[31] of an absent or blunted response of aldosterone in patients with essential hypertension in response to severe sodium restriction despite the "normal" increase in plasma renin activity [32–35]. Similar findings have been observed following severe hemorrhage or intravenous administration of furosemide [35].

A most important contribution has been made by Luetscher and his collaborators who have shown that during periods of salt loads of over 300 m-equiv of sodium per day, about 80% of patients with essential hypertension have only a partial suppression of aldosterone in contrast to normal subjects [36–38]. In fact, the daily secretion and excretion rates of aldosterone were at levels 3 times higher than those found in normal subjects receiving similar salt loads. Such results are consistent with our findings, especially in view of the fact that North American populations receive between 8 and 20 g of salt per day, especially in urban centers, and suggest a mild state of relative hyperaldosteronism in a good number of patients in the benign phase of essential hypertension.

3. *Plasma progesterone*

Three factors led us to measure plasma progesterone levels in patients with benign essential hypertension:

1. This hormone induces natriuresis by blocking

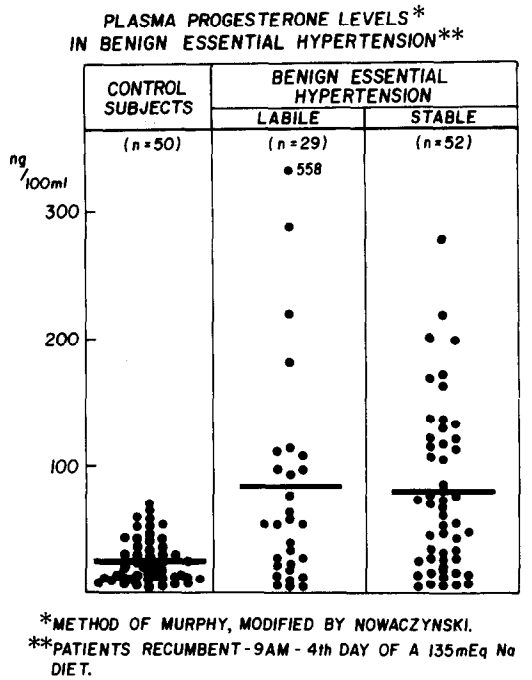

Fig. 7. Plasma progesterone concentration in patients in the early benign phase of essential hypertension and in control subjects, using the competitive protein binding assay of Murphy.

the sodium-retaining effect of aldosterone and is therefore an aldosterone antagonist [39].

2. Its administration to patients with essential hypertension or to renal hypertensive rats and dogs decreases blood pressure towards or at normal levels [40, 41].

3. Normal pregnancy is accompanied by high aldosterone secretion rate and plasma concentration.

Nevertheless, serum potassium and bicarbonates are normal and blood pressure is normal. Therefore, could the high progesterone levels in the normal pregnant woman protect against the biochemical and vascular effects of increased aldosterone secretion and plasma levels and could the absence of hypokalemia in essential hypertension be due to a similar increase in progesterone?

The mean plasma progesterone concentration in 20 patients with labile benign essential hypertension was 109 ng% (P vs N.S. $< 0{\cdot}02$) and 83 ng% (P vs N.S. $< 0{\cdot}01$) in the stable benign essential hypertensives in contrast to 35 ng% in normal subjects ($n = 21$) (Fig. 7). In response to severe sodium restriction to 10 m-equiv/day, normal subjects have a 700% increase in plasma progesterone levels in contrast to only 60% mean increase in patients with benign essential hypertension.

These observations suggest an homeostatic mechanism to prevent or decrease the severity of the biochemical and vascular consequences of increased plasma aldosterone levels or of hypermineralocorticoid activity observed in a number of patients in the early benign phase of essential hypertension.

CONCLUSIONS

Our findings indicate disturbances in aldosterone regulation and metabolism in the early benign phase of essential hypertension.

Acknowledgements—The authors wish to acknowledge the active collaboration of the following research fellows: Dr. Chikashi Sasaki; Dr. Marcel Lebel; Dr. Shinichiro Kubo; Dr. Ursula Ganten; Dr. John Grose; and the technical help of Misses Pauline Robinson, Diane Charbonneau, Mrs. Gaetane Castonguay and Mrs. Manon Monette.

