Effect of inhibin on rat testicular desoxyribonucleic acid (DNA) synthesis in vivo and in vitro

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Abstract. When injected in vivo 3 h before sacrifice or when incubated in vitro with testicular fragments for 3 h, triitated thymidine, a reliable index of DNA synthesis and of mitotic activity, was incorporated into the DNA of differentiated spermatogonia, as shown by autoradiography. The maximum DNA specific activity was obtained in pubertal rats aged 42 days, weight 150 g. Two preparations of inhibin extracted from ram rete testis fluid (RTF) of different molecular weight (> 10 000 for RTF₁ and < 5000 for RTF₃) but which possess the same biological properties were investigated for their effect on thymidine uptake in vivo and in vitro. In vivo both preparations specifically inhibited triitated thymidine incorporation into testicular DNA of pubertal animals (42 days). No change in thymidine uptake into hepatic DNA was observed. Triitated thymidine incorporation into testicular DNA was lower in normal adult rats and in hypophysectomized pubertal animals. RTF₁ and RTF₃ did not affect thymidine incorporation in either case. The reasons for this lack of effect are discussed. In vitro, both preparations induced a dose-dependent decrease in DNA synthesis in testis fragments from rats aged 42 and 49 days. The preparations lost their in vivo and in vitro inhibitory effects when denatured by heating and trypsin digestion. The inhibin preparations probably reduced testicular DNA synthesis and spermatogonial multiplication by reducing FSH secretion in vivo but also had a direct effect on the germ cells as shown by the in vitro experiments. These in vivo and in vitro actions of inhibin preparations are similar to those of the testicular chalones. The relationship which might exist between inhibin and the chalones is discussed.

Inhibin is a proteinaceous substance secreted by the testicular Sertoli cells (Steinberger & Steinberger 1976) and the ovarian granulosa cells (Eriksson & Hsueh 1978). Both in vivo and in vitro it specifically reduces basal FSH secretion and FSH release in response to GnRH administration (see review by Franchimont et al. 1979). By its effect on FSH secretion, it may alter spermatogenesis. In fact, De Jong et al. (1978) showed that bovine follicular fluid, devoid of steroids, given for 12 days to 21 day-old rats was able to induce a delay in pubertal development, characterized by a reduction of testicular weight, a retardation of spermatogenesis and a decrease in the number of pachytenes spermatocytes. In contrast, daily injection of ram rete testis fluid to adult male rats up to 70 days does not affect testicular fluid secretion, sperm counts, testis weights and fertility (Davies et al. 1979).

With the aim of defining the effect of inhibin on spermatogonial multiplication and on the first stages of meiosis, we have examined the results of its acute administration on the incorporation of triitated thymidine into rat testicular desoxyribonucleic acids (DNA) in vitro and in vivo under various experimental conditions. Tritium labelled thymidine is an extremely useful tool for the study of tissue mitotic activity. It has been well established

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that thymidine is incorporated almost exclusively into DNA during the brief period of DNA replication (Friedkin et al. 1956). In the testis of rats of more than two weeks of age, DNA synthesis and thus tritiated thymidine incorporation are confined to spermatogonia and to pre-leptotene spermatocytes (Courrot et al. 1970).

**Materials and Methods**

**a) Preparations used**

Gel filtration of ram rete testis fluid on Sephadex G100 (Franchimont et al. 1978) gives two fractions which have inhibin like activity, one of molecular weight greater than 10 000 (RTF1) and the other of molecular weight less than 5000 (RTF3). Neither fraction contains gonadal steroids as measured by radioimmunoassay or by gas chromatography.

The inhibin activity of both fractions was assessed by the inhibition of GnRH-induced FSH secretion by dispersed pituitary cells (Lee et al. 1979; Franchimont et al. 1979).

The reference preparations was derived from ovine testicular lymph (OTL) and was assigned an arbitrary potency of 1 U/mg. Inhibin standard was added to dispersed rat anterior pituitary cell cultures over a dose range of 250 to 2000 µg/ml.

The OTL standard (OTLS) and the two inhibin preparations gave parallel response lines when FSH release induced by GnRH was measured (Fig. 1). The mean specific activity of RTF1 was 80 U/mg and of RTF3 35 U/mg.

The proteinaceous nature of the substances tested was confirmed by the disappearance of biological activity following heating at 60°C for 1 h and subsequent treatment with trypsin (Franchimont et al. 1978, 1979).

