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HUMAN TESTICULAR SECRETION WITH INCREASING AGE*

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SUMMARY

Δ^4 and Δ^5 androgens were measured in spermatic venous blood of 25 subjects (age range 20–70) during surgical intervention for hernia repair. All androgens measured decreased significantly with age. However the androstenedione to testosterone ratio in spermatic venous blood of men aged between 60–70 was higher than that of subjects aged between 20–40.

17β-Oestradiol levels were unchanged in spermatic venous blood of the same subjects. These results seem to demonstrate that there is a general decrease with age of androgen secretion by the human testis and the increase of oestradiol in systemic blood in senescence is due to an increase in peripheral conversion of aromatizable androgens.

INTRODUCTION

In a society where the life-span of human beings is increasing, a need exists for more information on testicular function in old age. For this reason many studies on testicular function in aging males have been carried out in the last few years.

Decreased testicular function, characterized by a reduction of testosterone (T) concentrations in peripheral [1, 3] as well as in spermatic venous blood, has been demonstrated in elderly men [4, 5]. The human testis, however, does not secrete only T, but also other androgens, progestagens and oestrogens, which was demonstrated by the existence of a significant gradient between spermatic and peripheral venous blood by us and other authors [6] (see Fig. 1). Unfortunately, while T circulating in blood comes solely from testicular secretion, the other androgens, such as 5-androstene-3β,17β-diol (A-diol), androstenedione (Δ), dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA) also come from adrenal secretion and/or peripheral conversion of steroid precursors [7]. Dehydroepiandrosterone sulphate (DHEA-S) seems to originate entirely from the adrenal because a significant gradient between spermatic and peripheral venous blood has not been demonstrated [8, 9]. A schematic representation of the origin of androgens circulating in blood is shown in Fig. 2 [10–13].

Because of the different origin of various androgens the measurement of these steroids in peripheral venous blood cannot be considered to be a precise index of testicular secretion. For instance the response of each plasma androgen in systemic blood to HCG stimulation was different in young and in old men because of the different origin of the steroids.

The greatest increase was found for T which comes entirely from the testis, while A-diol showed a less important but significant response (Fig. 3). Δ and DHEA did not show any significant variation, originating mainly from the adrenal (Fig. 3).

The measurement of Blood Production Rate (BPR) is subjected to the same problems due to the different origin of circulating steroids [7]; moreover, the Metabolic Clearance Rate (MCR) of steroids is frequently decreased in senescence [1, 14].

ANDROGENS IN SPERMATIC VENOUS BLOOD

Because of the problems associated with the procedures mentioned above, the measurement of steroids in spermatic venous blood, taken during surgical intervention for hernia repair, seemed to us the best approach for studying *in vivo* testicular secretion in aging, in spite of some effects of anaesthesia on testicular function demonstrated in the dog [15].

Measurement of steroids in spermatic venous blood of anaesthetized subjects cannot be considered either a precise index of steroid synthesis by the human testis or an exact measure of steroid secretion by the gland [16]. It represents only a measure of the amount of steroid leaving the testis at the moment of sampling; it is however directly correlated with the secretion of steroid by the gland [16].

Using radioimmunoassay methods previously described [10, 11, 12, 17], we measured Δ_4 and Δ_5 androgens in spermatic venous blood of a group of 25 men ranging in age from 20 to 70 y. The patients were admitted to our hospital for operative repair of inguinal hernia. They were otherwise in good general health and received no medication. Varicocele

* This paper is dedicated to the memory of Enrico Greppi (President of Italian Society of Gerontology from 1950 till 1969 and President of International Society of Gerontology from 1957 till 1960) in occasion of the tenth anniversary of his death.

