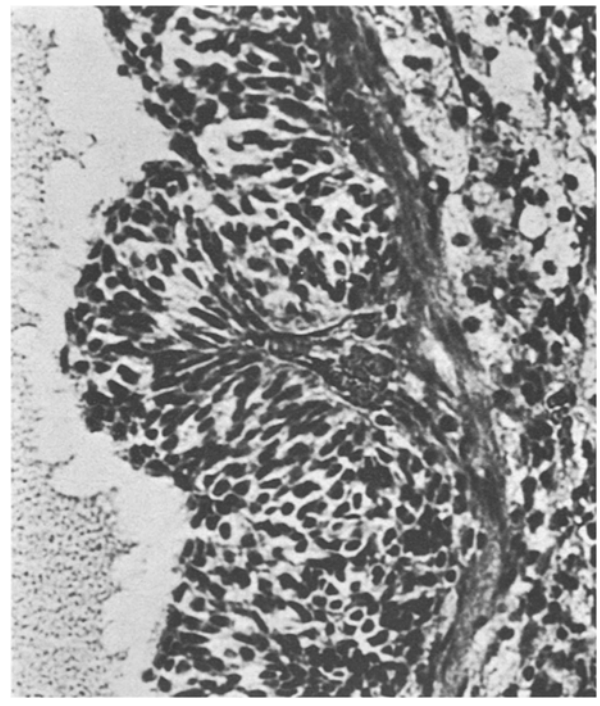
resulted in the early ovulatory failure only in some animals (experiment 4). Additional shortening of the progesterone treatment on days 10–26 with the daily dose of 100 µg during days 10–19 and 200 µg during days 20–26 did not induce an early ovulatory failure but resulted in a delayed failure (persistent vaginal estrus observed only after the 5th month of life) in all animals (experiment 5). Progesterone administration of 100 µg daily from days 1–19 was similarly effective (experiment 6). Shortening of this dosage only to the days 1–10 decreased the incidence of delayed ovulatory failure (experiment 7). Even less effective was a progesterone treatment during the days 10–19 with the daily dose of 100 µg (experiment 8). A single injection of 1200 µg progesterone on the 5th day of life followed up during days 20–26 with 200 µg daily, was completely ineffective (experiment 9). Equally ineffective was a progesterone treatment on days 20–26 with the daily dose of 200 µg (experiment 10) or a single dose of 1200 µg at the age of 5 days (experiment 11). A 26-day administration of the oil solvent only, beginning from the 1st day of life, was likewise without effect (experiment 12).

In all animals showing persistent vaginal estrus at decapitation, ovaries lacking corpora lutea and with the frequent presence of thecal hyperplasia were observed, the latter protruding into the large cavitated follicles with proliferating granulosa (figure).

During the period of protective action of progesterone, a significant uptake of ^{3}H-progesterone by the female rat hypothalamus could not be observed[13], nor could a decrease in ^{3}H-estradiol accumulation in the hypothalamus result from progesterone pretreatment in female rats during the first 10 days of life[14]. Therefore, a hypothesis has been expressed on the extracerebral mechanism of the protective effect of progesterone[14]. Present results demonstrating a delayed ovulatory failure induced by progesterone after the critical postnatal steroid sensitive period of sexual differentiation of the hypothalamus (experiment 5) are thought to be a further contribution to the above-mentioned hypothesis. An attempt to interpret the extracerebral mechanism of protective and/or noxious progesterone action is only speculative in the meantime; presumably, progesterone does impair the normal development of the relationship between the differentiating immune system and the ovary[15].



Theca interna growing into the antrum of the preovulatory follicle bedded with protruding granulosa. 6-month-old rat after long-lasting postnatal progesterone treatment. Papanicolaou stain, ×400.

1 R.A. Gorski, Endocrinology *82*, 1001 (1968).
2 N. Takasugi, J. Fac. Sci. Univ. Tokyo, Sect. IV Zool. *7*, 299 (1954).
3 R.I. Dorfman and F.A. Kincl, IV Reunión Anual Soc. Mex. Nut. Endocr. Ixtapan de la Sal, México, 6–7 December 1963, p. 171.
4 E.G. Shipley and R.K. Meyer, in: Hormonal Steroids, vol. 2, p. 293. Ed. L. Martini and A. Pecile. Academic Press, New York 1965.
5 J. Presl and A. Bukovský, Čslka Gynek. *41*, 449 (1976).
6 G. Shipley and K. Meyer, Int. Congr. Hormonal Steroids, Milan, Italy, 14–19 May, 1962, p. 86.
7 F.A. Kincl, A. Folch Pi and L. Herrera Lasso, Ciencia, Méx. *22*, 135 (1963).
8 J. Presl, J. Jirásek, I. Mikuláš and J. Horský, Čslka Gynek. *32*, 658 (1967).
9 R.R. Greene and M.W. Burrill, Am. J. Physiol. *133*, P302 (1941).
10 N. Takasugi, Annotnes zool. jap. *26*, 52 (1953).
11 N. Takasugi, Annotnes zool. jap. *26*, 91 (1953).
12 A. Bukovský, J. Presl and Z. Krabec, Čslka Gynek. *41*, 281 (1976).
13 J. Presl, V. Figarová, J. Herzmann and S. Röhling, Endocr. exp. *9*, 51 (1975).
14 J. Presl, J. Herzmann and S. Röhling, Čslka Gynek. *40*, 751 (1975).
15 A. Bukovský and J. Presl, Med. Hypotheses *4*, in press (1978).

