possible to elaborate such a conditioned reflex 1–3 h later (Fig. 1*B*). The signal stimuli evoked 50–60 per cent positive responses, that is, the same as in 1-day-old guinea pigs[2].

After tying the cord animals also showed increased motor activity and a strong reaction to indifferent external stimuli.

Independent rhythmic respiratory movements appear only in fœtuses shortly before term. Only animals aged 60–66 days survived.

The inability of fœtuses with an intact placental circulation to form temporary connexions is interesting in view of the fact that guinea pigs are born with a nearly completely differentiated cortex both morphologically[3] and enzymatically[4] and bioelectrically[5]. Probably the start of respiration is particularly important.

I thank Dr. J. Sedláček for his help in this work.

Vl. Hlaváčková *

Department of Physiology,
Charles University,
Prague.

* Present address: Institute of Physiology, Academy of Science, Salmovská 1, Prague 2.

[1] Sedláček, J., *Physiol. Bohemoslov.*, **11**, 313 (1962).
[2] Sedláček, J., and Hlaváčková, Vl., *Act. Nerv. Sup.*, **4**, 2 (1962).
[3] Peters, V. B., and Flexner, L. B., *Amer. J. Anat.*, **86**, 133 (1950).
[4] Kreps, E. M., *Zurn. vyss. nerv. dej.*, **2**, 46 (1952).
[5] Flexner, L. B., Tyler, D. B., and Gallant, L. J., *J. Neurophysiol.*, **13**, 427 (1950).

Hormonal Dependence of Oxidative Enzymes in the Testis of the Rat

Pituitary and chorionic gonadotrophins accelerate the synthesis of testosterone from acetate in dog testes *in vivo*[1] and in rabbit testes *in vitro*[2]. It is known that reduced triphosphopyridine nucleotide (TPNH) is required for steroid synthesis[3], and there is some evidence indicating that gonadotrophic hormones influence dehydrogenase activity in target glands. Thus, Samuels[4] showed stimulation of 3-β-ol-dehydrogenase by chorionic gonadotrophin in rat testis and Niemi[5] examined histochemically hormonal influences on *d*-1-β-hydroxybutyric and hydroxysteroid dehydrogenases.

In previous work[6], one of us found that addition of β-hydroxybutyrate brought about a marked increase in oxygen consumption in testis homogenates of postpuberal but not of pre-puberal rats. These changes coincide with the onset of testosterone synthesis.

In a series of experiments designed to elucidate the effect of gonadotrophins at the cellular level, we have examined various metabolic steps endeavouring to find the pathways concerned in the production and supply of energy for steroid synthesis. The experiments reported here concern the effect of chorionic gonadotrophin on isocitric, glucose-6-phosphate and lactic dehydrogenases.

Immature $A \times C$ male rats inbred weighing 23–25 g at the onset of the experiment were injected with chorionic gonadotrophin for 5 days, after which the rats were decapitated. Effectiveness of treatment was checked by the weight of the testes and seminal vesicles (Table 1). The testes were dissected free of their capsule, 100 mg of tissue were homogenized in 0·25 M sucrose in a motor-driven Potter–Elvehjem homogenizer at 2,000 r.p.m. for 90 sec in ice, and then centrifuged for 30 min at 14,000*g* under refrigeration. The oxidative enzymes examined here were located mainly in the post-mitochondrial fraction remaining in the supernatant.

Table 1. Effect of Gonadotrophic Hormone on Organ Weight and on Specific Activity of Dehydrogenase

No. of animals	Total dose 5 days (I.U.)	Testis weight	Seminal vesicle weight	Glucose-6-phosphate dehydrogenase acitivity	Isocitric dehydrogenase activity	Lactic dehydrogenase activity
12	500	79	715	141	67	0
12	100	43	570	123	61	0
6	1	38	430	132	44	8*
6	2·5	30	295	85	31	14*

* Not statistically significant.
All figures are expressed as percentage increase over those of untreated controls of the same age. Weights of organs were computed relative to 100 g body-weight.
Aconitase activity was not modified.

