# The Role of Zinc in the Posttesticular Antifertility Action of Monochlorhydrin[1] (36841)

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The actual mechanism of action of monochlorhydrin, a reversible antifertility agent effective in rats (1–7), guinea pigs (8), sheep (9), swine (10, 11) and monkeys (12), remains obscure, although it has been firmly established that its effect is posttesticular. While there is an interference with the absorption of water in the proximal head of the epididymis in rats (4), the early onset of sterility, within 3–5 days after the first oral administration of monochlorhydrin (2, 3, 6), pinpoints an antifertility action in the more distal epididymal tract. It has also been shown that the sperm removed from the vas deferens and cauda epididymis of monochlorhydrin-treated rats (6) and swine (11) have reduced fertilizing capacity.

In the course of investigations with $^{65}Zn$, which is taken up by developing spermatozoa within the testis (13–18), we observed that as the metal-laden sperm traversed the epididymal pathway, there was a distinctive pattern of depletion of zinc in the corpus and cauda region (16, 17). We questioned whether, in addition to interfering with water absorption in the proximal caput epididymis, monochlorhydrin might also interfere with the normal absorption of zinc from the sperm or seminal plasma during their sojurn in the more distal sperm transport pathway. To test this hypothesis, male rats treated with monochlorhydrin in doses sufficient to cause reversible antifertility, were given a single tracer dose of $^{65}Zn$ and the radioisotope uptake by the testis and various parts of the epididymis and vas deferens (as well as other tissues) in these monochlorhydrin-treated rats was compared with untreated controls.

*Materials and Methods.* A total of 30 male CD rats (Charles River Breeding Laboratories, Inc.), 14 wk of age, 400 g average body weight, were used. Fifteen rats were treated with monochlorhydrin; 15 served as untreated controls. Monochlorhydrin (3-chloro-1,2-propanediol) (sp gr 1.326) was purchased from K & K Laboratories, Inc. A 98% stock solution of monochlorhydrin (1.3 g/ml) was prepared in 0.025% aqueous methylcellulose and kept refrigerated. A working solution was made fresh weekly by diluting the stock solution approximately 1:87 with distilled water. The working solution (15 mg/ml) was kept under refrigeration and was used for daily oral administration by stomach tube to rats in volumes of 1 ml/kg. Treatment was continued for 9 days, a regimen which causes sterility but is reversible. Control groups of rats received water for 9 days by stomach tube.

*$^{65}Zn$ uptake studies.* $^{65}Zn$ (International Chemical & Nuclear Corp.) (sp act 0.267 $\mu$Ci/ug) was diluted to a concentration of 14 $\mu$Ci/ml. On day 9 of monochlorhydrin or water administration, each rat received a single 0.2 ml sc injection of radioisotope equivalent to 2.8 $\mu$Ci of $^{65}Zn$. The monochlorhydrin-treated and the untreated control rats were divided into 3 groups of 5 rats each. The groups were killed 1 hr, 1 day or 1 wk after administration of $^{65}Zn$. The following tissues were removed for determination of radioactive content: testis, caput epididymis, corpus epididymis, cauda epididymis, vas deferens, dorsolateral prostate, ventral prostate, seminal vesicles, liver, kidney and blood. Immediately after removal, the tissues were placed on tared aluminum foil and weighed. After wrapping in the foil, tissues were placed in 20-ml counting tubes and radioactivity was determined in a large well-type scintillation detector. Standards containing 1% of the administered dose of $^{65}Zn$ were always

[1] Supported by U.S. Public Health Service Research Grant HD-02999 from Nat. Inst. Child Health and Human Devel.

