hyperpolarization of the nervous terminal brings about an increase of the neurotransmitter release by the nerve impulses, and an increase of the synaptic potential amplitude[11]. Miledi and Slater[10] showed that it was sufficient to hyperpolarize the membrane of the pre-axon for 8 mV, in order to elicit the spike when a normal EPSP had been depressed to subthreshold level by prolonged repetitive stimulation. These results could shed some light on the effects of NAAA on the synaptic transmission. In fact, the hyperpolarization and consequentely the increase in AP amplitude of the 'main' pre-axon, during NAAA perfusion (mean value of 14 data, 10.5±0.5 mV), presumably produced an increase of the neurotransmitter release by nerve impulses with a consequent increase in AP amplitude of the postsynaptic fibre. In addition the PSP increase in naturally fatigued preparations (figure 2) could be explained by a larger release of neurotransmitter, not only at the giant synapse but also at the proximal synapses. This activation of proximal synapses is supported: a) by the decrease of synaptic delay and shape change of the orthodromic action potential[10]; b) by a relatively smaller increase of the orthodromic AP onset after the intracellular stimulation of the 'main' pre-axon, in respect to that one recorded after the extracellular preganglionic nerve stimulation. Therefore it is suggested that N-acetyl-L-aspartic acid facilitates the neurotransmitter release by a hyperpolarization of the presynaptic fibres, and consequentely by increasing the amplitude of the action potential. As for the membrane potential hyperpolarization observed in the giant fibre system, this effect agrees with previous results described on the mammalian brain[6]. At present, the role of NAAA on the synaptic transmission in the stellate ganglion remains speculative; nevertheless from the above data it is inconsistent with the usually suggested role of NAAA as inert passive anion balancing the high intracellular cation concentration of the nerve cells[2].

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Semen electrolytes in normal and infertile subjects. II. Zinc

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Summary. Zinc levels in seminal plasma of normal subjects are compared with those of oligospermic, asthenospermic and azoospermic. A linear direct relationship seems to exist between zinc in seminal plasma and motility of spermatozoans. The possible implications of this are discussed.

The high amount of zinc present in human semen is known since 1921[2]. The importance of this element in semen is not well known. As second in the series[3], we undertook this study to explore the role of zinc in male fertility.

Material and methods. Semen samples, collected after 10 days of abstinence into clean and dry glass bottles, included 37 normal, 34 oligospermic, 9 asthenospermic and 10 azoospermic. The term 'normal' was applied to the samples which fulfilled the requisites in the routine microscopic examination for semen quality and included few from proved fertile persons. Samples with sperm count of less than 40 million/ml were considered as oligospermic and those which showed absence of sperms in deposit despite centrifugation (10,000 rpm × 10) were termed azoospermic. Samples in which sperm motility was less than 50% were grouped as asthenospermic.

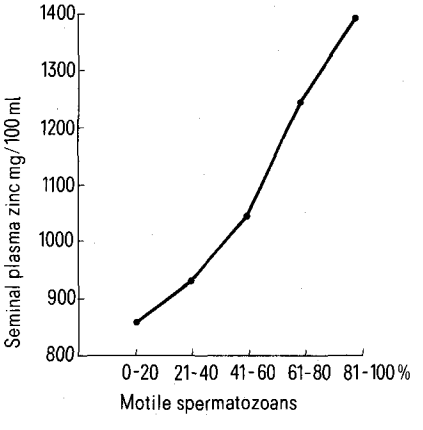
Zinc was estimated colorimetrically by the method described by Malmstrom[4]. For convenience we preferred to use iodine flask in place of separatory funnel. Vigorous shaking was done for 5 min. Organic phase was pipetted out with due care.

All necessary precautions were taken to avoid the infiltration of zinc from laboratory wares, distilled water and reagents. The whole procedure was carried out in dust free area.

Results. The motility of normal samples were fair or excellent. In asthenospermic the number of motile sperms varied from 0 to 30%. The results of the present study are given in the table. The values are plotted against the percentage of sperm motility in the figure.

Discussion. There are many reports about the high content of zinc in human semen[5–9], as well as in the semen of animals[10–14]. The values presently obtained in normal subjects (table) are lower than those reported by others[2,6,7]. Climatic difference can change the electrolyte composition of semen[15], and the racial differences and variations in food habits also might be important.