Sertoli cells of adult rats in vitro. II. Effect of different steroid precursors on estradiol 17β-synthesis[1]

M.L. Marcante, A. Floridi, P.G. Natali, G. Citro, V. Cioli and C. De Martino[2]

Regina Elena Institute for Cancer Research, Rome (Italy) and F. Angelini Research Institute, Rome (Italy), 9 June 1978

Summary. The estradiol 17β-synthesis by Δ^4 pathway has been studied in homogenous cultures of Sertoli cells isolated from adult rat testes. The data reported clearly demonstrate that progesterone, androstenedione, testosterone and estrone induce an increase of the estradiol 17β-production.

Previous studies[3–5] have demonstrated that the Sertoli cells of adult rats in vitro synthetize large amounts of cholesterol, estrone (E_1) and estradiol 17β (E_2), without the addition of a steroid precursor and/or FSH and cAMP to the culture medium. These findings seem to indicate that the Sertoli cells have the capacity to produce E_2 by the Δ^4 and/or Δ^5 metabolic pathways, which recognize progesterone (P) and pregnenolone, respectively, as the leading

precursors. Because the Δ^4 pathway seems to predominate in rat testis[6,7], special interest has been paid to the study of this metabolic route. This is also justified by finding that the granulosa cells, which have the same embryological origin as the Sertoli cells[8,9] synthetize E_2 preferentially through the Δ^4 pathway[10,11] and display a high content in Δ^5-3β-ol dehydrogenase activity[12].

The present paper reports the data obtained in E_2-biosynthesis after the addition of progesterone (P), androstenedione (A), testosterone (T) and estrone (E_1) to homogenous cultures of Sertoli cells.

Materials and methods. Sertoli cells of Long Evans rats were isolated and cultured as previously described[5]. On the 6th day of culture, a confluent monolayer was obtained. The cells were detached from the flasks by mild trypsinization (0.25%) and subcultured in new plates containing a constant number of cells (5.0×10^4/ml). The number of plates seeded was such that 3 samples of the cultures could be available for each time studied following addition of steroid precursors. 24 h after the subcultures have formed, a confluent monolayer T and E_1 was added at a final concentration of 5×10^{-7} M, while A and P were used at a concentration of 1×10^{-6} M. The higher concentrations of A and P used were justified by the knowledge that these 2 steroids can be transformed into several metabolites which cannot be converted into E_2. Addition of the hormones was repeated every 24 h until the 120th h. Because the steroid hormones were dissolved in absolute alcohol, the same amount (10 µl) of solvent was added to the cells. 24 h after addition of each steroid precursor, a duplicate of cultures was used to determine the amount of E_2 present in the nutritional medium, whereas 1 sample of culture was recovered every 24 h for the morphological study and cell count.

Estradiol was evaluted by the inhibition of a solid phase radioimmunoassay[13]. The antiserum employed was obtained in rabbits against E_2-6-O-carboxy-methyloxime-BSA (Ikapharm). The antiserum did not show any significant cross-reaction with E_1 (4% ± 2%), estriol (0.3% ± 0.3%), P (< 0.001%), A (< 0.01%), T (0.5% ± 0.2%) and with other steroid hormones such as cortisol (< 0.001%) and aldosterone (0.03%). In order to obtain a comparable estimation of E_2 among cultures containing different numbers of cells, the concentration of this steroid was always referred to 10^7 cells. Light and electromicroscopic studies were performed on cell pellets fixed in a picric acid-formaldehyde-osmium tetroxide solution[14] containing 0.8 g NaCl.

Results. The data concerning the E_2-synthesis by cultured Sertoli cells from the 48th to the 144th h are shown in the table. It can be seen that in hormone-treated cultures the E_2-concentration is significantly increased. However, the rate of this production is in relationship both to the steroid used and to the dose added in the culture medium (table). The fine morphology of the Sertoli cells does not differ from that described in our previous paper[3–5] and does not need to be elaborated further. In this context, it is important to report that no significant morphological changes have been observed in the control and in the steroid-treated cultures.

