New observations on the spermotoxic properties of normal rabbit serum

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'Natural' antibodies to spermatozoa have been detected in rabbit serum by a variety of techniques, summarized by Edwards (1967), and it would seem reasonable to attribute the spermotoxicity of fresh rabbit serum, described by Walsh (1925) and Chang (1947), to the activity of these antibodies in conjunction with serum complement. There have, however, been observations which do not support this hypothesis. Edwards (1960) found that the spermotoxic activity of rabbit serum, which disappears when the complement in the serum is inactivated by heating, was not restored by the addition of fresh complement (diluted guinea-pig serum). Similarly, in guinea-pigs, Spooner (1964) was unable to restore the sperm-immobilizing activity of heated guinea-pig serum by the addition of complement.

In contrast to these observations, I have found that heated rabbit serum showed pronounced spermotoxicity when used in conjunction with guinea-pig serum, in an assay based on one used by Menge (1970) to assay induced antibodies to rabbit spermatozoa.

Samples (0.25 ml) of diluted, heat-inactivated rabbit serum were incubated with 0.05 ml of a $\frac{1}{10}$ dilution of guinea-pig serum (Wellcome Reagents Ltd, Beckenham, Kent) and 0.05 ml of a suspension of ejaculated spermatozoa (30×10^6 /ml) collected, with an artificial vagina, from bucks selected for the high percentages of motile spermatozoa in their ejaculates. The ejaculates were diluted about 15 times, and centrifuged at 650 g for 5–10 min. The supernatant was discarded and the spermatozoa resuspended in fresh diluent (Dulbecco A: Oxoid). Tubes containing 0.25 ml diluent+0.05 ml complement and tubes containing 0.25 ml serum+0.05 ml heat-inactivated complement were included as controls. The tubes were incubated at 37°C for 1 hr and drops of each suspension were examined microscopically to determine the effects of the treatments on sperm motility. Nigrosin–eosin smears (Hancock, 1951) were made to determine the degree to which spermatozoa could exclude eosin. Single drops of sperm–serum suspension were mixed with equal volumes of nigrosin–eosin stain (prepared by refluxing 2 g eosin with 120 ml 10% nigrosin for 8 hr) for 5 min and drops of this suspension smeared. The spermatozoa were examined under oil-immersion.

The percentage of spermatozoa that were motile or able to exclude eosin was greatly decreased after incubation in serum+unheated complement compared with spermatozoa in the control tubes. Individual ejaculates varied in their sensitivity to a given batch of serum, but dilutions of serum from $\frac{1}{10}$ to $\frac{1}{40}$ usually reduced the percentages of motile spermatozoa to less than half those in the control tubes.

Using a procedure similar to that described by Edwards (1960), i.e. incubating 0.1 ml volumes of heated rabbit serum, complement and sperm suspension (20×10^6 /ml) for 1 hr with Dulbecco A (Oxoid) as diluent, higher percentages of eosin-stainable spermatozoa were found in suspensions containing rabbit serum+complement than in control tubes. This procedure was also tried with various sources of complement—freeze-dried guinea-pig serum supplied by Wellcome Reagents, guinea-pig serum which had been stored frozen at -80°C , or guinea-pig serum which had been absorbed with ejaculated rabbit spermatozoa in the absence of Ca^{++} and Mg^{++} ions (Boyse, Hubbard, Stockert & Lamm, 1970). Spermotoxicity of rabbit serum was obtained with all these procedures, but they were not used routinely. Spermotoxicity was observed in all sera tested from 2 male and 17 female rabbits.

I can only speculate about the reasons for the differences between my observations and those of Edwards (1960). These may have been due to slight differences in the procedures used, or differences

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Table 1. Percentages of unstained ('live') spermatozoa found when samples of spermatozoa from different ejaculates were incubated with samples of guinea-pig serum from different batches and rabbit serum or Dulbecco A

Experiment	Guinea-pig serum	Addition	Live spermatozoa (%)	95% confidence limits (%)
I*	Batch 5	Rabbit serum	64	50-77
		Dulbecco A	64	50-77
	Batch 6	Rabbit serum	24	13-38
		Dulbecco A	74	59-85
II†	Batch 7	Rabbit serum	16	7-29
		Dulbecco A	62	47-75
	Batch 5	Rabbit serum	78	64-88
		Dulbecco A	70	56-82
	Batch 1	Rabbit serum	52	37-66
		Dulbecco A	60	45-73
III‡	Batch 4	Rabbit serum	8	2-19
		Dulbecco A	78	64-88
	Batch 3	Rabbit serum	14	6-27
		Dulbecco A	44	30-59
	Batch 2	Rabbit serum	82	69-91
		Dulbecco A	74	59-85

* Sperm suspension (30×10^6 sperm./ml), 0.05 ml; guinea-pig serum (1/10), 0.05 ml; rabbit serum (1/40), 0.25 ml; Dulbecco A, 0.25 ml.

† Sperm suspension (20×10^6 sperm./ml), 0.1 ml; guinea-pig serum (1/15), 0.1 ml; rabbit serum (1/10), 0.1 ml; Dulbecco A, 0.1 ml.

‡ Sperm suspension (30×10^6 sperm./ml), 0.05 ml; guinea-pig serum (1/2.5), 0.05 ml; rabbit serum (1/10), 0.25 ml; Dulbecco A, 0.25 ml.

in the properties of the rabbit sera or complement sources. The effects described could be more readily demonstrated with some complement sources than with others (see Table 1). Where comparisons were made, the more satisfactory sources had higher haemolytic complement titres.

The spermotoxic activity of rabbit serum reported here, which is similar to the activity of mouse serum reported by Johnson & Edidin (1972), could be due to the activity of naturally occurring anti-sperm immunoglobulins, but the molecular basis for the phenomenon has not been fully worked out as only preliminary attempts have been made to isolate the spermotoxic activity of rabbit serum in a purified fraction. IgG fractions prepared by ammonium sulphate precipitation (Campbell, Garvey, Cremer & Sussdorf, 1964), absorption with DEAE Sephadex (Webb, 1972) and Sephadex gel filtration, all contained spermotoxic activity when tested using the first assay described in this communication, but the yield of activity was low, and it was not possible to conclude that the spermotoxicity of rabbit serum in this assay could be entirely attributed to IgG.

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