[^{14}C]sitosterol-fed insects indicated that extremely little of the 72,400 dpm of recovered radioactivity was associated with cholesterol ($< 1\%$ of the total). Sterols from an insect capable of dealkylating and converting phytosterols to cholesterol would have a major portion of the radioactivity in the cholesterol fraction[3,6]. In addition, fractionation of the steryl acetates (129,000 dpm total) from [^{14}C]desmosterol-fed insects by argentation chromatography provided no evidence for conversion of [^{14}C]desmosterol to [^{14}C]cholesterol. There was no detectable radioactivity associated with cholesterol acetate in these fractions, and insects that are capable of dealkylating readily convert desmosterol, the terminal intermediate in this pathway, to cholesterol[3,6]. It does appear that some selective uptake of the endogenous cholesterol in the diet occurred, as there was a higher percentage of cholesterol in the sterol samples from both insect samples than was found in the sterols from sitosterol-fortified medium. Selective uptake and lack of dealkylation of phytosterols were reported from studies using unlabeled sterols in which GLC analyses were done with several cactophilic *Drosophila* species by Kircher et al.[14]. The somewhat higher level of cholesterol in the sterols from [^{14}C]desmosterol-fed insects in our study is probably a reflection of the inability of the insect to utilize desmosterol as well as sitosterol and the overall inferior growth and development on desmosterol. Kircher et al. also reported poor development of several cactophilic species of *Drosophila* as well as *D. melanogaster* fed desmosterol-coated diets in growth studies using unlabeled sterols[15]. Apparently, with respect to dietary sterol utilization and metabolism, *D. melanogaster* is more similar to the house fly, *Musca domestica*, a more advanced member of the order Diptera, (which is unable to dealkylate phytosterols)[16] than it is to *Aedes aegypti*, a more primitive dipteran (which can dealkylate phytosterols)[6]. However, in agreement with Cook and Sang[7], sitosterol supported growth and development of *D. melanogaster* somewhat better than cholesterol in comparative studies that we conducted. This is contrary to what would be expected in an insect that cannot convert phytosterols to cholesterol. Perhaps, the very small amount of endogenous cholesterol in the diet components is adequate for specific needs such as ecdysone (molting hormone) synthesis to provide a sparing situation, or, alternatively, C_{28} or C_{29} phytosterols may serve as precursors for other ecdysteroids. Also, sitosterol must be taken up and/or incorporated as a membrane component more efficiently than is cholesterol in this insect. It is noteworthy that in vitro studies on ecdysteroids produced by *D. melanogaster* ring glands using high pressure liquid chromatography and radioimmunoassay analyses to determine ecdysteroid profiles showed variations in ecdysteroids secreted that correlated with different dietary sterols[17]. It will be of interest to examine the relative utilization of several radiolabeled C_{27}, C_{28}, and C_{29} dietary sterols as precursors of ecdysteroids in *D. melanogaster*.

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Toxic effects of methylmercury on spermatozoa in vitro

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Summary. In an in vitro investigation, methylmercury (MeHg) reduced the motility of rat spermatozoa probably by the inhibition of succinate dehydrogenase and ATPase activities. Concomitant morphological changes observed in the spermatozoa were coiled tails and kinks in midpiece and tail regions.
Key words. Methylmercury; sperm motility; enzymes; morphology; rats.

Methylmercury (MeHg) is reported to cause reproductive dysfunction in occupationally exposed men[1]. Recently, Mohamed at al.[2,3] investigated the toxic effects of this agent on ejaculated sperms in monkeys. However, the effects of MeHg on sperm enzymes and their relation to the inhibition of sperm motility is not understood. Hence, this study was undertaken on rat epididymal spermatozoa.

Healthy, adult male rats of the Charles Foster strain were used for the experiments. They were caged in an air-conditioned animal house and were fed with a standard diet and water ad libitum. Male rats of proven fertility were autopsied and the cauda epididymis was used for this study. An in vitro study on the epididymal sperm motility was performed, essentially following the method of Rao[4]. Aliquots of each sperm sample, containing 6–7 million sperms, were incubated with 1, 5, 10 and 20 ppm MeHg dissolved in distilled water, and the sperm motility was assessed[5] at 5- and 10-min intervals. After 1-h incubation, the sperm samples were washed twice with Krebs original Ringer phosphate buffer, pH 7.2 (100 ml 0.154 M NaCl; 4 ml 0.154 M KCl; 3 ml 0.11 M $CaCl_2$; 1 ml 0.154 M KH_2PO_4; 1 ml 0.154 M $MgSO_4$; and 21 ml 0.1 M Na_2HPO_4, pH 7.4) and used for the estimations of protein[6], succinate dehydrogenase (SDH)[7] and ATPase[8] activities. The sperm morphology was examined following the technique of Mann and Lutwak-Mann[9]. The data were statistically analyzed using Student's t-test.