TABLE I. Effect of Monochlorhydrin (15 mg/kg Orally for 9 days) on $^{65}Zn$ Uptake by Various Tissues of the Male Rat.

| | Change (%) in $^{65}Zn$ uptake, increase (+) or decrease (—), in tissues of monochlorhydrin-treated rats compared with untreated controls[a] | | |
|---|---|---|---|
| | Time following $^{65}Zn$ administration | | |
| | 1 hr | 1 day | 1 week |
| Testis | — | — | — |
| Caput epid. | — | — | — |
| Corpus epid. | 57.12 (+) | 91.94 (+) | 125.63 (+) |
| Cauda epid. | 44.24 (+) | 42.89 (+) | 28.06 (+) |
| Vas deferens | 20.49 (+) | 29.44 (+) | — |
| Dorsolat. pros. | — | — | — |
| Ventral pros. | — | — | — |
| Seminal ves. | — | — | — |
| Liver | — | — | — |
| Kidney | — | — | — |
| Blood | — | — | — |

[a] Listed only if statistically significant ($p < .02$).

counted simultaneously with the tissue samples. Calculations of radioisotope concentration per gram of tissue (based on % of dose administered as indicated by the standard) were thereby automatically corrected for decay. Comparisons were made between the $^{65}Zn$ uptake of monochlorhydrin-treated rats and the untreated controls. The radioisotope uptake in tissues of monochlorhydrin-treated rat is expressed as percentage change (increase or decrease) over the untreated controls. Results are cited only if there was a statistically significant change ($p < .02$, using the method of Student's $t$ test).

*Results.* Of all the tissues examined, only the corpus and cauda epididymis and the vas deferens of monochlorhydrin-treated rats showed significant changes (increases) in $^{65}Zn$ uptake (Table I). Figure 1 shows both the increases in weight and $^{65}Zn$ concentration (1-day uptake) in the sperm transport pathway after monochlorhydrin. The increase in weight is most pronounced in caput epididymis, becoming progressively less in the corpus and cauda, with no change in the vas deferens. In contrast, the increase in $^{65}Zn$ concentration from monochlorhydrin is most pronounced in corpus epididymis, nearly double that of the untreated controls. Significant increases were also evident in cauda epididymis and vas deferens.

*Discussion.* The functions of zinc in sperm motility and fertilization are unknown, but there is considerable evidence to suggest that the role of zinc may be inhibitory, geared to hold the energy systems in check until the actual time that fertilization is to take place. The fact that the sperm of some marine forms do not become motile until their zinc concentration has been diminished (19) suggests an inactivating role for zinc in primitive forms with external means of fertilization. In mammals, sperm acquire some of their zinc during various phases of their development within the testis (13–18). Former observations show that as the zinc-laden sperm traverse the epididymis, the concentration of zinc is diminished (16, 17) suggestive of a built-in mechanism of depletion of zinc in animals with internal fertilization. At the time of ejaculation, mammalian sperm are endowed with another rich source of zinc contributed by secretions from the lateral lobes of the prostate (20–23). As the sperm traverse the female reproductive tract, the zinc concentration is gradually diminished, being absorbed into the circulation of the female, ultimately appearing in other tissues, such as the liver

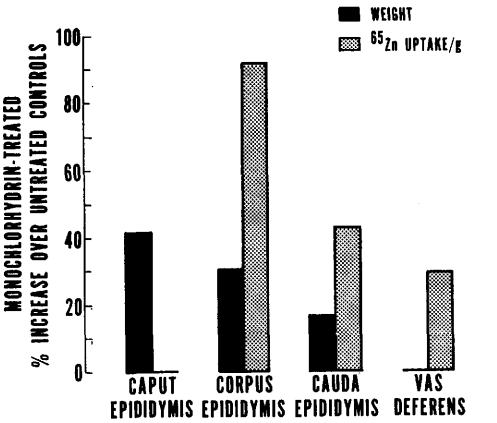


FIG. 1. Comparison of effect of monochlorhydrin (15 mg/kg orally for 9 days) on organ weight and on $^{65}Zn$ concentration in epididymis (caput, corpus and cauda) and vas deferens of the rat. Results expressed as percentage increase over untreated controls.

(24). While the function of the prostate has long been an enigma, its contribution of zinc may be another inactivator, serving to postpone the sperm's final metabolic expenditure of energy until it is needed, some hours later, in the oviducts where fertilization actually takes place.

The recent report of Eliasson, Johnson and Lindholmer (25) lends weight to our thesis that zinc in the seminal plasma may be inhibitory to sperm. The characteristically low oxygen consumption of human sperm in seminal plasma is increased 2- or 3-fold when sperm are transferred to artificial salt solution. These investigators also noted a leakage of zinc when human sperm were washed and suggested that this may be responsible for the increased respiratory activity of washed sperm compared to that in zinc-rich seminal plasma. In addition, they found that zinc inhibited the succinate-induced increase in respiration of human sperm. There have been other reports that zinc, in high concentrations, was detrimental to the motility of human sperm (26, 27). Rosado *et al.* (27) also demonstrated that zinc significantly inhibited the highly active pyruvate kinase and adenylate kinase activity of human sperm.