Two criteria of assay specificity were used (Franchimont et al. 1979). Firstly, there was required to be a preferential inhibitory effect on FSH secretion after stimulation of pituitary cells by GnRH. In fact, more than 0.5 mg OTLS was needed to induce a reduction of LH release whilst 0.25 mg produced a significant decrease in FSH secretion. Secondly, the secretion of other pituitary hormones such as prolactin and TSH was not affected by inhibin preparations.

**b) Rats**

Intact male Sprague Dawley rats were used at the following ages and weights: 25 days, mean weight 60 g, 35 days, 120 g, 42 days, 150 g, 49 days, 200 g and 56 days, 250 g. There was a minimum of 5 animals in each group.

Another group of rats aged 42 days were hypophysectomized and treated immediately with testosterone propionate 2 mg/100 g body weight daily, divided into two im injections, so as to maintain spermatogenesis (Steinberger & Chowdhury 1974). A week later, when the animals were 49 days of age, the experiment itself was commenced.

**c) Experimental protocol in vivo**

On the first day of the experiment, the rats were given 3 ip injections, at 9 a.m., 1 p.m. and 5 p.m. either of NaCl 0.9% (control group), or preparations of inhibin either untreated or destroyed by heat and trypsin, in a dose of 100 µg/100 g body weight. On the second day of the experiment, at 6 a.m., the rats were given a further ip injection, either of NaCl 0.9% or of the same inhibin preparations, in a dose of 50 µg/100 g body weight.

This dose was chosen as it had previously been shown capable of reducing FSH levels without altering those of LH in 35 day old rats which had been castrated 24 h earlier (Franchimont et al. 1978).
Four hours later, each animal was given tritiated thymidine ip [\(^{3}H\)-thymidine: specific activity: 27 000 mCi/mM] in a dose of 100 \(\mu\)Ci/100 g body weight, diluted in 1 ml of NaCl 0.9%.

Three hours later, at 1 p.m., the animals were killed with ether and the testes were removed, one for autoradiography and the other for measurement of DNA and of the amount of tritiated thymidine which had been incorporated.

The interval of 3 h between the injection of thymidine and the sacrifice of the animals was chosen on the basis of the experiments of Messier & Leblond (1960), Courrot et al. (1970) and Clermont & Mauger (1976) who showed that maximum thymidine incorporation into spermatogonia and pre-leptotene spermatocytes occurs 1 to 3 h after the injection of tritiated thymidine.

d) **Experimental protocol in vitro**

Tritiated thymidine incorporation in vitro was examined in segments of the testes from rats aged 42 to 49 days. Once the testes had been excised, the tunica albuginea was removed and the testes were sliced so as to obtain small homogeneous pieces. The testicular tissue was placed on filter paper (Whatman n° 3) on a culture grid (60 mesh, Falcon, Cal., USA) in dishes containing the culture medium and the substances to be tested.

The culture medium was made up to DMEM (Dulbecco modified Eagle medium) without L-glutamine containing 10% horse serum, 5% foetal calf serum, 1% L-glutamine and 1% non essential amino-acids (Flow Laboratories). Tritiated thymidine was added to the medium in order to achieve an activity of 2 \(\mu\)Ci/ml. The final incubation volume was 2 ml.

Incubation was carried out at 31°C for 3 h in a controlled moist air incubator in an atmosphere of 5% CO2 and 95% air. Following the period of incubation, the testicular pieces were washed twice with 2 ml of a solution of unlabelled thymidine (Sigma Chemical Co., St. Louis, Mo) at 10 mg/100 ml in physiological saline and were centrifuged at 1200 g.

e) **Measurement of DNA and incorporation of tritiated thymidine**

The isolation and the assay of DNA were carried out according to the method of Schneider (1977), used in the study of various chalones (Verly et al. 1971). The testicular homogenate was centrifuged at 4000 r.p.m. for 10 min. The pellet was first washed twice with a solution of 10 mg/100 ml of unlabelled thymidine, twice with 10% trichloroacetic acid and finally three times with 95% ethanol. The pellet was re-suspended in 3 ml of 5% trichloroacetic acid and taken to 90°C in a water bath for 15 min. The tubes were left to cool at room temperature and were centrifuged at 4000 r.p.m. for 10 min. The supernatant was retained for radioactivity counting and measurement of DNA. 0.5 ml of the supernatant was placed in the counting vials and mixed with 10 ml of scintillation fluid. All measurements were made in duplicate.

