of receptors, without their being saturated with exogenous gonadotropin, as shown by Sharpe[9] in the immature rat testis.

It is generally accepted that the action of gonadotropin on steroidogenesis is mediated via formation of cAMP. Testicular gonadotropin-binding in rats neonatally treated with sex hormones underwent variation which depended on the functional state of the testis[2], and represents a suitable model for studying its relationship to changes in synthesis of cAMP and testosterone. The results of the present study show that neither plasma and testis levels of cAMP, nor responsiveness of testis to LH in cAMP synthesis, correlate with testicular gonadotropin receptors and steroidogenesis. It is possible that formation of cAMP occurred in cells not involved in testosterone production, or that neonatal estrogenization may have uncoupled cAMP synthesis from formation of testosterone. Moyle et al.[14] observed that LH binding to receptors involved in cAMP synthesis may be different than those necessary for testosterone production in rat Leydig cells. Moreover, Catt and Dufau[10] showed that, with increasing gonadotropin-binding in rat testis, there is a progressive rise in cAMP synthesis, despite the fact that above 1% occupancy there is no further increase in testosterone production. In our experiments only maximal stimulatory dose of LH was used[13], and so it is difficult to explain difference between responsiveness in cAMP and gonadotropin bindig sites. However, the pattern of changes of testicular receptors in estrogenized and PMSG-treated rats is related to plasma levels of testosterone, which points to a close relationship between gonadotropin-binding capacity and steroidogenesis in the modified gonadal function.

1 F. A. Kincl, A. Folch Pi and L. H. Lasso, Endocrinology *72*, 966 (1963).
2 J. Kolena, Endocrinologie, *69*, 266 (1977).
3 J. Kolena, Endocr. exp. *10*, 113 (1976).
4 A. G. Gilman, Proc. nat. Acad. Sci. USA *67*, 305 (1970).
5 J. Kolena and C. P. Channing, Endocrinology *90*, 1543 (1972).
6 F. Stahl, Endokrinologie *65*, 288 (1975).
7 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
8 F. A. Kincl, M. Maqueo and A. Folch Pi, Acta endocr. *47*, 200 (1964).
9 R. M. Sharpe, Nature *264*, 644 (1976).
10 K. J. Catt and M. L. Dufau, Nature New Biol. *244*, 219 (1973).
11 K. Brown-Grant, G. Fink, Fenella Greig and M. A. F. Murray, J. Reprod. Fert. *44*, 25 (1975).
12 C. P. Channing, Proc. Soc. exp. Biol. Med. *149*, 238 (1975).
13 J. Kolena, Archs int. Physiol. Biochim. *83*, 627 (1975).
14 W. R. Moyle, O. P. Bahl and L. März, J. biol. Chem. *250*, 9163 (1975).

Influence of temperature and gonadal steroids on the testis composition in *Rana esculenta*[1]

M. Di Meglio and R. K. Rastogi

Institute and Museum of Zoology, Faculty of Sciences, via Mezzocannone, 8, I–80134 Napoli (Italy), 5 July 1977

Summary. Male frogs at 28 °C show an increase in the testis weight and a decrease in its protein content. At 15 °C and 4 °C the testis lipids increase. Testosterone administration nullifies the low temperature-induced changes in the testis.

Several encyclopedic accounts have been published on the influence of temperature and hormonal compounds upon the anuran testis[2–4]. The available literature indicates that adverse environmental, hormonal and nutritional factors notably affect the anuran testis and the severity and time relationship of the response of the testis differs from species to species. It is also well understood that the spermatogenic tissue responds more readily to such factors than the interstitial tissue. Very little information is, however, available regarding the relationship between the changing testis composition and testis function.

Adult male frogs were procured from the surroundings of Naples and divided into 5 batches of 10 frogs each. 3 batches were kept at 28 °C, 15 °C and 4 °C, on a 12 h light: 12 h dark schedule, for 7 days. The remaining 2 batches of frogs, kept at 15 °C (12 h dark: 12 h light), were treated respectively with 50 µg testosterone and 50 µg estradiol-17β. 3 equal fractions of the total dose of hormones, dissolved in the physiological solution, were injected into dorsal sac on alternate days. At the time of sacrifice, frogs were killed with chloroform and the b.wt and fresh testes weight were determined. 1 testis from each animals was used for the determination of total lipids (according to de La Huerga[5]) and proteins (according to Lowry et al.[6]), whereas the contralateral testis was used for obtaining dry weight.