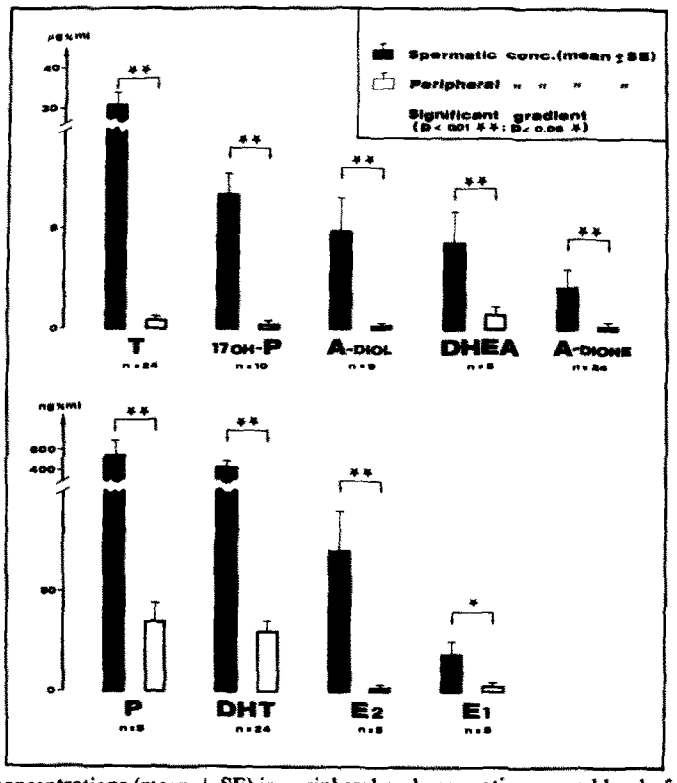

Fig. 1. Steroid concentrations (mean ± SE) in peripheral and spermatic venous blood of normal males. T = testosterone, 17-OH-P = 17-hydroxyprogesterone, A-diol = 5-androstene-3β,17β-diol, DHEA = dehydroepiandrosterone, A-dione = androstenedione, P = progesterone, DHT = dihydrotestosterone, E_2 = 17β-oestradiol, E_1 = estrone.

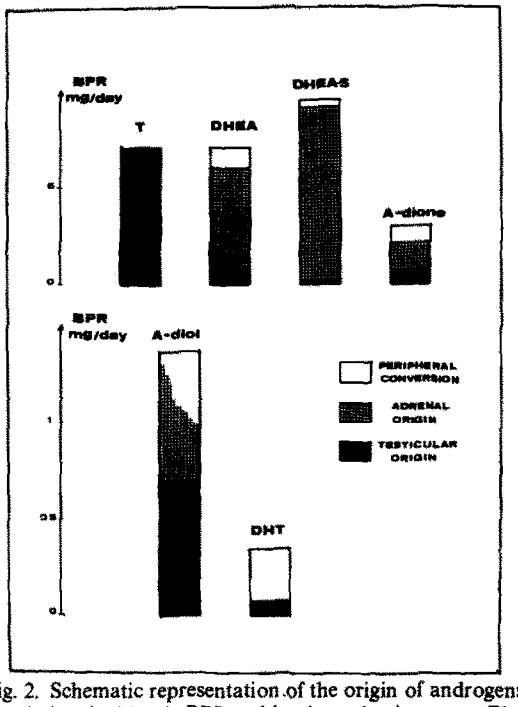

Fig. 2. Schematic representation of the origin of androgens circulating in blood. BPR = blood production rate. The steroids are testosterone (T), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), androstenedione (A-dione), 5-androstene-3β,17β-diol (A-diol) and dihydrotestosterone (DHT). The relative contribution of adrenal secretion and peripheral conversion to blood production rate of 5-androstene-3β,17β-diol is unknown.

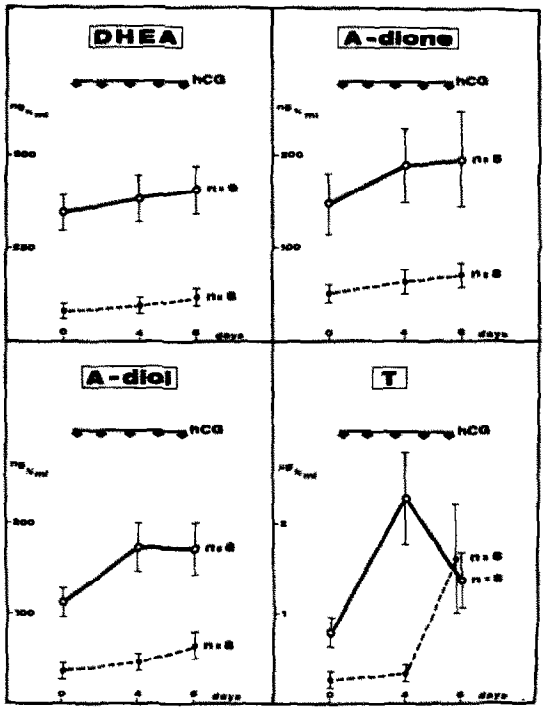

Fig. 3. Δ_4 and Δ_5 androgens (mean ± SD) in peripheral venous blood of normal adults (continuous line) and old subjects (dotted line) under HCG stimulation (4.000 I.U./day)

