PITUITARY GONADOTROPHIN ACTIVITY IN MALE RATS WITH DAMAGE OF THE GERMINAL EPITHELIUM INDUCED BY BUSULPHAN

L. DEBELJUK* AND R. E. MANCINI

*Centro de Investigaciones sobre Reproduccion, Facultad de Medicina,
Universidad de Buenos Aires, Buenos Aires, Argentina*

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It is well known that steroids secreted by the Leydig cells inhibit gonadotrophin secretion (Ramirez & McCann, 1965; Bogdanove, 1967). Three decades ago, it was suggested that some cellular component of the seminiferous tubule might regulate the secretion of pituitary gonadotrophins (Vigdoft, Hill, Vehrs & Kubin, 1939; McCullagh & Schaffenburg, 1952). Although later findings (Allanson & Deanesly, 1962; Katsh & Duncan, 1968) seemed to offer some support to this hypothesis, no conclusive proof has yet been presented. Moreover, testicular extract or a glycoprotein substance related to germinal cells administered to intact and castrated male rats, failed to demonstrate an inhibitory effect on pituitary gonadotrophin concentrations (Debeljuk & Mancini, 1970). The purpose of the present work was to achieve selective damage of spermatogonia in rat testes, temporarily arresting the normal progression of spermatogenesis, in order to establish a possible correlation between the lack of given cellular types in the seminiferous tubules with changes in the concentrations of pituitary gonadotrophins in adult male rats. For selective damage of spermatogonia, busulphan, a well-known alkylating agent (Jackson, Fox & Craig, 1961), was chosen.

One hundred adult male Wistar rats weighing 250 to 320 g were treated with a single intraperitoneal injection of busulphan finely suspended in arachis oil. The dose administered was 10 mg/kg body weight, as in the work of Jackson *et al.* (1961). The animals were killed weekly and the weights of adenohypophyses and testes were recorded. Pituitaries were then quickly homogenized in saline, pooled, frozen and stored at -20°C , pending assay for gonadotrophins. Pituitary FSH concentrations were determined by means of the method described by Steelman & Pohley (1953), as modified by Parlow & Reichert (1963). Ovine NIH-FSH-s4 was used as the standard preparation. Pituitary LH concentrations were measured by means of the ovarian ascorbic acid depletion method of Parlow (1961) with ovine NIH-LH-s12 used as the standard. Relative potency, parallelism, precision index and 95% confidence limits were calculated according to Gaddum (1953). Testes were fixed in Bouin's solution and stained by means of the PAS-haemalum technique. Body weights showed no significant

* Present address: Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, and Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana, U.S.A.

changes during the experiment. Testis weights declined progressively from the 1st to the 8th week after treatment, with a small increase during the 9th week (Table 1).

In agreement with previous reports (Jackson *et al.*, 1961), no apparent abnormalities were detected in the cellular composition of seminiferous tubules during the 1st week after treatment with busulphan. During the 2nd week, all the tubules lacked spermatogonia as in the 3rd week, when half of the tubules showed a decreased number of spermatocytes. By the 4th week, very few spermatocytes were to be seen and none was observed in Week 5 although small numbers of spermatogonia were present. During the 6th week, many spermatogonia could be seen but very few young spermatocytes and no immature spermatids. By the 8th week, spermatocytes were present in almost normal numbers but spermatids were still absent. In the 9th week, lowered numbers of young spermatids and very scarce mature spermatids could be seen.

Concentrations of LH in pituitary glands of busulphan-treated rats were quite stable during all the experiments, with only one exception during the 3rd week, when a moderate decrease was noticed. On the other hand, pituitary FSH concentrations underwent more significant changes. A moderate decrease was noted during the 1st week with a subsequent increase, while a new significant decrease appeared during the 5th and 6th weeks. Thereafter, FSH concentrations were in the normal range (Table 1).