High temperature (28 °C) stimulates testis weight ($p < 0.05$) but decreases the water and protein contents ($p < 0.001$ and $p < 0.001$; table). The testis lipids show no significant alteration at 28 °C as compared with the initial value ($p = 0.2$). High temperature influence is always accompanied by the stimulation of spermatogenesis and regression of the interstitial tissue[3]. Frogs kept at lower temperatures (i.e. 15 °C and 4 °C) do not show any change in their testis weight but the testicular lipid concentration rises significantly ($p < 0.02$ and $p < 0.01$ respectively). At 15 °C no alteration is found in the testicular protein concentration, whereas at 4 °C a significant decrease is observed ($p < 0.001$). As far as the testis function is concerned, low temperatures usually impair spermatogenesis and stimulate the interstitial tissue[3]. Administration of testosterone to frogs at 15 °C greatly stimulates the testis dry weight (i.e. a decrease in water content) as compared with frogs at 15 °C ($p < 0.001$). On the other hand, the protein content of the testis in testosterone-treated frogs greatly decreases, and both the dry weight and protein concentra-

1 We are thankful to Prof. G. Chieffi for his criticism. Work done under the C.N.R.-financed finalized project 'Biologia della Riproduzione'.

2 B. Lofts, in: Physiology of Amphibia, vol. II, p. 107. Ed. B. Lofts. Academic Press, New York & London 1974.
3 R. K. Rastogi, Boll. Zool. *43*, 151 (1976).
4 R. K. Rastogi, L. Iela, P. K. Saxena and G. Chieffi, J. exp. Zoll. *196*, 151 (1976).
5 J. De la Huerga, in: Tecnica e diagnostica di laboratorio, vol. I, p. 287. Ed. D. D. USES, Bologna 1969.
6 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).

Effects of temperature, testosterone and estradiol-17β on the testis composition in frog

Treatment	Gonosomatic index	Dry weight (%) (% water)	mg protein/g dry tissue	mg lipids/g dry tissue
Initial value	0.26 ± 0.02	7.5 ± 2.5 (92.5)	1485 ± 237	12.7 ± 3.1
28 °C	0.32 ± 0.02*	20.7 ± 2.6 (79.3)*	541 ± 46*	18.1 ± 4.9
15 °C	0.20 ± 0.06	12.4 ± 1.5 (87.6)*	1108 ± 154	23.7 ± 5.8*
4 °C	0.23 ± 0.02	10.9 ± 4.3 (89.1)	1089 ± 91*	27.3 ± 7.2*
15 °C + testosterone	0.21 ± 0.04	22.5 ± 1.9 (77.5)*, **	544 ± 69*, **	10.3 ± 2.6**
15 °C + estradiol-17β	0.22 ± 0.04	14.5 ± 0.4 (85.5)*	818 ± 67*	12.8 ± 3.3**

*Significantly different (student's t-test) from the initial value;
**significantly different from values obtained in 15 °C-treated frogs.

tion of the testis of these frogs are similar to the values obtained in frogs at 28 °C (table). Similar changes are observed for the testis lipids also. Thus testosterone inhibits the low temperature-induced increase in the testis lipids and proteins. Administration of estradiol-17β, on the other hand, does not alter the testis weight (wet or dry) but impedes the rise in testicular lipid concentration. It may not be out of line to mention that high temperature provokes degranulation of the pituitary gonadotrops whereas low temperature induces hypertrophy[3,4]. Thus in future, consideration must be given to the testis composition which, at least in part, reflects the influence of environmental and hormonal factors upon the testis.