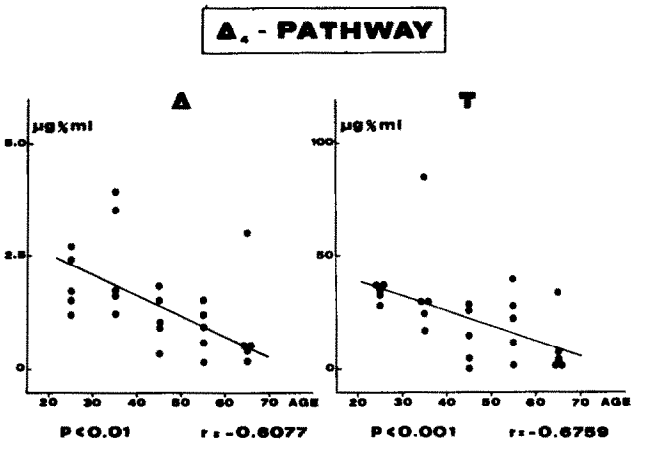

Fig. 4. Androstenedione (Δ) and testosterone (T) concentrations in spermatic venous blood of normal subjects as a function of age.

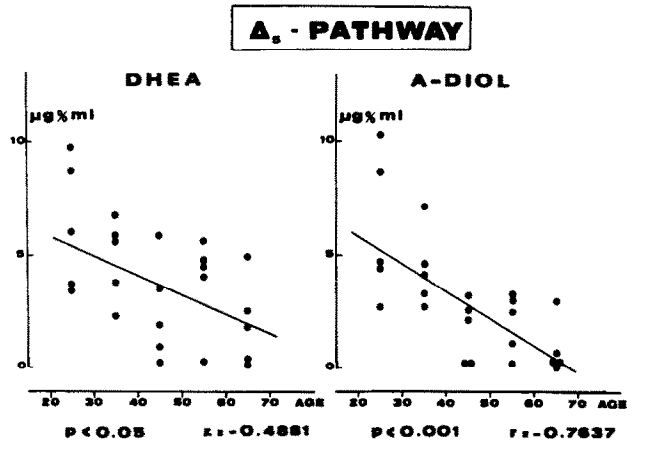

Fig. 5. Dehydroepiandrosterone (DHEA) and 5-androstene-3β,17β-diol (A-DIOL) in spermatic venous blood of normal subjects as a function of age.

was not present. Anaesthesia was obtained by premedication with 100 mg of mephedine and 1 mg of atropine and by injection of 5 mg of buvocaine and 0.005 mg of epinephrine into the epidural space.

A significant negative correlation of all steroids with age was found using a weighted regression analysis (Figs 4 and 5). These findings lead us to the conclusion that Δ_4 and Δ_5 androgens are secreted in reduced amount by the human testis with advancing age.

ANDROSTENEDIONE TO TESTOSTERONE RATIO

The ratio between the absolute concentrations of androgens in spermatic venous blood may be interesting from a biological point of view. It has been demonstrated that the ratio Δ to T in systemic venous blood changes markedly from the prepubertal stage to that in sexual maturity. In fact the Δ level is higher than the T level in the prepubertal stage [18, 19] and seems to increase again in old age [20]. In a group of elderly subjects previously studied [5] we observed an increase in the Δ/T ratio in spermatic venous blood similar to that reported by Vermeulen and Verdonk[20] in systemic blood. Therefore we also calculated the ratio of Δ to T in the spermatic venous blood of three different groups of subjects, i.e. prepubertal boys (age range 3–6), young normal men (age range 20–40) and elderly men (age range 60–70). The ratio of Δ to T in spermatic venous blood of prepubertal boys is higher than the ratio found in young men (Fig. 6). In the young men the major steroid secreted by the testis is largely T. In elderly men the ratio Δ to T seems to increase again in comparison to young normals.