DNA was assayed by the method of Burton (1956). 0.2 ml of the supernatant was mixed with 0.8 ml of 5% trichloroacetic acid to which was then added 2 ml of diphenylamin reagent which was prepared freshly. The reference curve was established using high molecular weight calf thymus DNA (Boehringer, Manhain). The tubes were heated at 100°C for 10 min and were then returned to room temperature. Tubes were read in a colorimeter at 600 nm using a Beckmann instrument.

f) **Histological and autoradiographic studies**

After fixation in Bouin’s fluid for 48 h, the testes were dehydrated and embedded in Paraplast\textsuperscript{®}, and then sliced at 5 \(\mu\)m.

The sections were freed from paraffin, rehydrated and covered with photographic emulsion (Ilford, liquid autoradiographic emulsion) in a dark room. The preparations were then kept in sealed black dishes at 4°C under a dry atmosphere (Silica gel) for 4 weeks; they were then developed at room temperature (Kodak Microdol) and fixed. Counterstaining with Harris haematoxylin was subsequently undertaken.

The following parameters were assessed in the preparations which had been treated as above:

a) mean tubular diameters;

b) state of spermatogenesis, defined as the percentage of seminiferous tubules containing spermatooza and/or spermatids beyond stage 12 of development (Clermont 1972); calculations of a) and b) were made on the basis of more than 240 seminiferous tubule cross-sections;

c) identification of germ cells labelled with reduced silver grains and

d) the percentage of these as compared to the total number of cells of the same type using the classification of Chowdhury (1979) which classifies spermatogonia into two groups: undifferentiated spermatogonia corresponding to types A\(_n\), A\(_1\) to A\(_4\) and differentiated spermatogonia corresponding to intermediate spermatogonia and type B.

**Results**

A. **Tritiated thymidine incorporation as a function of age**

As shown in Table 1, tritiated thymidine incorporation (expressed in DPM/mg DNA) varied as a function of age and reached a maximum in animals aged 42 days and weighing 150 g. At that age, spermatogenesis was still incomplete as only 40% of tubules contained spermatooza or advanced stages of spermatogenesis (spermatids from stage 12 to 19). The seminiferous tubules had not yet reached their maximum diameter.
In autoradiography, it was essentially the differentiated spermatogonia which had been labelled with tritiated thymidine although some pre-leptotene spermatocytes were labelled in animals aged 42 and 49 days.

B. Effects of inhibin in intact pubertal rats (42 days) and intact adult rats (56 days)

In intact 42 day old rats, the two inhibin preparations caused a significant reduction in tritiated thymidine incorporation per mg of DNA whilst the same preparations which had previously been denatured by heat and trypsin digestion produced no reduction as compared with controls (Table 2). In contrast, in adult 56 day old animals, neither preparation of inhibin at the doses used produced any significant reduction in DNA synthesis (Table 2).

In 42 day old rats, tritiated thymidine incorporation into liver DNA was not changed by treatment with RTF3 (196 ± 6.5 DPM/mg DNA \( \times 10^{-3} \)) as compared with treatment with NaCl 0.9% (184 ± 10.6 DPM/mg DNA \( \times 10^{-3} \)).

Autoradiography showed that in pubertal 42 day old animals, labelling took place in differentiated spermatogonia (intermediate and type B) and much less frequently in undifferentiated (type A) spermatogonia. Some pre-leptotene spermatocytes were also labelled. Treatment with inhibin preparations decreased the percentage of spermo-