Addition of 5–20 ppm MeHg to aliquots of sperm suspension resulted in a concentration-related inhibition of sperm motility. An instantaneous immobilization of sperm occurred in the samples containing 20 ppm MeHg, whereas at least 5-min incubation was necessary at 5 and 10 ppm (table 1). These data show that MeHg has toxic effects on spermatozoa. It is known that organic mercury compounds can bind to protein -SH groups located on membranes that encapsulate the nucleus, midpiece and tail as well as the flagellar matrix and outer longitudinal fibers of spermatozoa[9]. MeHg is also known to inhibit microtubular assembly by reacting with -SH groups on microtubules[10].

Table 1. Percentage motility of the cauda epididymal sperms in samples incubated with MeHg

Time (min)	Control	1 (ppm)	5	10	20
0*	85 ± 10	80 ± 3.1	50 ± 6.2	25 ± 0.7	0
5	82 ± 12	78 ± 3.9	34 ± 2.1**	15 ± 0.9 $^+$	
10	75 ± 10	74 ± 13	27 ± 1.3**	12 ± 1.5 $^+$	
15	71 ± 10	72 ± 8.2	10 ± 1.2 $^+$	5 ± 0.8 $^+$	
20	69 ± 5.2	67 ± 2.8	6 ± 2.7 $^+$	0	
25	61 ± 6.1	60 ± 4.2	3 ± 0.5 $^+$		
30	61 ± 4.8	58 ± 4.1	0		
40	59 ± 3.8	57 ± 1.2			
50	55 ± 1.8	50 ± 2.5			
60	50 ± 4.6	47 ± 3.9			

Values are mean ± SE of 10 observations; *Motility was determined immediately after addition of MeHg; **$p < 0.01$; $^+ p < 0.001$.

Table 2. Sperm enzymes and protein levels in samples incubated with MeHg

Dose (ppm)	Protein (mg/ml sperm suspension)	SDH (µg formazan formed/15 min/ml)	ATPase (µmoles of i.p. released/h/ml)
Control	0.51 ± 0.2	21 ± 1.7	0.50 ± 0.06
1	0.52 ± 0.2	19 ± 0.6	0.51 ± 0.11
5	0.53 ± 0.1	19 ± 0.8	0.52 ± 0.10
10	0.53 ± 0.3	12 ± 0.2*	0.40 ± 0.05*
20	0.49 ± 0.6	8.5 ± 0.3 $^+$	0.23 ± 0.04 $^+$

Values are mean ± SE of 10 observations; *$p < 0.05$; $^+ p < 0.01$.

It is also possible for -SH dependent enzymes that are involved in sperm respiration and motility to be affected; in this study, there was a reduction in the activities of SDH and ATPase in the presence of MeHg at 10 and 20 ppm levels, indicating alterations in sperm energy metabolism (table 2). Mohamed et al.[2,3] investigated the interference of MeHg with dynein ATPase activity in monkey sperms, leading to a decrease in spermatozoal motility. This enzyme is of primary importance in the transduction of chemical energy provided by ATP hydrolysis into mechanical energy for flagellar motility[9,11]. Hence, the decline in the sperm ATPase activity in the present study is related to loss of sperm motility at higher concentrations. Webb et al.[12] also reported that MeHg and other mercurials inhibit or stimulate the activity of mitochondrial and myosin ATPase depending on the dose and experimental conditions. In this study, we observed a reduction in the SDH activity in sperm aliquots treated with MeHg, which indicated that the oxidative metabolism of sperms was also affected. This is in contrast to the results of Mohamed et al.[3], who could not find MeHg-induced alterations in mitochondrial function of sperms in monkeys, and, moreover, observed an increased oxygen consumption rate as a result of MeHg addition to the ejaculated sperm samples. The discrepancy between these results is probably due to the difference in animal species, test system and experimental conditions. The observation of an inhibition of oxidative phosphorylation and of electron transport by MeHg in human liver mitochondria[13] is in agreement with our data. The appearance of coiled tails and kinks in the midpiece and tail regions of sperms at higher dose levels was also evident and these data are comparable with those of others[3]. Thus, it is concluded that the sperm immobilization effect of MeHg is correlated with alterations in the energy pool and the morphology of spermatozoa.