The people of this part of our country (Gujarat) are mostly



Results of the present study

	Normal (37)*	Oligospermia (34)	Asthenospermia (9)	Azoospermia (10)
Range	1030–1780	720–1740	250–750	960–1740
Mean ± SE	1413.0 ± 2.5	1205.0 ± 3.4	477.33 ± 5.1	1249.30 ± 5.7

Comparison	p-value	Remarks
Normal v/s oligospermia	$0.05 > p > 0.02$	Significant
Normal v/s azoospermia	$0.10 > p > 0.05$	Non-significant
Normal v/s asthenospermia	< 0.0001	Highly significant
Azoospermia v/s oligospermia	> 0.10	Non-significant
Azoospermia v/s asthenospermia	< 0.001	Highly significant
Oligospermia v/s asthenospermia	< 0.001	Highly significant

Values are given in mg/100 ml. *Numbers in parentheses indicate number of samples.

vegetarians and do not consume the best sources of zinc, like meat, eggs and shell fish. Milk products do contain zinc, but, here, milk is usually consumed along with lunch or dinner consisting mainly of wheat preparations. So, less zinc may be absorbed from the intestine because of formation of zinc phytate, a nonabsorbable complex with phytic acid present in wheat. This may lead to less zinc in semen. Bondani et at.[16] believed that electrolytes in seminal plasma are excretory substance and so their value may change in tune with the concentration in blood.

However, all agree that high amount of zinc present in genital tract and semen may have a prominent role to play in fertility. In oligospermia, the zinc concentration in seminal plasma is much lower than normal (table). Marmar et al.[8] reported the same. A decreased intake of zinc can lead to retarded sexual maturity[17] and may cause less release of zinc in semen. Supplementary zinc sulphate given to oligospermic patients lead to increased zinc level in semen and improved qualities of semen[8]. Hormonal stability is an important factor for normal uptake or zinc by prostate glands[18,19] and the prostate gland is believed to be the main contributor of zinc to semen.

Zinc is present in azoospermic semen (table) also. Mawson and Fischer[6] reported the same earlier in one case. This finding together with the reports about presence of zinc in post-vasectomy semen[8], in prostatic fluid[20] and in split ejaculation[5,7], tend to confirm that the main source of zinc is prostate gland. Zinc may have an important role in spermatogenesis, since its deficiency leads to aspermia in rats[21]. Marmer et al.[8] observed that less release of zinc and acid phosphatase from the prostate gland is associated with asthenospermia and teratospermia. In cases of asthenospermia the zinc level is found to be the lowest (table). In the present study a linear relationship is observed between zinc content of seminal plasma and the percentage of motile spermatozoans (figure). Can this be evidence of the influence of zinc in sperm motility?

Better sperm motility is seen in first split of ejaculation[7] which also shows high content of zinc[5,7]. Saito et al.[22] have shown increased activity of dog epidydimal sperms after addition of zinc to the medium. All the above observations seem to show a direct relation ship between the zinc content of seminal plasma and sperm motility.

Spermatozoans also contain zinc[11,12,23]. Lindholmer and Eliasson[24] found more zinc in the spermatozoans of last split of ejaculate which shows minimum motility. They[25] observed a significant decrease of zinc content in sperm 1 h after ejaculation. Fujii et al.[26] could induce motility to starfish spermatozoa, in vitro, by adding histidine to the surrounding fluid. They explained that the imidazole group of the histidine behaved as chelating agent and fetched out zinc from cells into the surrounding medium and made the cells motile. Zinc is not present in the washed sperm[27] and they are immotile[28].

All these in vitro studies seem to show that spermatozoans liberate zinc from within after ejaculation, since they gain in motility. It looks as though there is a reciprocal relation between zinc within and without the spermatozoans as far as motility is concerned. Can it lead to a new concept; the sperms release zinc to the medium while gaining motility, after maturation? Can it be presumed that a similar process occurs while they traverse through the female genital tract? Zinc is known to be a co-factor in many energy-yielding metabolic reactions. To assess the validity of these questions and to answer them, further detailed study alone can help.

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