### Table 1.
Effects of age and pubertal maturation on the incorporation of tritiated thymidine into testicular DNA.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Weight (g)</th>
<th>n</th>
<th>Percentage of seminiferous tubules containing spermatogonia and/or spermatids from stage 12 to 19</th>
<th>Seminiferous tubules diameter ( \mu m ) mean ± SEM</th>
<th>Incorporation of ([^{3}H])-thymidine into testicular DNA DPM/mg DNA ( \times 10^{-3} ) mean ± SEM</th>
<th>Percentage of labelled spermatogonia mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>60</td>
<td>9</td>
<td>0</td>
<td>120 ± 4.6</td>
<td>105 ± 5.5</td>
<td>17.4 ± 2.2</td>
</tr>
<tr>
<td>35</td>
<td>120</td>
<td>9</td>
<td>31</td>
<td>173 ± 7.35</td>
<td>89 ± 7.5</td>
<td>21.5 ± 4.5</td>
</tr>
<tr>
<td>42</td>
<td>150</td>
<td>10</td>
<td>40</td>
<td>186 ± 3.8</td>
<td>117 ± 5.03</td>
<td>26.3 ± 1.2</td>
</tr>
<tr>
<td>49</td>
<td>200</td>
<td>6</td>
<td>91</td>
<td>225 ± 6.78</td>
<td>43 ± 3.5</td>
<td>9 ± 1.4</td>
</tr>
<tr>
<td>56</td>
<td>250</td>
<td>6</td>
<td>100</td>
<td>230 ± 7.03</td>
<td>23 ± 1.7</td>
<td>3.1 ± 0.4</td>
</tr>
</tbody>
</table>

### Table 2.
Effects of inhibin preparations of DNA synthesis in vivo.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>([^{3}H])-thymidine incorporation into testicular DNA (DPM/mg DNA ( \times 10^{-3} ))</th>
<th>42 day old rats</th>
<th>56 day old rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SEM</td>
<td>n</td>
</tr>
<tr>
<td>NaCl 0.9%</td>
<td>10</td>
<td>117 ± 5.03</td>
<td>6</td>
</tr>
<tr>
<td>RTF3*</td>
<td>5</td>
<td>64.3 ± 4.8</td>
<td>6</td>
</tr>
<tr>
<td>Denatured RTF3*</td>
<td>5</td>
<td>118.2 ± 4.6</td>
<td>N.D.</td>
</tr>
<tr>
<td>RTF3*</td>
<td>5</td>
<td>53.4 ± 5.7</td>
<td>6</td>
</tr>
<tr>
<td>Denatured RTF3*</td>
<td>5</td>
<td>126.8 ± 5.9</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

*: 150 μg/100 g body weight

a: \( P < 0.01 \) compared to animals of the same age treated with NaCl 0.9%.

b: not significantly different from the values obtained in animals of the same age treated with NaCl 0.9%.

N.D.: Not determined.
**Table 3.**
Effects of inhibin preparations on the labelling of differentiated spermatogonia.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage of labelled differentiated spermatogonia (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>42 day old rats</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>NaCl 0.9%</td>
<td>10</td>
</tr>
<tr>
<td>RTF₁</td>
<td>5</td>
</tr>
<tr>
<td>Denatured RTF₁</td>
<td>5</td>
</tr>
<tr>
<td>RTF₃</td>
<td>5</td>
</tr>
<tr>
<td>Denatured RTF₃</td>
<td>5</td>
</tr>
</tbody>
</table>

*a: P < 0.01 compared with the values observed in animals treated with NaCl 0.9%.

*b: Not significantly different from the values observed in animals of the same age treated with NaCl 0.9%.

N.D.: Not determined.

gonia labelled with tritiated thymidine whilst the same preparations which had previously been denatured had no significant effect (Table 3).

In 56 day old animals, the same cells were labelled but to a much smaller degree. Inhibin treatment had no effect (Table 3).

C. Tritiated thymidine incorporation in hypophysectomized rats

The experiment itself was commenced when the rats were 49 days of age, although the growth of the hypophysectomized animals had stopped and their weight had remained at about 150 g like that of 42 days old non-operated animals. Testicular histology showed marked Leydig cell atrophy whilst the germ cells, spermatogonia to type II spermatocytes appeared to be unchanged as compared with intact 42 day old animals. Spermatogenesis had not yet developed completely and about 40% of the seminiferous tubules contained spermatids or stage 12 to stage 19, in contrast to the normal 49 day old animals, in which 91% of the seminiferous tubules showed advanced or complete spermatogenesis. Thus, it appeared that no further spermatogenesis had occurred.

No intra-tubular cell labelling was observed nei-

**Table 4.**
Effects of hypophysectomy on [3H]thymidine incorporation in 49 day old rats.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>DPM/mg DNA × 10⁻³ mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal 49 day old rats</td>
<td>10</td>
<td>43 ± 3.5a</td>
</tr>
<tr>
<td>Hypophysectomized rats* treated with NaCl 0.9%</td>
<td>10</td>
<td>2.25 ± 0.3b</td>
</tr>
<tr>
<td>Hypophysectomized rats treated with RTF₁</td>
<td>10</td>
<td>2.18 ± 0.25c</td>
</tr>
<tr>
<td>RTF₃</td>
<td></td>
<td>2.8 ± 0.4c</td>
</tr>
</tbody>
</table>

* The rats were hypophysectomized at the age of 42 days and the experiment itself began at the age of 49 days.