The higher concentrations of Δ found in spermatic venous blood of prepubertal boys do not allow the conclusion that Δ is the major androgen synthetized and secreted by the prepubertal human testis, as we did not find any significant gradient between the spermatic and the systemic venous blood for Δ, T and 17OH-progesterone (see Fig. 7).

However we cannot exclude the possibility that the steroids entering the testis may be metabolized by the gland. Therefore the absence of a gradient neither demonstrates nor excludes Δ secretion by prepubertal human testis.

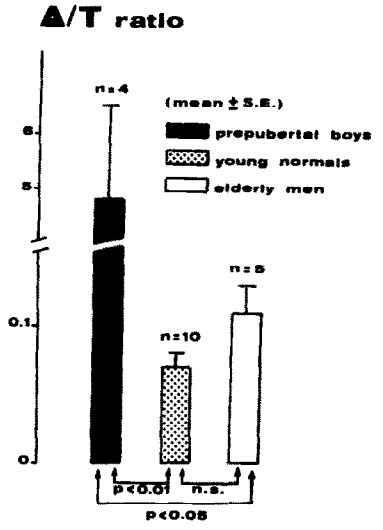

Fig. 6. Androstenedione/testosterone (Δ/T) ratio (mean ± SE) in spermatic venous blood of prepubertal boys, normal adult subjects and elderly men.

The increase of the Δ/T ratio in spermatic venous blood of elderly men remains unexplained. While Sciarra *et al.*[21], using radioactive pregnenolone as precursor, found a synthesis of Δ greater than T by the testis of an elderly man, Bell and Lacy[22] did not confirm these data because they did not find any age effect on the conversion of Δ to T by human isolated interstitium and seminiferous tubules.

OESTRADIOL IN SPERMATIC VENOUS BLOOD

Lastly, the decrease of Δ_4 and Δ_5 androgens by the human testis with advancing age indicated another endocrine problem of male senescence. In fact, while T decreases in systematic blood, several authors have found a pronounced increase in circulating 17β-oestradiol (E_2) in old age [3, 23, 24, 25].

The majority of circulating E_2 does not originate from testicular secretion (which accounts for about

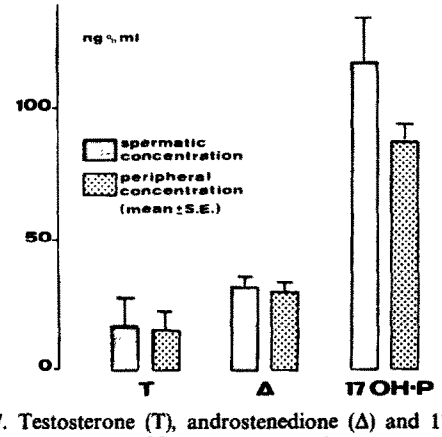

Fig. 7. Testosterone (T), androstenedione (Δ) and 17-hydroxyprogesterone (17OH-P) concentrations (mean ± SE) in peripheral and spermatic venous blood of 4 prepubertal boys (age range = 3–6 years). The gradients are not statistically significant.

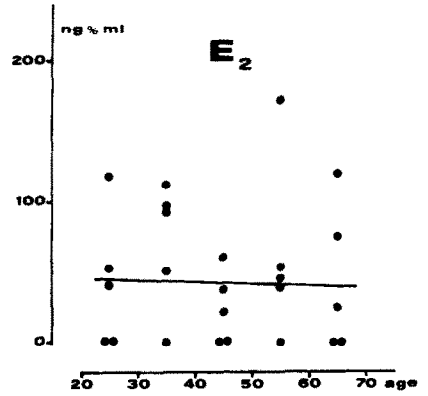

Fig. 8. 17β-oestradiol (E_2) concentrations in spermatic venous blood of normal subjects as a function of age.

25–40% of E_2 blood production rate) but comes mainly from peripheral conversion of T [27, 28].

The increase of E_2 plasma concentrations with age is probably due to a decrease in MCR [14] and an unchanged BPR [14].