Isocitric dehydrogenase was determined by the method of Ochoa[7], glucose-6-phosphate dehydrogenase by the method of Kornberg and Horecker[8], aconitase by Anfinsen's[9] and lactic dehydrogenase by Neilands's[10] procedure. Proteins were estimated by the biuret method as described by Layne[11].

Chorionic gonadotrophin caused a significant increase in the specific activity of testis isocitric and glucose-6-phosphate dehydrogenases (Table 1). Lactic dehydrogenase activity was not increased. All figures were well beyond statistical error at a probability-level of 0·001 by Student's *t* test, except the lowest figure for isocitric dehydrogenase which was in the limit between $P = 0{\cdot}05$ and 0·1.

Doses as small as 0·5 I.U. a day were effective; smaller doses have not been tried. The effect appears to be organ-specific since it could not be obtained in liver homogenates. Our results also showed that in rat testis these enzymes are TPN dependent. No reduction of DPN could be obtained with testis homogenates using isocitrate and glucose-6-phosphate as substrates.

These findings provide evidence that enzymes belonging to the respiratory system and to the hexose monophosphate shunt are stimulated in the testis by chorionic gonadotrophin; but we cannot say at the moment whether the specific action of the hormone is mediated by these systems. The gonadotrophic stimulation of dehydrogenases may be indirect, since Field[12] found no stimulation *in vitro* of glucose oxidation by luteinizing hormone or human chorionic gonadotrophin.

If the hexosé monophosphate shunt is involved in steroid synthesis in the testis it may be stimulated by a different mechanism from that postulated by Haynes[13] in adrenal cortex, since cyclic adenosine monophosphate, which mediates the action of ACTH on phosphorylase in the adrenal cortex, does not stimulate the conversion of ^{14}C-acetate to ^{14}C-testosterone *in vitro*, according to Brinck-Johnsen and Eik-Ness[1].

Further experiments are in progress to elucidate some of the points raised here and to investigate other systems in rat testis which may be influenced by gonadotrophic hormones.

One of us (N. A. S.) received financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.

N. A. Schor
J. Cara
A. Perez

Institute of Cellular Biology,
Universidad Nacional de Córdoba,
Argentina.

[1] Mason, N. R., and Samuels, L. T., *Endocrinol.*, **68**, 899 (1961). Brinck-Johnsen, T., and Eik-Ness, K., *ibid.*, **61**, 676 (1957).
[2] Hall, P. F., and Eik-Ness, K., *Biochim. Biophys. Acta*, **63**, 411 (1962).
[3] Lynn, jun., S. W., and Brown, R. H., *J. Biol. Chem.*, **232**, 1015 (1958). Pincus, G., *Proc. Fourth Intern. Cong. Biochem.*, **4**, 61 (1958).
[4] Samuels, L. T., and Hemreich, M. L., *Endocrinol.*, **58**, 435 (1956).
[5] Niemi, M., and Ikonen, M. I., *Endocrinol.*, **70**, 167 (1962).
[6] Schor, N. A., *Rev. Soc. argent. Biol.* (in the press).
[7] Ochoa, S., *Methods in Enzymology*, edit. by Colowick, S. P., and Kaplan, N. O., **1**, 699 (Academic Press, New York, 1957).
[8] Kornberg, A., and Horecker, B. L., in *Methods in Enzymology*, **1**, 323 (Academic Press, New York, 1957).
[9] Anfinsen, C. A., in *Methods in Enzymology*, **1**, 695 (Academic Press, New York, 1957).
[10] Neilands, J. B., in *Methods in Enzymology*, **1**, 449 (Academic Press, New York, 1957).
[11] Layne, E., in *Methods in Enzymology*, 450 (Academic Press, New York, 1957).
[12] Field, J. B., Pastan, I., Herring, B., and Johnson, P., *Endocrinol.*, **67**, 801 (1960).
[13] Haynes, R. C., *J. Biol. Chem.* **233**, 1220 (1958).