These results confirm that busulphan selectively damages spermatogonia. No other cells appeared to have been damaged as far as their morphological aspect was concerned. Pituitary gonadotrophins underwent changes shortly after treatment. A moderate decrease in pituitary FSH concentration was noted during the 1st week, and in pituitary LH during the 2nd week. Since these changes occurred shortly after the administration of busulphan and were associated with normal testicular histology during the 1st week, it is probable that they reflect a direct effect of the alkylating agent at the pituitary level. It was, however, surprising that during the 5th and 6th weeks, when a new generation of spermatogonia appeared indicating that the effect of busulphan had stopped, a decrease in pituitary FSH concentration was again noted. It is not possible to know from the present data whether this decrease is an index of a depressed synthesis or of an increased release of the hormone by the pituitary gland. The decrease might indicate that certain cells within the seminiferous tubules could have some influence in regulating the process of synthesis or release of pituitary FSH. As the seminiferous tubules lacked spermatocytes during the 5th week after treatment, and had very few spermatocytes and lacked young spermatids in the 6th week, it is possible that some of these cells could be involved in pituitary FSH regulation. Recently, Johnsen (1970) proposed the hypothesis that, in man, the final steps of spermatogenesis (mature spermatids and spermatozoa) are involved in the control of FSH secretion. The finding that certain dichloroacetyl diamines inhibit spermatid maturation and may induce a rise in total urinary gonadotrophins seems to be in good agreement with the hypothesis of Johnsen (Heller, Flageolle & Matson, 1963). In the rat, the present results do not support the hypothesis that mature spermatids are involved in the control of gonadotrophin secretion since, in their absence, pituitary

TABLE I
EFFECTS OF THE TREATMENT WITH A SINGLE DOSE OF BUSULPHAN

	Body weight (g) (Mean $\pm$ S.D.)	Pituitary weight (mg) (Mean $\pm$ S.D.)	Testis weight (g) (Mean $\pm$ S.D.)	Pituitary FSH concentration* (mU/g)	Pituitary LH concentration† (mU/g)	Germinal epithelium‡
Control	328 $\pm$ 30.5	6.4 $\pm$ 0.88	2.543 $\pm$ 0.320	76.3 (61.8 to 90.5)	5.2 (4.6 to 8.5)	Normal
1st week	314 $\pm$ 29.5	7.4 $\pm$ 0.87	2.730 $\pm$ 0.159	46.2 (33.6 to 56.9)	6.8 (4.2 to 9.1)	Apparently normal
2nd week	286 $\pm$ 28.2	6.6 $\pm$ 1.04	2.365 $\pm$ 0.255	54.7 (44.4 to 63.2)	3.4 (3.2 to 3.5)	No spermatogonia
3rd week	317 $\pm$ 29.2	7.8 $\pm$ 0.97	2.458 $\pm$ 0.214	61.0 (53.2 to 65.4)	4.8 (2.3 to 7.4)	No spermatogonia; spermatocytes decreased
4th week	318 $\pm$ 40.5	7.3 $\pm$ 1.16	2.132 $\pm$ 0.308	§	6.6 (4.8 to 10.0)	No spermatogonia; very few spermatocytes
5th week	327 $\pm$ 43.1	8.2 $\pm$ 1.16	1.795 $\pm$ 0.409	41.4 (23.0 to 55.8)	6.0 (5.1 to 7.0)	Few spermatogonia; no spermatocytes
6th week	319 $\pm$ 42.8	7.4 $\pm$ 0.87	1.612 $\pm$ 0.120	49.3 (36.1 to 59.8)	5.5 (4.8 to 6.3)	Some spermatocytes; no young spermatids
7th week	324 $\pm$ 37.6	7.7 $\pm$ 0.90	1.374 $\pm$ 0.113	64.8 (43.9 to 95.7)	7.2 (5.4 to 8.5)	Few spermatocytes; no young spermatids
8th week	337 $\pm$ 23.0	7.1 $\pm$ 1.00	1.262 $\pm$ 0.779	63.9 (61.8 to 66.2)	§	No spermatids
9th week	255 $\pm$ 29.5	6.6 $\pm$ 1.07	1.350 $\pm$ 0.303	78.1 (72.2 to 83.5)	6.8 (6.4 to 7.3)	Young spermatids decreased; few mature spermatids

Number of animals used: twenty rats for control group and ten rats for the remaining groups.

* Expressed as μ equiv. NIH-FSH-s4/mg wet weight of anterior pituitary gland. 95% confidence limits in parentheses.

† Expressed as μ equiv. NIH-LH-s12/mg wet weight of anterior pituitary gland. 95% confidence limits in parentheses.

‡ The cells not mentioned are considered to be in normal number.

§ Assays lacking parallelism.

gland FSH concentrations were in the normal range. These present findings are regarded as preliminary results, since plasma assays of gonadotrophins in rats treated with busulphan are required to interpret the precise meaning of the pituitary changes already described.

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