PURIFICATION OF ASPERMATOGENIC SUBSTANCE IN BULL SEMINAL VESICLE FLUID

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Damage to spermatogenesis in mice, rats, guinea-pigs, rabbits and rams after the intramuscular or subcutaneous administration of bull seminal vesicle fluid was reported by Matoušek (1966, 1969). Matoušek & Petrovská (1969) also observed an inhibitory effect of bull seminal vesicle fluid on the fertility of female mice and hens. The present report deals with the separation and purification of a substance that causes aspermatogenesis in mice.

Seminal vesicle fluid obtained from mature bulls at slaughter was diluted 2.5-fold with 2% acetic acid and mixed for 30 min at room temperature. The precipitate was separated from the supernatant fluid by centrifugation at 7000 *g* for 45 min. The aspermatogenic substance remained in the supernatant fluid. The bulk of the protein was then precipitated by 3 M-ammonium sulphate and separated from the supernatant by further centrifugation at 7000 *g* for 15 min. The aspermatogenically active supernatant was dialysed against water and freeze dried. This material was found to have a higher activity than the seminal vesicle fluid.

The freeze-dried protein material was chromatographed on a column (2 × 25 cm) of CM Sephadex C-50 (Pharmacia, Uppsala, Sweden) in 0.05 M-sodium phosphate buffer, pH 8.0, with a linear gradient of NaCl. The majority of the aspermatogenic activity occurred in a peak eluted at 0.36 M-NaCl. This protein material was dialysed, freeze dried and separated on a column (3 × 50 cm) of Sephadex G-100 (Pharmacia, Uppsala, Sweden) in 0.1 M-tris-HCl buffer, pH 7.5. The material from the large peak was found to be homogeneous chromatographically, electrophoretically (starch gel and polyacrylamide disc electrophoresis) and immunoelectrophoretically (antibodies from four rabbits injected with four to eighteen 1-ml doses of the supernatant fraction resulting from precipitation with 2% acetic acid and 3 M-ammonium sulphate—10 mg) and to possess high aspermatogenic activity, and, hence, was called the 'aspermatogenic substance' (AS).

The absorption maximum of AS was 278 to 280 nm. The AS was stained by Amido Black 10B. The molecular weight of AS was determined on Sephadex G-100 in 0.1 M-sodium acetate with 8 M-urea at pH 5.5 and on Sephadex G-75 at pH 3.5, 5.5, 7.6, 9.1 and 10.5. The results of the molecular weight determination are shown in Table 1.

The molecular weight behaviour of AS was the same before and after chromatography on Sephadex G-100 in 0.1 M-sodium acetate with 8 M-urea at

pH 5.5. On the basis of the results presented in Table 1, it would appear that changes in the molecular weight of AS are dependent on pH, so that the molecule appears as a dimer or monomer according to the pH of the solvent immediately after solution.

The presence of aspermatogenic activity in the individual fraction was tested on male mice, 0.02 ml of the dialysed and freeze-dried material (10 mg/ml 0.9% NaCl concentration) being injected into the left testis of four males. On the 10th day after injection, the mice were killed and both testes were weighed, the right testes being used as controls. The degree of damage was determined histologically by the method described by Matoušek (1969). When purified AS was used, the concentration required to produce a 50% decrease in testis weight and to abolish spermatogenesis within 10 days decreased from 10 to 1 mg/ml. Such an effect could be observed using 0.06 to 0.08 mg AS for the injection into one testis.

TABLE 1
MOLECULAR WEIGHT DETERMINATION OF ASPERMATOGENIC SUBSTANCE IN
BULL SEMINAL VESICLE FLUID

Buffer	pH	Molecular weight
Sephadex G-100		
0.1 M-sodium acetate-acetic acid+8 M-urea	5.5	22,000
Sephadex G-75		
0.1 M-citric acid-sodium phosphate	3.5	44,000
0.1 M-sodium acetate-acetic acid	5.5	44,000
0.1 M-tris-hydrochloric acid	7.6	29,000
0.05 M-boric acid+0.05 M-potassium hydroxide+0.025 M-sodium hydroxide	9.1	22,000
0.05% ammonium hydroxide	10.5	22,000

Aspermatogenesis in mice can also be provoked by subcutaneous injections of AS. By this method, it is necessary to use five daily injections totalling 2.5 to 3 mg AS per mouse. Following the injections, the mice were killed and examined on the 28th day after the first injection.

It has been found that AS also causes embryonic mortality in pregnant mice. A statistically significant decrease in the number of offspring was observed using 0.5 mg AS injected subcutaneously in 0.1 ml 0.9% NaCl per mouse. Total embryonic mortality was provoked in twenty-four mice by the injection of 2 mg AS per mouse. The AS was divided into four doses injected daily, starting on the 4th day after mating.

REFERENCES

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