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Microcalorimetry study of seminiferous tubules in vitro

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Abstract

The metabolic activity of seminiferous tubules in vitro has been determined by use of the LKB-2277 Bioactivity Monitor. The experimental results indicate that, although tubule cultures in F_{12} medium containing 20% of serum maintain higher metabolic activity than cultures in F_{12} medium alone, and the thermogenesis curve falls more slowly, the power output is also larger. The metabolic activity of seminiferous tubules in vitro is inhibited by gossypol and $CdCl_2$, the thermogenesis curves falling faster than those for a normal culture in F_{12}; the heat of metabolism is also smaller. These results for metabolic activity are consistent with the results of respirometry. Thus these results as physiogenic factors are very significant in explaining the function of inhibitors (such as gossypol, etc.) towards the spermatogenesis process and spermicide activity.

INTRODUCTION

Some chemical compounds can influence spermatogenesis; thus, in 1957 Lui Boa-Shan pointed out that the oil of cotton-seed exerts a spermicidal function [1], and in the 1960s some doctors in China widely researched this subject and indicated that prolonged ingestion of unrefined cotton-seed oil could induce male sterility. Experiments on animals have proved that gossypol (a component of cotton-seed oil) is an effective spermicidal agent. Now some contraceptive preparations containing gossypol have been used clinically.

At present, most work on the effect of physiogenic factors on spermatogenesis has concentrated on in vivo experiments in animals, but testis culture experiments in vitro have not been reported. In this work, the seminiferous tubules of male mouse (Wistar) were used for culture experiments in vitro in F_{12} medium, and corresponding experiments involved the addition of serum or some inhibitor (gossypol or $CdCl_2$) to the F_{12}. At the

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same time, the thermograms of the culture process were determined using an LKB-2277 Bioactivity Monitor, and respiratory intensity was measured with a microrespirometer. These experiments indicate that serum exerts a function in maintaining metabolism, but that gossypol and $CdCl_2$ can noticeably inhibit the metabolic processes of seminiferous tubules.

EXPERIMENTAL

Instruments and materials

Male mice (Wistar) were provided by the animal farm of the Department of Biology, Wuhan University. The F_{12} medium was produced by GIBCO.

We prepared the following mixtures: (1) F_{12} medium containing 20% of calf serum; (2) F_{12} medium containing gossypol at 10 $\mu g\ ml^{-1}$; (3) F_{12} medium containing gossypol at 40 $\mu g\ ml^{-1}$; (4) F_{12} medium containing $CdCl_2$ at 2.5 $\mu g\ ml^{-1}$; (5) F_{12} medium containing $CdCl_2$ at 10 $\mu g\ ml^{-1}$.

Calf serum was provided by Shenzhen Kuangming Biochemical Reagent Factory, Shenzhen, China. Gossypol ($C_{30}H_{30}O_8$, MW = 518.54) was obtained from Xian Oil-Chemical Factory, Xian, China. Cadmium chloride (A.R.) was produced by Shanghai Chemical Reagent Factory, Shanghai, China.

The instruments used were the LKB-2277 Bioactivity Monitor (LKB, Sweden) and the SHW-2 microrespirometer made at Shanghai University of Science and Technology, Shanghai, China.

Methods

Isolation of seminiferous tubules (S.T.)

The testis was removed (in a bacteria-free operation) from a male mouse (Wistar) and put in F_{12} medium. After removal of the membrane the seminiferous tubules were dispersed then washed with F_{12} medium; the process was repeated twice. The resulting sample was used for the experiments.

Measurement of metabolic thermogenesis

The various metabolic events which occur within organ culture processes are all reactions producing heat, and the intensity of the power output will reflect the level of metabolic activity. An LKB-2277 Bioactivity Monitor was used to record the thermograms for the metabolic processes of S.T. in vitro. The performance of this instrument and the details of its construction have been described previously [2]. In this experiment the ampoule operating mode was used, and the procedure was as follows.

(1) The monitor temperature was controlled at 32.00°C. (2) A suitable amount of S.T. sample and 1.5 ml of F_{12} medium were sealed in a 3 ml