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Calcium release from frog sarcoplasmic reticulum by an imidazolyl reagent

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Summary. Calcium is released from the isolated heavy sarcoplasmic reticulum (SR) of frog skeletal muscle upon application of 0.1–1 mM diethylpyrocarbonate (DEP, an imidazolyl reagent). The Ca-ATPase activity of SR was suppressed by 20% in the presence of 1 mM DEP. More than 1 mM of free magnesium ion or 5 μM ruthenium red eliminated the effect of DEP on calcium release but not on Ca-ATPase activity. A plausible site of DEP action is on the calcium channel.
Key words. Fragmented sarcoplasmic reticulum; diethylpyrocarbonate; calcium release; Ca-ATPase.

Excitation-contraction coupling in skeletal muscle is accompanied by a rapid release of calcium from the sarcoplasmic reticulum (SR), in response to depolarization of the transverse tubular membrane. However, the investigation of molecular mechanisms involved in release of calcium from the SR requires further attention. Several methods are used to induce the release of calcium from the SR[1–3]. One is to chemically modify calcium channel proteins on the SR. Much attention has recently focused on the sulfhydryl group[4–7]. Sulfhydryl reagents appear to release calcium at lower concentrations than those that inhibit Ca-ATPase. On the other hand, other reagents, such as methionyl, imidazolyl, carboxyl, guanidyl or amino reagents can induce contraction of skinned fibers; an imidazolyl reagent, diethylpyrocarbonate (DEP), was most effective[8]. A clarification of the site of action would help in understanding the calcium release mechanism. We have now obtained evidence which suggests that DEP releases calcium by binding to the calcium release channel or to a regulatory structure of the channel of the heavy fraction of the SR, quite independently of the effect on Ca-ATPase.

Materials and methods

Fragmented heavy SR was prepared from leg muscle of *Rana catesbeiana* by the method of Koshita and Hotta[9]. The muscle was homogenized in a blender for 10 s in three volumes of 5 mM PIPES/Tris buffer (pH 6.8) 3 times with 40-s intervals, and then centrifuged for 1 h at 4000 × g. The supernatant was filtered through gauze and was centrifuged for 1 h at 10,000 × g. The pellet was suspended in 50 mM KCl, 20 mM Tris/maleate (pH 6.8), and recentrifuged for 10 min at 5000 × g. The pellet obtained by centrifugation for 60 min at 30,000 × g was stored in a small amount of 50 mM KCl, 20 mM Tris/maleate (pH 6.8) at − 70 °C. The protein concentration of the sample was determined by the Biuret method, standardized against bovine serum albumin.

Extravesicular calcium was monitored using a calcium selective electrode prepared by the method of Nakamura et al.[10], with some modification. A Pipetman polyethylene tip for 1–200 μl was dipped in the sensor cocktail (8.8% ETH1001, 0.88% sodium tetraphenylborate, 78.32% o-nitrophenyloctylether, 12% polyvinylchloride (n = 1000)). The sensor column was overlaid with an internal solution containing 100 mM KCl, 5 mM $CaCl_2$, 5 mM EGTA, 5 mM $MgCl_2$, and 20 mM MOPS (pH 7). The reference electrode of a glass pipette was filled with 1.5% agar in the internal solution, without $CaCl_2$ and EGTA. Electrical signals were fed to a high impedance differential amplifier (MEZ 7101, Nihon Kohden) and displayed on a conventional recorder. The calcium electrode gave a slope of 27–30 mV per pCa unit in a calibration solution containing calcium-EGTA between pCa 2 and 6.4 at experimental temperature (25–27 °C).

Solutions were sequentially poured into the chamber: 374 μl of base solution, 25 μl of 1 mM $CaCl_2$, 50 μl of 15 mg protein/ml SR, 6 μl of 0.5 M phosphoenolpyruvate (PEP), 5 μl of 1000 U pyruvate kinase, 20 μl of 0.1 M ATP, and 20 μl of several concentrations of DEP. The final concentration was 1.5 mg protein/ml SR,