Effect of Castration on $^{14}CO_2$ Production from Glucose-U-^{14}C in the Hypothalamus and Cerebral Cortex[1] (35429)

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(Introduced by Virgilio G. Foglia)

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In previous papers we have demonstrated that sexual activity modifies hypothalamic metabolism (1, 2). For example, castrated male rats show a decrease in the oxygen uptake of anterior and posterior hypothalamus and the administration of testosterone restores values to normal (3). In addition, incorporation of labeled phenylalanine into hypothalamic proteins is less in 3-week castrated rats than in normal animals (4).

In the present investigation, the production of $^{14}CO_2$ from glucose-U-^{14}C was studied in normal and castrated rats, to determine if the decrease in the hypothalamic metabolism observed after gonadectomy is related to alterations in the glucose oxidation.

Material and Methods. Experiments were performed on male rats from the strain of the Institute of Physiology, weighing 150–170 g. They were housed under conditions of constant temperature (23 ± 2°) and lighting (12 hr of light and 12 hr of darkness), fed *ad libitum* with a standard diet and allowed free access to drinking water.

Orchiectomy was carried out under ether anesthesia. The following groups of rats were studied: (A) control; (B) castrated; and (C) castrated injected with testosterone. The hormone was injected subcutaneously at the dose of 150 μg in 0.5 ml of sesame oil twice a week starting 1 day after operation.

Animals were decapitated 1 week after castration and the whole hypothalamus was removed according to the limits given by De Groot (5). The sample was limited rostrally by the anterior commissure; and caudally by the end of the mammillaris bodies; the superior border was limited by the thalamus-hypothalamic sulcus and the inferior border was taken as the line passing through the hypothalamic-pituitary junction. The following nucleus and areas were included in the studied sample: the preoptic and anterior hypothalamic areas, the paraventricular, suprachiasmatic, mammillaris, posterior, arcuate, ventromedial and dorsomedial nucleus, as well as the median eminence. A slice of cerebral cortex was also taken from the frontal lobe. The samples were weighed on a torsion balance and transferred to glass bottles sealed with rubber stopper for incubation following the technique described by Goodner and Freinkel (6). The stopper supported a small plastic cup containing paper strips (1 × 10-cm Whatman No. 40) into which 0.2 ml of 2 *N* sodium hydroxide solution (7) was injected at the end of incubation period for collection of $^{14}CO_2$ as outlined below. All incubations were performed at 37° for 60 min in a Dubnoff metabolic shaker (shaking rate, 100 cycle/min), in 1 ml of Krebs Ringer phosphate buffer pH 7.4, with 3.3 m*M* unlabeled glucose and 0.25 μCi of glucose-U-^{14}C (39 Ci/mmole) obtained from the Commisariat a l'Energie Atomique (France). In the experiments in which time-course of $^{14}CO_2$ production was studied only 0.10 μCi of glucose-U-^{14}C was used. The closed flasks were gassed with 100% O_2 for 5 min prior to introduction of the tissue and for 5 min after tissue placement and re-

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sealing. At the end of incubation period, 0.25 ml of 50% trichloacetic acid were injected into each bottle and 0.2 ml of 2 *N* sodium hydroxide solution in the plastic caps, after which, shaking was continued for 2 hr.

$^{14}CO_2$ evolved during incubation was assayed by placing the paper strips at 60° for 48 hr and then transferring them directly into counting vials containing 12 ml of Bray solution. Radioactivity was determined in a Packard Tri-Carb liquid scintillation counter and each sample was counted long enough to give a random error less than 1%. Counts were corrected to 100% efficiency by the channel's ratio method. The radioactivity incorporated into CO_2 was expressed as disintegrations per minute per milligram of wet weight.

Lactic acid accumulation was determined by measuring the initial and final concentration in the incubating media by the method of Baker and Summerson (8) and expressed as micromoles produced per gram of wet tissue.

All results are presented as the means ± SEM, and were compared statistically by means of Student's *t* test. Differences were considered significant when *p* value were less than 0.05.

Results. The production of $^{14}CO_2$ from labeled glucose as well as the lactic acid accumulation were linear during the 2 hr of incubation studied (Figs. 1 and 2). There is apparently a more active oxidation of glucose in the cerebral cortex than in the hypothalamus, as shown by the higher ratio of incorporation of labeled C into respiratory CO_2. Since similar curves were found in pilot experiments carried out in castrated rats, 60 min of incubation was selected for further work.

