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Effect of Diisopropyl Fluorophosphate on Gastric Secretion and Gastric ATPase.* (30630)

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Diisopropyl fluorophosphate (DFP) was first used as an inhibitor of cholinesterase(1). Since that time its inhibitory action has been extended to several other enzyme systems(2). In general, its mechanism of action is to form a diisopropyl phospho derivative of the enzyme, presumably at or near the active site. In many instances this combination has been shown to occur at a serine residue forming a diisopropyl phosphoryl serine derivative(3). Recently, Hokin and Yoda(4) described the inhibition of kidney ATPase (Na^+ and K^+ dependent) due to the phosphorylation of the serine residue. With this in mind, the effect of diisopropyl fluorophosphate on acid secretion and on the gastric ATPase was tested.

Methods. Rana pipiens were decapitated and the stomach was removed. The muscle layer was stripped off and the membrane was mounted into lucite chambers as previously described(5). Potential difference was measured using a vacuum tube voltmeter and short circuit current was defined as the current required to reduce the potential difference to zero after about 2 minutes. Resistance was calculated from the change of potential difference obtained by sending 10 microamps of current in either direction. Acid secretion was measured using the pH stat method of Durbin and Heinz(6). The nutrient solutions contained Na^+ 118 mM, K^+ 4 mM, Mg^{++} 0.8 mM, Ca^{++} 1.7 mM, HCO_3^- 18 mM, Cl^- 109 mM, glucose 10 mM, and the secretory solutions contained Na^+ 105 mM, K^+ 4 mM, and Cl^- 109 mM. For Cl^--free experiments, sulfate was substituted for chloride. DFP was added to the nutrient or secretory side and usually at 2×10^{-2} M final concentration.

Enzyme preparations were obtained by placing mucosae in 0.25 M sucrose, 0.1 M tris-HCl at pH 7.4 and 0°C. After scraping off the mucosal cells with the edge of a glass slide, the standard homogenization and fractionation techniques of Schneider and Hogeboom(7) were carried out and the microsomal fraction was retained for further study. In experiments where the effects of DFP on the intact tissue were studied, the enzyme was prepared from tissue in which DFP had been added on the nutrient or on the secretory side and incubated for 30 minutes or one hour. The protein analysis was performed by Lowry method(8), and enzyme activities were expressed per mg protein. For direct inhibition studies of microsomal enzymes, the microsomal fraction was incubated with 10^{-2} M DFP for varying times and the DFP was removed by centrifugation. A second washing was performed and the enzyme was resuspended in sucrose for assay. The ATPase system contained 3 mM ATP, 3 mM $MgCl_2$, 20 mM tris-HCl buffer pH 8.4; incubations were carried out for 20 minutes at 37° and results expressed as μM Pi/mg protein/hr.

Results. From Table I it can be seen that 2×10^{-2} M DFP within 30 minutes inhibited acid secretion, P.D., short circuit current and increased the resistance of the frog gastric mucosa. In Fig. 1, the time course

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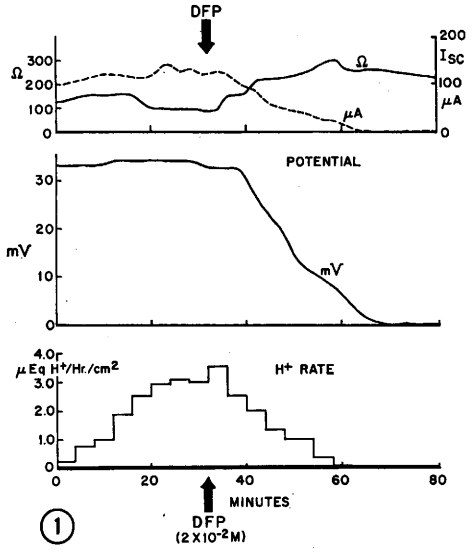
TABLE I. Effect of DFP on Secretory Parameters of the *in vitro* Frog Gastric Mucosa.

	No. of exp	H^+, μEq/hr/cm^2	Potential, mV	Resistance, ohms cm^2	Short circuit current, μa cm^{-2}
Normal membrane	7	2.11 ± .23	24.3 ± 2.2	172.1 ± 23	82.8 ± 9.8
30 min after adding DFP (2×10^{-2})	7	.2 ± .20	1.3 ± 1.2	236.4 ± 39	1.4 ± 3.3
Change	—	-1.90*	-23.02*	+64.3*	-81.4*

*P <.01 by t test.

of these effects can be seen. The P.D. with this inhibitor fell clearly to zero as did acid rate. In occasional experiments although the resistance rose initially, the final resistance was lower. There were no transient rises in the P.D., showing that probably both Cl^- and H^+ mechanisms were inhibited simultaneously. When DFP was added to the secretory side, inhibition was also obtained, with the same change in the parameters. In sulfate solutions (Fig. 2) the inverted P.D. was also reduced to zero and in contrast to many other inhibitors (*e.g.*, dinitrophenol(9)) the straight line obtained in the plot of P.D. *versus* acid secretion intersected the P.D. axis at zero. DFP, therefore, apparently inhibits all active transport in the gastric mucosa (Table I).