Discussion. The results obtained in this study confirm previous data concerning the E_2 production by cultured Sertoli cells[3–5] and demonstrate that the in vitro administration of P, A, T and E_1 to these cultures promotes an increase in E_2-synthesis.

The data presented show that P is able to increase the E_2-synthesis in cultured Sertoli cells, suggesting that they are capable of carrying out the 17α-hydroxylation and of converting 17α-hydroxyprogesterone into A by the 17–20 desmolase activity. The previous[3–5] and the present findings indicate that the Sertoli cells in vitro have the capacity to convert A into E_2 by 2 distinct pathways. The first involves the transformation of A into E_1 by aromatizing enzymes and, after dehydrogenation at position 17β, the conversion of E_1 into E_2. The existence of this metabolic route is also supported by the finding that E_1 can be recovered from the nutritional medium of control Sertoli cells[3,4]. The 2nd metabolic pathway involves the conversion of A into T by 17β-dehydrogenase and subsequently the T into E_2 by aromatizing enzymes. However, since equivalent doses of E_1 and T have been used in the stimulation of cell cultures, the different levels of E_2 recovered from the culture medium suggest that E_1 is easily metabolized in E_2, while a large amount of T is presumably converted to DHT and/or 5α reduced metabolites. Moreover, because the transformation of E_1 into E_2 requires only the 17β-dehydrogenation step, the data reported in the present paper show that the Sertoli cells contain a considerable amount of 17β-dehydrogenase. The low rate of utilization of T by the Sertoli cells for the E_2-synthesis, in comparison with that obtained with the E_1, seems to indicate that the enzymatic reactions involving aromatizing enzymes in the conversion of C_{19}-steroids to C_{18}-compounds may be a rate limiting step of the E_2-synthesis in cultivated Sertoli cells.

The present data, which confirm that the Sertoli cells in vitro are able to produce E_2 also in the absence of steroid precursors, gonadotrophins and cAMP[3–5] raises the question about the factors responsible for the continuous stimulation of these cells in the E_2-production. The presence, however, of fetal bovine serum in the culture medium suggests that the lack of gonadotrophins may be only apparent. In fact, gonadotrophin hormones have been detected in the pituitary and blood of various mammalian fetuses such as pig[8], rat[15,16] and man[17]. The fetal bovine serum also contains pituitary gonadotrophins, which may stimulate the Sertoli cell cultures. This possibility is further supported by the demonstration of an extensive cross-reaction in the biological activity of gonadotropins of

Effect of steroid precursors on estradiol-17 β-synthesis (pg/10^7 cells) at different times

	24 h	48 h	72 h	96 h	120 h
C	0.20 ± 0.02	0.40 ± 0.04	0.48 ± 0.09	0.54 ± 0.02	0.60 ± 0.05
P	0.60 ± 0.05	0.72 ± 0.02	0.81 ± 0.05	0.94 ± 0.05	1.02 ± 0.02
A	0.70 ± 0.03	0.84 ± 0.01	1.02 ± 0.05	1.26 ± 0.02	1.54 ± 0.06
T	0.50 ± 0.02	0.66 ± 0.03	0.80 ± 0.02	1.00 ± 0.04	1.15 ± 0.02
E_1	6.00 ± 0.5	6.70 ± 0.09	7.30 ± 0.08	7.80 ± 0.05	8.30 ± 0.05

Each value ± SD was the average of 5 determinations performed in duplicate. The differences between control and treated cultures at various times are statistically significant ($p < 0.001$). C: Control; P: progesterone; A: androstenedione; T: testosterone; E_1: estrone.

different animal sources[18-20]. Experiments in order to demonstrate the capacity of the Sertoli cells to synthesize pregnenolone and P by cholesterol are in progress and the results will be reported in a forthcoming paper.

1 This work was partially supported by grants No. 77.01322.04 and No. 77.01958.04 from the Consiglio Nazionale delle Ricerche.
2 The excellent technical assistance of Antonio Muller and Massimo Rosati is deeply appreciated.