Since the testicular and adrenal secretion of aromatizable androgens decreases with age, the unchanged BPR of E_2 may be due to an increase in direct testicular secretion or to an increase in peripheral aromatization of T. The latter possibility is strongly supported by the finding of increased transfer constants of Δ to E_1 [26] and of T to E_2 with age [14].

In an attempt to clarify this problem we measured E_2 levels in spermatic venous blood of the above mentioned 25 subjects using a radioimmunoassay previously described [31]. We did not find significant variation of E_2 concentrations with age (Fig. 8). These results strongly suggest that the increase of E_2 levels in systemic blood in aging is in part due to the reduction of MCR and in part to an increase in peripheral aromatization of androgens. An increase in testicular secretion of E_2 by the human testis seems to be excluded.

CONCLUSION

The reduction of testicular androgen secretion in aging can not be easily explained.

An enzymatic defect should be excluded. As we have demonstrated a significant reduction of all testicular androgens with advancing age, it is reasonable to suggest the presence of factors capable of impairing all the steps of androgen synthesis in the human testis. One of the major factors involved in the impairment of androgen secretion may be the reduction of testicular blood flow due to the damage of the arterial system in aging [30]; another cause may be the reduction in the number of Leydig cells. However, concerning the latter problem, the results are contradictory [30]. In fact, a proper morphological study in a large group of healthy elderly men is not easy to carry out. Moreover, other causes such as nutritional factors and impairment of LH action at the testicular level, cannot be excluded.