REFERENCES

1. Genest J., Lemieux G., Davignon A., Koiw E., Nowaczynski W. and Steyermark P.: *Science* **123** (1956) 503–505.
2. Genest J., Koiw E., Nowaczynski W. and Leboeuf G.: *Proc. exp. biol. Med.* **97** (1958) 676–679.
3. Genest J., Koiw E., Nowaczynski W. and Sandor T.: *Acta endocr., Copenh.* **45** (1960) 413–425.
4. Genest J. *et al.*: (abstract) *Circulation* **20** (1959) 700.
5. Nowaczynski W., Koiw E. and Genest J.: *Can. J. biochem. Physiol.* **35** (1957) 425–448.
6. Laragh J. H. *et al.*: *J. clin. Invest.* **39** (1960) 1091–1106.
7. Laragh J. H., Sealey J. E. and Sommers S. C.: *Circ. Res.* **18** (Suppl. 1) (1966) I—158, I—174.
8. Kaplan N. M.: *J. clin. Invest.* **46** (1967) 728–734.
9. George J. M., Gillespie L. and Bartter F. C.: *Ann. Int. Med.* **69** (1968) 693–701.
10. Biglieri E. G. *et al.*: *Am. J. Med.* **45** (1968) 170–175.
11. Laragh J. H: *Fedn Proc.* **26** (1967) 39–41.
12. Conn J. W.: 1954–1967. Harvey Lect. **63** (1966–1967) 257–291.
13. Nowaczynski W., Silah J. and Genest J.: *Can. J. Biochem.* **45** (1967) 1919–1936.
14. Nowaczynski W., Sasaki C. and Genest J.: *J. steroid Biochem.* **5** (1974) 123–131.
15. Tait J. F., Bougas J., Little B., Tait S. A. S. and Flood C.: *J. clin. Endocr. Metab.* **25** (1965) 219–228.
16. Tait J. F., Little B., Tait S. A. S. and Flood C.: *J. clin. Invest.* **41** (1962) 2093–2100.
17. Murphy B. E. P.: *J. clin. Endocr.* **27** (1967) 973–990.
18. Boucher R., Veyrat R., de Champlain J. and Genest J.: *Can. med. Ass. J.* **90** (1964) 194–201.
19. Stewart G. N.: *J. Physiol., Lond.* **22** (1897) 159–183.
20. Hamilton W. F., Moore J. W., Kinsman J. M. and Sturling R. G.: *Am. J. Physiol.* **99** (1932) 534–551.
21. Bradley S. E., Ingelfinger F. J., Bradley G. P. and Curry J. J.: *J. clin. Invest.* **24** (1945) 890–897.
22. Genest J. and Nowaczynski W.: *J. Roy. Coll. Phys., Lond.* **5** (1970) 77–86.
23. Nowaczynski W., Kuchel O. and Genest J.: In *Hypertension '72* (Edited by J. Genest and E. Koiw). Springer-Verlag, Heidelberg (1972) pp. 244–255.
24. Genest J., Nowaczynski W. J., Kuchel O. and Sasaki C.: *Trans. Am. clin. Climatol. Ass.* **83** (1971) 134–146.
25. Nowaczynski W., Kuchel O. and Genest J.: *J. clin. Invest.* **50** (1971) 2184–2190.
26. Kornel L., Miyavos S., Saito Z. and Fu-Tzu W.: *Res. Steroids* **5** (1973) 413–427.
27. Kornel L.: Commentary, in the *Yearbook of Endocrinology* (Edited by T. B. Schwartz). Chicago, Yearbook Medical Publishers, Inc. (1971) pp. 303–304.
28. Wilkins R. W., Culbertson J. W. and Rymut A. A.: *J. clin. Invest.* **31** (1952) 529–531.
29. Wollheim E.: Symposium in Freiburg 1964 (Edited by L. Heilmeyer and H. J. Holtmeier). Stuttgart, Georg Thieme Verlag (1965) pp. 136–152.

30. Messerli F., Genest J., Nowaczynski W., Kuchel O., Honda M., Latour Y. and Dumont S.: *Circulation*, June 1975.
31. Helmer O. M. and Judson W. E.: *Circulation* **38** (1968) 965–976.
32. Streeten D. H. P., Schletter R. E., Clift G. V. *et al.*: *Am. J. Med.* **46** (1969) 844–861.
33. Jose A., Crout J. R. and Kaplan N. M.: *Ann. Int. Med.* **27** (1970) 9–16.
34. Driessen W. M. M., Benraad T. J. and Kloppenborg P. W. C.: (abstract) *Acta endocr., Copenh.* **138** (1969) 126.
35. Williams G. H., Lauler D. P. and Dluhy R. G.: In *Hypertension '72* (Edited by J. Genest and E. Koiw). Springer-Verlag, Heidelberg (1972) pp. 277–285. Also: Williams G. H. *et al.*: *Ann. int. Med.* **72** (1970) 317–326.
36. Luetscher J. A. *et al.*: (abstract), in the *Endocrine Society*, Program of the Fifty-Second Meeting, St Louis (1970) p. 65.
37. Luetscher J. A. *et al.*: In *Hypertension '72* (Edited by J. Genest and E. Koiw). Springer-Verlag (1972) pp. 286–292.
38. Luetscher J. A. *et al.*: *J. clin. Endocr. Metab.* **29** (1969) 1310–1318.
39. Landau R. L. and Lugihbihl K.: *J. clin. Endocr. Metab.* **18** (1958) 1237–1245.
40. Armstrong J. G.: *Proc. Soc. exp. Biol. Med.* **102** (1959) 452–455.
41. Genest J., Nowaczynski W. and Koiw E.: An International Symposium (Edited by K. D. Bock and P. T. Cottier). Springer-Verlag, Berlin (1960) pp. 126–146.