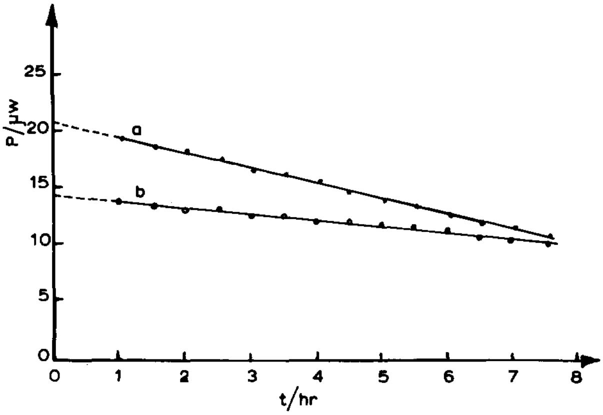

Fig. 1. Thermograms of S.T. culture in F_{12} medium in vitro at 32°C: plot a, S.T. + F_{12} medium (1.5 ml); plot b, S.T. + F_{12} medium (1.5 ml) containing 20% of serum.

glass ampoule, and the same volume of F_{12} medium was sealed in another ampoule as a reference. (3) The sample and reference ampoules were loaded into the pre-heating position, and after waiting for the appropriate time to allow thermal equilibrium to be reached and for the pen of the chart recorder to stabilize at the baseline, both sample and reference were lowered slowly (at the same time) to the measurement position. (4) Measurements were then made at the 30 μW range setting of the amplifier.

The resulting metabolic thermogenesis curves are shown in Figs. 1–3.

Measurement of the respiratory activity of S.T.

In general, the metabolism of living organs is accompanied by respiration, and oxygen will be consumed, so that measurements can be made with

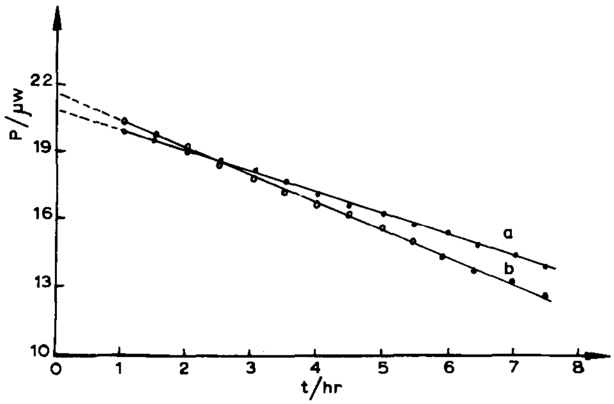

Fig. 2. Thermograms of S.T. culture in F_{12} medium containing gossypol in vitro at 32°C: plot a, S.T. + F_{12} (1.5 ml), gossypol concentration 10 μg ml^{-1}; plot b, S.T. + F_{12} (1.5 ml), gossypol concentration 40 μg ml^{-1}.

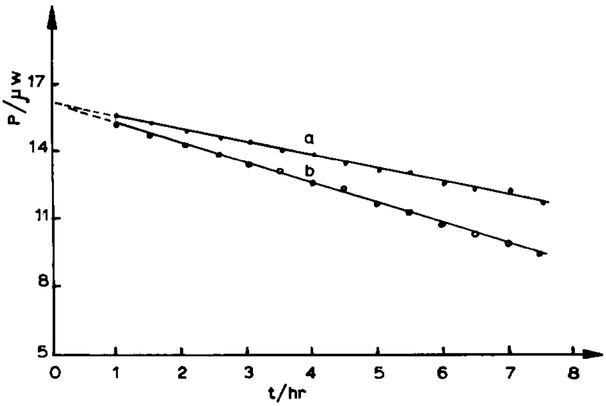

Fig. 3. Thermograms of S.T. culture in F_{12} medium containing $CdCl_2$ in vitro at 32°C: plot a, S.T.+F_{12} (1.5 ml), $CdCl_2$ concentration 2.5 μg ml^{-1}; plot b, S.T.+F_{12} (1.5 ml), $CdCl_2$ concentration 10 μg ml^{-1}.

a microrespirometer. In our experiments the SHW-2 Microrespirometer was used.

The rate of consumption of oxygen by the sample is

$$X = \frac{k \, \Delta h}{\Delta t} \tag{1}$$

where k is an apparatus constant, Δh is the difference in pressure in a given time interval ($\Delta t = 30$ min).

For the same apparatus the k value is constant, so that the ratio of consumption of oxygen both before and after the time of addition of an inhibition reagent can be calculated as

$$\frac{X_2}{X_1} = \frac{\Delta h_2}{\Delta h_1} \tag{2}$$

where Δh_1 and Δh_2 correspond to the difference in the pressures (1) before the reaction chamber solution and the side chamber solution (containing the inhibition reagent) are mixed and (2) after mixing, determined over the same interval time (30 min). We can thus use the ratio $\Delta h_2/\Delta h_1$ to evaluate the effect of inhibition on the respiratory intensity of the S.T.

RESULTS AND CONCLUSIONS

The metabolic thermograms of seminiferous tubules in vitro have been determined.