3 C. De Martino, M.L. Marcante, A. Floridi, G. Citro, A. Cantafora, M. Bellocci and P.G. Natali, Atti X Congr. Soc. It. Microsc. Elettr. Ostuni, 1-4 October 1975.
4 C. De Martino, M.L. Marcante, A. Floridi, G. Citro, A. Cantafora, M. Bellocci and P.G. Natali, J. submicrosc. Cytol. *8*, 250 (1976).
5 C. De Martino, M.L. Marcante, A. Floridi, G. Citro, A. Cantafora, M. Bellocci and P.G. Natali, Cell Tissue Res. *176*, 69 (1977).
6 J.B.G. Bell, Experientia *28*, 212 (1972).
7 P.F. Hall, in: The testis, vol. II, p. 1. Ed. A.D. Johnson, W.R. Gomes and N.L. Van Demark. Academic Press, New York and London 1970.
8 R.A. Willis, The Boederland of Embryology and Pathology. Butterworth and Co., London 1958.
9 L.B. Arey, Developmental anatomy. Saunders, Philadelphia and London 1965.
10 K.J. Ryan and O.W. Smith, Recent Prog. Horm. Res. *21*, 367 (1965).
11 K.J. Ryan and L. Petro, J. clin. Endocr. Metab. *26*, 46 (1966).
12 R.E. Schully and R.B. Cohen, Obstet. Gynec., N.Y. *24*, 667 (1964).
13 A. Floridi, G. Citro, E. Sega and P.G. Natali, Immun. Commun. *4*, 603 (1976).
14 C. De Martino, M. Stefanini, M. Bellocci and G. Quintarelli, J. submicrosc. Cytol. *4*, 111 (1972).
15 A. Jost, in: The pituitary gland, vol. II, p. 299. Ed. G.W. Harris and B.T. Donovan. University of California Press, Berkeley and Los Angeles 1966.
16 M. Chowdhury and E. Steinberger, J. Endocr., Great Br. *69*, 381 (1976).
17 M.M. Grumbach and S.L. Kaplan, in: Foetal and Neonatal Physiology, p. 462. Ed. K.S. Comline, K.W. Cross, G.S. Dawas and P.W. Nathanielsz. Cambridge University Press, Cambridge 1973.
18 K.J. Catt, M.L. Dufau and T. Tsuruhara, J. clin. Endocr. Metab. *34*, 123 (1972).
19 T. Braun and S. Sepsenwol, Endocrinology *94*, 1028 (1974).
20 V. Hansoon, S.L. Weddington, E. Petrusz, E. Martin Ritzen, S.N. Nayfeh and F.S. French, Endocrinology *97*, 469 (1975).

DISPUTANDUM

Theoretical prediction of carcinogenicity: Quasi-quantification by quasi-valence[1]. A reply to V. Veljkovic and D.I. Lalovic

W.S. Barnes and D.E. Levin

Department of Botany, University of Massachusetts, Amherst (Massachusetts 01003, USA), 11 September 1978

Summary. We have shown the recently proposed method for prediction of carcinogenicity by 'average quasi-valence number' to be neither a good predictor of carcinogenicity, nor of non-carcinogenicity.

Veljkovic and Lalovic have recently proposed that a quantity termed the 'average quasi-valence number' can be used as a predictor of carcinogenicity[2]. This is defined as:

$$Z^* = \sum_{i=1}^{m} N_i Z_i \Big/ \sum_{i=1}^{m} N_i,$$

where N_i is the number of atoms of the i^{th} kind present in a molecule, Z_i is the number of valence electrons in the i^{th} element (except for the halogens where $Z=1$ rather than $Z=7$ is used) and m is the number of elements present in the molecule. As an example, the molecular formula for carbon tetrachloride is CCl_4. Consequently:

$$Z^* = \frac{(1\times 4)+(4\times 1)}{5} = {}^{8}/_{5} = 1.60$$

The authors state that a low value for Z* is necessary but not sufficient for carcinogenicity, while a high value is both necessary and sufficient to indicate non-carcinogenicity. The borderline between these 2 states is supposed, on empirical evidence, to occur at $Z^* = 3.2$. Before such a method is accepted as valid, it is reasonable to expect that it satisfy the following criteria. 1. Few predictions of carcinogenicity when the molecule is non-carcinogenic (false positives) or predictions of non-carcinogenicity when the molecule is carcinogenic (false negatives). 2. A correlation between Z* value and carcinogenic potency. 3. A large change in Z* value between a procarcinogen and the activated, ultimate carcinogen.

False positives and false negatives. The table illustrates that method of average quasi-valence number misclassifies many compounds. False positives include cyclohexane, ethyl alcohol and glucose. False negatives are acetylaminofluorene-N-sulfate (the ultimate carcinogen after activation of 2-acetylaminofluorene), the ultimate metabolite of benzo(a)pyrene, 4-NQO and all but one of the aflatoxins and the nitrosoguanidines. Such glaring inconsistencies in themselves are enough to raise grave doubts about the ability of average quasi-valence number to predict carcinogenicity. Our list, however, shows that average quasi-valence number does not even fulfill the criteria set up by the authors in that a value of $Z^* > 3.2$ is not necessary and sufficient for non-carcinogenicity. Moreover, if the entire list is considered, it can be seen that there is no correlation of Z* with carcinogenicity. Carcinogens and non-carcinogens are found throughout the entire range of Z* values. It is true that the authors claim only that Z* is necessary but not