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REFERENCES

1. Vermeulen A., Rubens R. and Verdonk L.: Testosterone secretion and metabolism in male senescence. *J. clin. Endocr. Metab.* **34** (1972) 730–735.
2. Nieschlag E., Kley H. K., Wiegelmann W., Solbach H. G. and Kruskemper H. L.: Age and endocrinal function of the testis in adult men. *Btsch. Med. Wockenschr.* **98** (1973) 1281–1284.
3. Baker H. W. G. and Hudson B.: Male gonadal disfunction. *clin. Endocr. Metab.* **3** (1974) 507–532.
4. Giusti G., Gonnelli P., Borrelli D., Fiorelli G., Forti G., Pazzagli M. and Serio M.: Age related secretion of androstenedione, testosterone and dihydrotestosterone by the human testis. *Exp. Geront.* **10** (1975) 241–245.
5. Serio M., Cattaneo S., Borrelli D., Gonnelli P., Pazzagli M., Forti G., Fiorelli G., Giannotti P. and Giusti G.: Age-related changes in androgenic hormones in human spermatic venous blood. In *Androgens and Antiandrogens* (Edited by Martini L. and Motta M.). Raven Press, New York (1977) pp. 67–75.
6. Vikho R. and Hammond G.: Spermatic vein steroids. In *Endocrinology* (Edited by V. H. T. James). Excerpta Medica, Amsterdam–Oxford, Vol. I (1977) pp. 423–428.
7. Lipsett M. B.: Steroid secretion by the testis in man. In *The endocrine function of the human testis* (Edited by V. H. T. James, M. Serio and L. Martini). Academic Press, London–New York, Vol. II (1974) pp. 1–12.
8. Nieschlag E., Loriaux D. L., Ruder H. J., Zucker I. R., Kirschner M. A. and Lipsett M. B.: The secretion of dehydroepiandrosterone and dehydroepiandrosterone sulphate in man. *J. Endocr.* **57** (1973) 123–134.
9. Vikho R. and Ruokonen A. J.: Steroid sulphates in human adult testicular steroid synthesis. *J. steroid Biochem.* **6** (1975) 353–356.
10. Fiorelli G., Cattaneo S., Forti G. and Serio M.: Adrenal androgen secretion. *J. Nucl. Biol. Med.* **18** (1974) 26.
11. Fiorelli G., Borrelli D., Forti G., Gonnelli P., Pazzagli M. and Serio M.: Simultaneous determination of androstenedione, testosterone and 5α-dihydrotestosterone in human spermatic and peripheral venous blood. *J. steroid Biochem.* **7** (1976) 113–116.
12. Forti G., Calabresi E., Giannotti P., Borrelli D., Gonnelli P., Barbieri U. and Serio M.: Measurement of 5-Androsten-3β,17β-diol in spermatic and peripheral venous blood samples from the same human subjects by a radioimmunoassay method. *Hormone Res.* **9** (1978) 194–200.
13. Vermeulen A. and Ando S.: Prolactin and adrenal androgen secretion. *Clin. Endocr.* **8** (1978) 295–303.
14. Baker H. G. W., Burger H. G., de Kretser D. M., Hudson B., O'Connor S., Wang C., Mirovics A., Court J., Dunlop M. and Rennie G. C.: *Clin. Endocr.* **5** (1976) 349.
15. Eik-nes K. B.: Secretion of testosterone in anaesthetized dog. *Endocrinology* **71** (1962) 101–106.
16. Eik-nes K. B.: Biosynthesis and secretion of testicular steroids in *Handbook of Physiology*, section 7: *Endocrinology* (Edited by D. W. Hamilton and R. O. Greep). Williams and Wilkins, Baltimore, Vol. V (1975) pp. 95–109.
17. Forti G., Pazzagli M., Calabresi E., Fiorelli G. and Serio M.: Radioimmunoassay of plasma testosterone. *Clin. Endocr.* **3** (1974) 5–17.
18. Frasier S. D., Gafford F. and Horton R.: Plasma androgens in childhood and adolescence. *J. clin. Endocr. Metab.* **29** (1969) 1404–1408.
19. Migeon C. J.: Adrenal androgens in man. *Am. J. Med.* **53** (1972) 606–626.
20. Vermeulen A. and Verdonk L.: Radioimmunoassay of 17β-hydroxy-5α-androstan-3-one, 4-androstene-3,17-dione, dehydroepiandrosterone, 17-hydroxyprogesterone and progesterone and its application to the human male plasma. *J. steroid Biochem.* **7** (1976) 1–10.
21. Sciarra F., Piro C. and Sorcini G.: Aspetti della biosintesi steroidea nel testicolo umano normale. *Folia Endocr.* **21** (1968) 412–422.
22. Bell J. B. and Lacy D.: Studies on the structure and function of the mammalian testis. V. Steroid metabolism by isolated interstitium and seminiferous tubules of human testis. *Proc. R. Soc. Lond. (Biol)* **186** (1974) 99–102.
23. Pirke K. M. and Doerr P.: Age related changes and interrelationships between plasma testosterone, oestradiol and testosterone-binding-globulin in normal adult males. *Acta Endocr., Copenh.* **74** (1973) 792–798.
24. Kley H. K., Nieschlag E., Bidlingmaier F. and Kruskemper H. L. Possible age dependent influences of estrogen on the binding of testosterone in plasma of adult men. *Horm. Metab. Res.* **6** (1974) 213–218.
25. Rubens R., Dhont M. and Vermeulen A.: Further studies on Leydig cell function in old age. *J. clin. Endocr. Metab.* **39** (1974) 40–45.
26. MacDonald P. C., Rombant R. P. and Siiteri P. K.: Plasma precursors of estrogen. I. Extent of conversion of plasma Δ_4-androstenedione to estrone in normal males and nonpregnant normal, castrate, and adrenalectomized females. *J. Clin. Endocr. Metab.* **27** (1967) 1103–1111.
27. Longcope C., Layne D. S. and Tait J. F.: Metabolic clearance rates and interconversion of estrone and 17β-estradiol in normal males and females. *J. Clin. Invest.* **47** (1968) 93–106.
28. Weinstein R. L., Kelch R. P., Jenner M. R., Kaplan S. L. and Grumbach M. M.: Secretion of unconjugated androgens and estrogens by the normal and abnormal human testis before and after human chorionic gonadotrophin. *J. Clin. Invest.* **53** (1974) 1–6.
29. Calabresi E., De Giuli G., Becciolini A., Giannotti P., Lombardi G. and Serio M.: Plasma estrogens and androgens in male breast cancer. *J. steroid Biochem.* **7** (1976) 605–609.
30. Suoranta H. and Kormano M.: Age changes in the microvascular supply of human testis. *Morphol. Aspects Androl.* **1** (1970) 90–96.
31. Leathen J. H.: Aging and the testis in *The Testis* (Edited by A. D. Johnson and W. R. Gomes). Academic Press, London–New York, Vol. IV (1977) pp. 547–563.