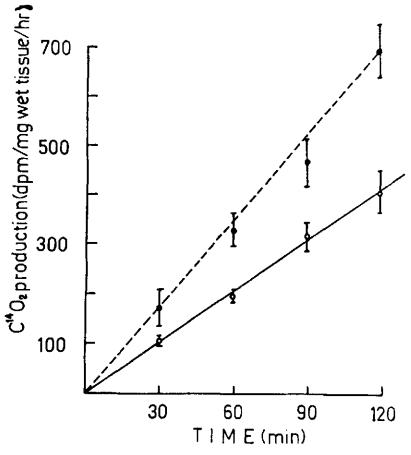

FIG. 1. Time course of production of $^{14}CO_2$ from glucose -U-^{14}C: The results are the mean ± SE of 5 separate determinations. (—) hypothalamus; (- - -) cerebral cortex.

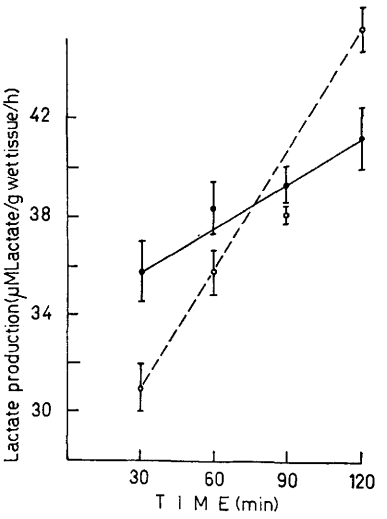

FIG. 2. Time course of lactate accumulation. The results are recorded as the mean ± SE of 5 separate determinations. (—) hypothalamus; (- - -) cerebral cortex.

TABLE I. $^{14}CO_2$ Production from Glucose-U-^{14}C in Cerebral Cortex and Hypothalamus of Castrated Rats.

	$^{14}CO_2$ production (dpm/mg of wet tissue/hr)	
	Hypothalamus	Cerebral cortex
A. Control	520.7 ± 22.6[a] (10)	711.4 ± 44.5 (10)
B. Castrated	392.1 ± 18.8 (9)	667.5 ± 40.4 (11)
C. Castrated + testosterone	544.8 ± 36.7 (10)	708.1 ± 90.6 (10)
	p value	*p* value
A vs B	<0.001	NS[b]
A vs C	NS	NS

[a] Mean ± standard error; number of determinations is given in parentheses.

[b] NS, not significant.

TABLE II. Lactate Production in Cerebral Cortex and Hypothalamus of Castrated Rats.

	Lactate (μmoles/g of wet tissue/hr)	
	Hypothalamus	Cerebral cortex
A. Control	38.6 ± 3.1[a] (8)	35.0 ± 2.9 (9)
B. Castrated	43.2 ± 2.0 (10)	35.9 ± 1.8 (10)
C. Castrated + testosterone	39.0 ± 3.9 (10)	35.0 ± 1.7 (10)

[a] Mean ± standard error; number of determinations is given in parentheses. The differences between control and experimental groups were not significant.

As shown in Table I, castration produces a significative depression in the production of $^{14}CO_2$ from glucose-U-^{14}C in the hypothalamus. The administration of testosterone to gonadectomized rats corrected this modification. No changes were found in the $^{14}CO_2$ production in the cerebral cortex of castrated rats as compared with the control.

Similar values of lactic acid production (Table II) were found in the cerebral cortex and hypothalamus between the different studied groups.

Discussion. In previous papers (3, 9) it has been demonstrated that experimental and physiological modifications in the secretion of gonadotropins and sexual hormones are accompanied by changes in the oxidative metabolism of hypothalamus. Gonadectomy performed in male rats produces a depression in the oxygen uptake of anterior and posterior hypothalamus (3), as well as in the oxidation of several substrates of Krebs cycle (10). On the other hand suppression of gonadotropin secretion, as in hypophysectomized rats, produces an elevation in the oxidative activity of these hypothalamic areas (11). Studies performed in hypophysectomized–castrated rats have shown that the metabolic modifications of hypothalamus in connection with sexual activity are related to a direct effect of gonadotropins on this nervous structure (9).