DFP was shown to inhibit irreversibly the ATPase of the frog gastric mucosa at 2×10^{-2} M concentration and approximately 50% inhibition was obtained after preincubation. The time course of this inhibition is shown in Fig. 3. In contrast to Hokin and Yoda's results on kidney ATPase, this inhibition was not Mg^{++} dependent nor was it antagonized by excess ATP, or K^+ activated. When DFP was placed on the nutrient or secretory side and the microsomal fraction prepared from the mucosal cell, there was clear inhibition even after 30 minutes incubation (Table II),



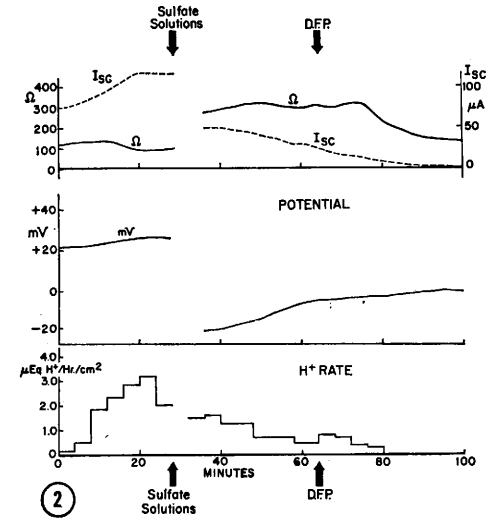


FIG. 1. Effect of DFP on secretory parameters of the frog gastric mucosa in Cl^- solutions.

FIG. 2. Effect of DFP on secretory parameters of the *in vitro* frog gastric mucosa in SO_4^{--} solutions.

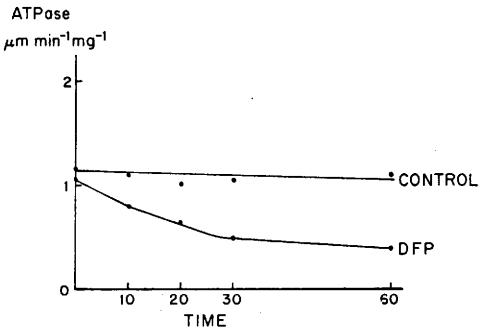


FIG. 3. Time course of inhibition of frog gastric microsomal ATPase by DFP.

TABLE II. ATPase Activity (μm Pi mg^{-1} hr^{-1}).

	Control membrane	DFP-treated membrane
1.	1.56	.71
2.	1.42	.68
3.	1.18	.91
4.	1.27	.89
5.	1.34	.54
	Control microsomes	DFP microsomes
1.	1.87	1.01
2.	1.71	1.00
3.	1.11	.72
4.	1.31	.83
5.	1.20	.88

ATPase activity of microsomal fraction from intact membrane treated with 2×10^{-2} M DFP for 30 min and ATPase activity of microsomal fraction preincubated with 2×10^{-2} M DFP for 30 min compared with control activity (5 typical experiments in each group).

This inhibition is also apparently irreversible. Various compounds which have been observed to reactivate DFP treated cholinesterase(9) were used in an attempt to show a similar reactivation for the microsomal ATPase. Thus 1, 1′ trimethylene bis (hydroxyiminomethyl) pyridinium bromide, pyridine-2-aldoxime methiodide and pyridine-2-aldoxime were added to the inhibited *in vitro* preparation. No reactivation of transport was observed. In the microsomal ATPase preparation, no reactivation was found by any of these compounds. From the time course of inhibition, it would appear that there is an initial site of action of DFP, different from the irreversible site.

Discussion. It appears from previous work that DFP has significant inhibitory actions on the 'transport ATPase' of rat kidney and rat brain. In this paper it has been shown that DFP inhibits the gastric musocal transport of H and Cl ions at 2×10^{-2} M concentration. This inhibition is irreversible, and since the resistance of the mucosa rises and then shows a terminal fall, it seems clear that there is a two stage effect. In terms of the enzymes known to be sensitive to DFP, none of these, except perhaps phosphoglucomutase could account for the observed transport inhibition. The frog microsomal ATPase has heretofore not been directly implicated in mucosal transport. However, since inhibition is observed with DFP both in the fractionated and intact system, it appears likely that at least some of the inhibitory action of DFP is due to irreversible inhibition of the ATPase. In most experiments, the maximal inhibition of the ATPase observed was about 50%. Since there is apparently no Na^+ and K^+ dependent activity of the ATPase in the frog gastric mucosa(10), and in kidney DFP specifically inhibits the Na^+ and K^+ ATPase, without affecting the non-transport ATPase (4), the significance of the DFP sensitive fraction of the frog ATPase is obscure. In analogy to other transport ATPases sensitive to DFP, however, a possible conclusion is that about 50% of the frog gastric microsomal ATPase activity is related to transport of H^+ or Cl^-. DFP, therefore, might be a useful tool in eliciting information about the active site of this enzyme, as has been done for cholinesterase.

Summary. DFP has been shown to inhibit the transport of H and Cl ions in frog gastric mucosa. It has also been shown that the microsomal ATPase is inactivated by DFP both in fractionated and intact tissue.

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