*a-b: Significantly different P < 0.001.

a-b: Not significantly different.
ther in animals treated with NaCl 0.9% nor with inhibin preparations.

Tritiated thymidine incorporations into testicular DNA was very low in untreated hypophysectomized animals and was not altered by pre-treatment with inhibin (Table 4).

D. Thymidine incorporation in vitro

As shown in Table 5, the inhibin preparations RTF₁ and RTF₃ produced a dose-dependent inhibition of tritiated thymidine incorporation into testicular DNA in vitro (Table 5). Thus, RTF₃ significantly inhibited thymidine incorporation at concentrations of 80 and 40 µg/ml but had no significant effect at concentrations of 20 and 10 µg/ml. RTF₁ inhibited tritiated thymidine incorporation to a greater degree as its concentration in the culture medium was increased. The preparations denatured by heat and trypsin digestion do not affect the thymidine incorporation in vitro.

<table>
<thead>
<tr>
<th>Amount added (µg) per ml of culture medium</th>
<th>RTF₁ Mean ± SEM</th>
<th>%</th>
<th>RTF₃ Mean ± SEM</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.7 ± 3.8 (6)</td>
<td>100</td>
<td>21.5 ± 0.86 (6)</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>30.9 ± 3.1 (5)</td>
<td>69⁺</td>
<td>22.3 ± 1.6 (6)</td>
<td>103</td>
</tr>
<tr>
<td>20</td>
<td>23.9 ± 2.6 (5)</td>
<td>53⁺</td>
<td>22.1 ± 1.4 (6)</td>
<td>102</td>
</tr>
<tr>
<td>40</td>
<td>21.6 ± 1.9 (5)</td>
<td>48⁺</td>
<td>17.8 ± 2 (6)</td>
<td>82⁺</td>
</tr>
<tr>
<td>80</td>
<td>12.5 ± 1.2 (6)</td>
<td>58⁺</td>
<td>20.6 ± 1.1 (6)</td>
<td>95</td>
</tr>
</tbody>
</table>

* Donor animals 42 days of age for the experiment using RTF₁ and 49 days for RTF₃.
** Denatured by heat and trypsin.
⁺ P < 0.05. ( ) Number of culture dishes.

Table 5.
Incorporation of [³H]thymidine into testicular DNA in vitro DPM/mg DNA × 10⁻³.*

Discussion

Tritiated thymidine incorporation represents DNA synthesis and is thus an index of mitotic division (Friedkin et al. 1956). When tritiated thymidine is injected 3 h prior to the determination of its specific activity, in rats more than 40 days of age, it is only the germ cells which incorporate the label (Courot et al. 1970). Under our experimental conditions, it was effectively only differentiated spermatogonia (type B and intermediate) which were labelled. If the timing between thymidine injection and sacrifice is raised to 9 or 10 h, thymidine incorporation is greater and the frequency of labelling of all types of spermatogonia and pre-leptotene spermatocytes is increased (Clermont & Mauger 1974, 1976). Sertoli cells do not take up tritiated thymidine in animals of this age as no DNA synthesis takes place in the cells of that time (Steinberger 1971; Clermont & Percy 1957; Nagy 1972; Griswold et al. 1977).

Testicular DNA synthesis is raised in pubertal animals (rats aged 25 to 42 days) and represents increased mitotic activity in seminiferous tubules corresponding to the establishment of complete spermatogenesis. When this is achieved (rats 56 days of age), tritiated thymidine incorporation is five or six times lower. The number of spermatogonia (A, B and intermediate) per seminiferous tubule cross-section is greater in 33 day old rats during puberty as compared with the adult rat (Clermont & Mauger 1976).