Studies on $^{14}CO_2$ production from glucose-U-^{14}C provide information on the participation of glucose in the total respiration of the tissue. The fate of glucose-U-^{14}C has been studied with slices of rat cerebral cortex (12). The results indicated that 60 to 70% of the glucose metabolized was converted to lactate through the Embden Meyerhof pathway, 20% to CO_2 by the tricarboxylic acid cycle [the pentose phosphate shunt, the alternative path for glucose oxidation that produces CO_2, apparently does not operate in brain under normal conditions (13)] and most of the remainder to amino acid and derivates.

The results of the present experiments show that the production of $^{14}CO_2$ from glucose-U-^{14}C is less in the hypothalamus of castrated rats than in normal animals and that the administration of testosterone to gonadectomized rats corrected such modifications. No changes were found in the lactic acid accumulation of hypothalamus of castrated rats compared with the control. These findings confirm previous studies indicating that castration depresses the oxidative degradation of glucose at the level of the tricarboxilic acid cycle (14). It is probable that this metabolic effect is connected with the action of gonadotropins, which are elevated in gonadectomized rats (9).

It has been recently demonstrated that after 3 weeks of castration there is a decrease in the *in vivo* incorporation of 1, 4(^{3}H)-phenylalanine into hypothalamic proteins (4). Glucose metabolism is the principal source of the high energy intermediaries involved in protein synthesis in the central nervous system. It is probable that the decrease in the glucose oxidation in the hypothalamus of castrated rats as seen from the appearance of labeled carbon in the respiratory CO_2 described in the present paper is directly connected with the alterations in the hypothalamic protein synthesis found in castrated rats.

It is interesting to note that castration increased the oxygen uptake of cerebral cortex (3). The present results showed no changes in the $^{14}CO_2$ production from labeled glucose nor in lactic acid production in castrated rats. It is probable that the mechanisms involved in the cerebral cortex modifications of oxygen uptake in gonadectomized rats are different than those that take place in the

hypothalamus, especially taking into account that different hormonal mechanisms are responsible for the metabolic changes in these nervous structures in castrated rats (15). Further experimental evidence is needed before a conclusion can be reached in this respect.

Summary. The production of $^{14}CO_2$ from glucose-U-^{14}C was studied in normal and castrated rats. Gonadectomy produced a significative depression in the production of $^{14}CO_2$ from glucose -U-^{14}C in the hypothalamus. The administration of testosterone to gonadectomized rats corrected this modification. No changes were found in the $^{14}CO_2$ production in the cerebral cortex of castrated rats compared with the control. Similar values of lactic acid accumulation were observed in the hypothalamus and cerebral cortex between the different groups studied.

1. Moguilevsky, J. A., and Malinow, M. R., Amer. J. Physiol. **206**, 855 (1964).

2. Moguilevsky, J. A., Libertun, C., Schiaffini, O., and Szwarcfarb, B., Neuroendocrinology **3**, 193 (1968).

3. Moguilevsky, J. A., Schiaffini, O., and Foglia, V. G., Life Sci. **5**, 447 (1966).

4. Moguilevsky, J. A., Kalbermann, L. E., Libertun, C., and Gomez, C. J., Proc. Soc. Exp. Biol. Med. **136**, 1115 (1971).

5. De Grott, J., "The rat forebrain in stereotaxic coordinates." North-Holland, Amsterdam (1959).

6. Goodner, C. J., and Freinkel, N., J. Clin. Invest. **40**, 261 (1961).

7. Davidson, E. A., "Techniques for paper strip counting in a scintillation spectrometer." Packard Tech. Bull. No. 4 (1962).

8. Baker, J. B., and Summerson, W. H., J. Biol. Chem. **138**, 535 (1941).

9. Moguilevsky, J. A., Libertun, C., Schiaffini, O., and Scacchi, P., Neuroendocrinology **4**, 264 (1969).

10. Schiaffini, O., Acta Physiol. Lat. Amer. **18**, 345 (1968).

11. Libertun, C., Moguilevsky, J. A., Schiaffini, O., and Foglia, V. G., Experientia **25**, 196 (1969).

12. Beloff-Chain, A., Catanzaro, R., Chain, E. B., Masi, L., and Pochiari, F., Proc. Roy. Soc., Ser. B **152**, 283 (1960).

13. DiPietro, D., and Weinhouse, S., Arch. Biochem. Biophys. **80**, 268 (1959).

14. Schiaffini, O., Moguilevsky, J. A., Garcia Argiz, C. A., and Libertun, C., Acta Physiol. Lat. Amer. **18**, 351 (1968).

15. Moguilevsky, J. A., Acta Physiol. Lat. Amer. **16**, 353 (1966).

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