Purification of the cytosol oestradiol-receptor complex from foetal guinea-pig uterus using electrofocusing on polyacrylamide plates[1]

J. R. Pasqualini and C. Cosquer-Clavreul

C.N.R.S. Steroid Hormone Research Unit, Foundation for Hormone Research, 26, boulevard Brune, F-75014 Paris (France), 8 June 1977

Summary. The ^{3}H-oestradiol receptor complex obtained from the cytosol fraction of foetal guinea-pig uterus was purified by the following steps: column chromatography in Sephadex G-15 and Ultrogel, and by electrofocusing on polyacrylamide plates. In the final step a concentration of 15–17% of the foetal uterine oestradiol receptor protein was obtained. The isoelectric point (pl) of this receptor was determined to be 6.1–6.2.

Recently it has been found that the foetal uterus of guinea-pig contains specific cytosol and nuclear receptors for oestradiol[2]. These specific oestradiol binding sites increase significantly during foetal developement, particularly at the end of gestation when values reach 500–800 fmoles/mg cytosol protein[3]. Purification of the oestradiol receptors from the uteri of immature calves or rats was obtained by ammonium sulfate precipitation, gel filtration and ion exchange chromatography[4] or by affinity chromatography[5]. In this paper the purification of ^{3}H-oestradiol receptor complex from the foetal uterus of guinea-pig by electrofocusing on plates is described.

Materials and methods. The uteri of Hartley Albino guinea-pig foetuses (55–62 days of gestation) were used. 500–600 mg (10–14 fetal uteri) were homogenized in 4 ml of 0.01 M Tris, HCl–0.001 M $CaCl_2$ (pH 7.4), centrifuged at 900 × g and the cytosol fraction was obtained after ultracentrifugation of the supernantant at 200,000 × g. The ^{3}H-oestradiol receptor complexes were prepared by incubation of this cytosol fraction (containing 4 mg protein/ml) with 9×10^{-8} M, 6,7-^{3}H-oestradiol (s.a. 60 Ci/mmole). Parallel incubations were carried out using the same concentration of ^{3}H-oestradiol plus a 100fold excess of non-radioactive oestradiol. The incubations were carried out at 25 °C for 20 min.

The ^{3}H-oestradiol macromolecule complexes were first obtained after column chromatography on Sephadex G-15 (Pharmacia, Uppsala, Sweden) (30 cm high, 0.9 cm diameter). These ^{3}H-oestradiol protein complexes were re-chromatographed in a column of Ultrogel AcA-34 (L. K. B., Broma, Sweden, 0.30 cm high, 0.9 cm diameter). Both chromatographies were carried out at 2 °C. The peak containing the oestradiol receptor was submitted to thin layer gel electrofocusing in a polyacrylamide gel plate (L. K. B. 2117 Multiphor, Broma, Sweden) containing ampholytes with a pH range of 3.5–9.5 for 90 min at 2 °C. The isoelectric point (pl) was calculated by measuring the pH in different gel sections, using a pH meter (Radiometer PHM 62). Furthermore, in order to estimate the sedimentation coefficient of the ^{3}H-oestradiol receptor complex, aliquots (0.2 ml) of the cytosol fraction from the Sephadex and Ultrogel columns were layered on a sucrose density gradient (5–20% w/v) in a 0.01 M Tris, HCl (pH 7.4) solution, containing 0.0005 M dithiothreitol and 0.01 M EDTA and centrifuged at 200,000 × g for 18 h at 2 °C.

1 The expenses of the investigation were partially defrayed by a grant from the Centre National de la Recherche Scientifique (CNRS), France (Equipe de Recherche CNRS No. 187.)

2 J. R. Pasqualini and B. L. Nguyen, C. r. Acad Sci. (Paris) Serie D *283*, 413 (1976).
3 J. R. Pasqualini, C. Sumida, C. Gelly and B. L. Nguyen, J. steroid Biochem. *7*, 1031 (1976).
4 E. R. De Sombre, J. P. Chabaud, G. A. Puca and E. V. Jensen, J. steroid Biochem. *2*, 95 (1971).
5 V. Sica, I. Parikh, E. Nola, G. A. Puca and P. Cuatrecasas, J. biol. Chem. *248*, 6543 (1973).