The thermogram of S.T. culture in F_{12} medium is shown in Fig. 1, plot a, and that of S.T. culture in F_{12} medium plus 20% of serum is shown in Fig.

TABLE 1

Results of metabolic power of seminiferous tubules [a]

Exp. no.	Composition of medium	Curve slope (μW h^{-1})	Max. power (μW)	Soluble peptone of sample (mg)	Max. power per unit of peptone (μW mg^{-1})
1-1	F_{12}	−1.40	21.0	0.18	117
1-2	F_{12} (1)	−0.58	14.5	0.11	132
2-1	F_{12} (2)	−0.93	21.0	0.25	86
2-2	F_{12} (3)	−1.20	21.5	0.27	80
3-1	F_{12} (4)	−0.56	16.0	0.16	100
3-2	F_{12} (5)	−0.87	16.0	0.18	86

[a] In each group of experiments the samples of S.T. were taken from separate mice.

1, plot b. The thermograms of S.T. culture in F_{12} medium plus different concentrations of gossypol are shown in Fig. 2, plots a and b. The thermograms of S.T. culture in F_{12} medium plus various concentrations of $CdCl_{12}$ are shown in Fig. 3, plots a and b.

All of these thermogenesis curves approximate to straight lines, but the slopes of the lines differ. From these thermograms we can obtain two parameters which clarify the influence of an inhibitor on metabolic activity. One is the slope of the line; the second is the intercept (P_m) when the straight line is extrapolated to the P axis (at $t = 0$), so that P_m is the maximum power output and indicates the power output at the moment the experiment began. We can then calculate

$$P_0 = P_m / W$$

where W is the weight of soluble peptone in the S.T. sample and P_0 indicates the metabolic power of unit sample. The corresponding values are shown in Table 1.

From Table 1 we can see the following.

(1) In the first group of experiments, the P_0 value of an S.T. sample cultured in F_{12} plus 20% of serum is greater than that of an S.T. sample cultured in pure F_{12} medium, and the linear slope of the thermogram (the absolute value) in experiment 1-1 is less than in experiment 1-2. This indicates that the serum has some maintainable action on the metabolic activity of S.T.

(2) In the second group of experiments, the results indicate that the slope of the thermograms increases with the concentration of gossypol in the F_{12} medium but the change in the value of P_0 is slight. From these results we can anticipate that gossypol exerts an inhibiting function on the metabolic activity but the toxicity will be slight.

(3) In the third group of experiments (nos. 3-1 and 3-2), the results similarly indicate that the slope of the thermograms increment in line with the concentration of $CdCl_2$ in the F_{12} medium, and the change in the value

TABLE 2

Respiratory suppression of seminiferous tubules by inhibitor (at 32°C)

Exp. no.	Side chamber solution (1.5 ml)	Reaction chamber solution (1.5 ml)	Δh_1 (mm)	Δh_2 (mm)	$\Delta h_2/\Delta h_1$	Average
1-1	F_{12}	F_{12}	31	30	0.97	0.99
1-2	F_{12}	F_{12}	28	28	1.00	
2-1	F_{12} (3)	F_{12}	30	25	0.83	0.825
2-2	F_{12} (3)	F_{12}	28	23	0.82	
3-1	F_{12} (5)	F_{12}	40	36	0.90	0.89
3-2	F_{12} (5)	F_{12}	35	31	0.88	

of P_0 is large; we can thus conclude that $CdCl_2$ has an inhibiting effect on metabolic activity and its toxicity is greater than that of gossypol.

Because the physiological state of each animal's testes will be slightly different, the experimental results for the three groups in Table 1 cannot be compared, but within the same group of experiments these conclusions are valid.

The results of respiratory suppression of seminiferous tubules by the inhibitors are shown in Table 2.

From Table 2, we can see that in the first group of experiments the average value of $\Delta h_2/\Delta h_1 = 0.99$, that is, approximately equal to unity. This indicates that if the F_{12} medium in the side chamber does not contain an inhibitor it will not affect the respiratory intensity of the S.T. sample when the medium is mixed with the sample solution in the reaction chamber.

However, in the second and third groups of experiments, in which the F_{12} medium in the side chamber contains the inhibitor gossypol or $CdCl_2$, the respective ratio of $\Delta h_2/\Delta h_1$ is 0.8 or 0.9. This indicates that gossypol and $CdCl_2$ have an inhibiting effect on the metabolic activity of seminiferous tubules, a conclusion consistent with that based on the results from the thermograms.

Thus, the application of these methods to determine the metabolic activity and respiratory intensity of S.T. in vitro is very significant and is useful for studies concerning the effect of inhibitors on spermatogenesis.

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