Two inhibin preparations were examined; both were derived from gel filtration of rete testis fluid on Sephadex G100 and were characterized by identical biological characteristics but different molecular weights (Franchimont et al. 1978, 1979). In fact, fraction RTF₁ was eluted with molecules of molecular weight greater than 10 000 and RTF₃ with molecules of molecular weight less than 5000 Daltons. However, both preparations decreased in
parallel the secretion of FSH induced by GnRH in vitro (Fig. 1), had a lesser effect on LH secretion and did not alter the secretion either of prolactin or of TSH under the same conditions. Their biological activity was destroyed when they were subjected to heat treatment and trypsin digestion. The relationship between these two forms of inhibin both of which were extracted from the same biological fluid (RTF) remains to be clarified.

The inhibin preparations at the dose of 150 μg/100 g body weight were able to reduce testicular DNA synthesis in 42 day old animals during puberty. This inhibitory effect was specific for tritiated thymidine incorporation into testicular DNA as these preparations did not produce any significant change in the specific activity of liver DNA.

The effects seen in pubertal rats could be due to the reduction of FSH secretion which gives rise to spermatogonial multiplication and which is necessary for the initiation and development of spermatogenesis (Means 1975; Sivashankar et al. 1977; Hochereau de Reviers & Courrot 1978). Furthermore, FSH receptors are found on the surface of spermatogonia (Orth & Christensen 1978).

In adult rats, inhibin preparations did not induce any reduction in testicular DNA synthesis. The reason for a lack of inhibitory effect in adult animals is not obvious. It may be postulated that the turn-over of the germ cells is insufficient to allow inhibitory effects of inhibin to be detectable. However, it must also be remembered that in adult rats spermatogenesis can be maintained in the absence of FSH almost exclusively under the influence of testosterone (Steinberger 1971; Ahmad et al. 1973).

With a view to determining whether the effect of inhibin preparations is mediated by FSH or whether there is in fact a direct effect on the testis, we examined the effect of inhibin preparations on tritiated thymidine incorporation into testicular DNA of hypophysectomized rats and in vitro.

No conclusion could be drawn from the experiment in hypophysectomized animals. Seven days after hypophysectomy, testicular DNA synthesis was considerably reduced as indicated by a lack of spermatogonial labelling measured by autoradiography and by extremely low tritiated thymidine incorporation per mg of DNA despite testosterone treatment which lead to the maintenance of a normal histological appearance in the seminiferous tubules. Thus, no significance could be attached to the fact that inhibin did not appear to exert any effect on the specific activity of testicular DNA already markedly reduced by hypophysectomy.

The experiments in vitro showed that both inhibin preparations also had direct inhibitory effects on the division of germ cells. This effect was dose-dependent. Moodbidri et al. (1980) showed that there was another direct effect of an inhibin preparation extracted from RTF: inhibition of FSH binding to its testicular receptors.

In the absence of the availability of absolutely pure inhibin preparations, it is impossible to say whether the reduction in testicular DNA synthesis in vivo and in vitro was due to the same substance or to different factors in each of the two preparations of inhibin which were studied. However, the inhibition of tritiated thymidine incorporation was undoubtedly not due to gonadal steroids as neither preparations contained these (Franchimont et al. 1978). Furthermore, it was due to proteinaceous constituents as the inhibitory property disappeared when the two inhibin preparations were treated with heat and trypic digestion.

The specific inhibitory effect on testicular DNA synthesis observed with the inhibin preparations in the pubertal rats both in vivo and in vitro should be correlated with the findings on the spermatogonial chalone described by Clermont & Mauger (1974, 1976) and Thumann & Bustos-Obregon (1978). These investigators showed that a saline extract of the normal adult testis specifically reduced tritiated thymidine incorporation into testicular DNA of growing and pubertal rats whilst it was without effect in the adult rat. Furthermore, inhibin and the testicular chalone have a number of common physical properties: they are water soluble, heat labile, precipitated by ethanol and not destroyed by lyophilisation (Thumann & Bustos-Obregon 1978; Franchimont et al. 1979).

However, the testicular chalone preparation of Clermont & Mauger (1976) appears to act on DNA synthesis in type A spermatogonia whilst our preparations were active on intermediate and type B spermatogonia. This difference in target cells could be due to the differences in the experimental conditions: Clermont & Mauger (1976) used Sherman rats at 33 days of age which were different from ours (Sprague Dawley rats at 42 days); furthermore, the interval between thymidine injection and sacrifice was 3 h in our experiments and 10 h in those of Clermont & Mauger (1976).

One could thus postulate that both substances
are the same. However, until the testicular chalone and inhibin have been isolated in a pure state and their biological, biochemical and biophysical properties established such identity can not be proved.

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References


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