The mechanism of action of monochlorhydrin (3-chloro-1,2-propanediol), an orally effective antifertility agent, has not yet been established. Its chemical structure, differing from glycerol only in the substitution of a chlorine for a hydroxyl group, suggested an action as a metabolic analogue of glycerol (3, 4), interfering with the synthesis or utilization of one of the main secretory products of the epididymis, glycerylphosphorylcholine (28). Proof is still lacking for interference at this site (29, 30). The suggestion that monochlorhydrin exerted its action by selective injury to the vasculature of the caput epididymis (31), has been disproved (32).

Previous experiments from this laboratory established that monochlorhydrin caused an interference with absorptive mechanisms in the epididymis; increased tissue weight and microscopic evidence of edema were most pronounced in the head of the epididymis, the site of greatest water absorption (4). The studies reported in this paper bear out earlier predictions (4, 32, 33) that absorption of another chemical constitutent in more distal parts of the sperm transport pathway may also be altered. The increased $^{65}Zn$ uptake in monochlorhydrin-treated rats was most pronounced in the region of the corpus, the site where, in normal rats, the depletion of zinc first becomes apparent (16, 17). Under monochlorhydrin treatment, $^{65}Zn$ may be taken up normally by the sperm and seminal plasma, but its normal egress may be blocked. It is of interest that spermatozoa removed from the vas deferens of monkeys treated with monochlorhydrin have an increased $^{65}Zn$ uptake (29), an observation which came to our attention after our own experimental studies were completed.

Several investigators noted that the motility of sperm from monochlorhydrin-treated animals was decreased (3, 5, 7, 9, 10). Erickson and Bennett (7) found normal amounts of sperm in the uterus, but only a few sperm in the oviduct. Ericsson and Youngdale (2) observed that sperm congregated around the egg, but that they did not penetrate. It is known that sperm are passively pushed up the uterine tract by the contractile movements of the uterus and it is not until the sperm are in the oviducts that they actually become motile (34, 35). It is clear that monochlorhydrin causes some interference with the final motility of sperm, perhaps with the energy systems needed for the ultimate penetration of the ovum. That this inhibition of fertilization may be mediated, at least in part, through a change in the constitutents of semen of treated males was demonstrated by the following experiments of Erickson and Bennett (7). When semen was collected from the uterus of a female rat 3–4 hr after mating with a monochlorhydrin-treated male and transferred directly into the ovarian bursa of an unmated female, the spermatozoa were found to be capable of fertilization; semen from untreated males, transferred in the same manner, caused an acceleration of egg transport in the oviducts which was not observed with the semen from treated males.

In view of the accumulating evidence that zinc may be inhibitory to sperm, the increased concentration of zinc in the epidid-

ymal tract in monochlorhydrin-treated rats becomes a significant observation. The mode of action of monochlorhydrin may be in preventing the normal depletion of zinc in the spermatogenic pathway and/or allowing too high a concentration of zinc to exist in the seminal fluid, monochlorhydrin has blocked the expenditure of energy within the sperm for their final surge of motility requisite for traversing the oviducts and for the ultimate penetration of the ovum.

*Summary.* Treatment of male rats with monochlorhydrin (15 mg/kg administered orally for 9 days) caused an increased uptake of $^{65}Zn$ by the epididymis and vas deferens. In no other tissues examined was the $^{65}Zn$ uptake altered. The effect was most pronounced in corpus (92–126% increase in $^{65}Zn$ concn), with substantial increases also in cauda (44%) and vas deferens (29%), all statistically significant changes. Zinc is normally diminished as sperm traverse the epididymal pathway (16). The possibility is considered that these increased concentrations of zinc following monochlorhydrin treatment may be inhibitory, preventing sperm from attaining their final surge of motility in the oviducts requisite to penetration and fertilization of the ovum.

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Received June 26, 1972. P.S.E.B.M., 1972, Vol. 141.