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DDT inhibition of (Na+, K+)-activated ATPase in gill tissue of Fundulus heteroclitus

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DDT Inhibition of (Na^+, K^+) - activated ATPase in Gill Tissue of *Fundulus heteroclitus*

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ABSTRACT

The DDT inhibition of (Na^+, K^+) - activated ATPase in gill preparations of the euryhaline teleost, Fundulus heteroclitus, was measured. The (Na^+, K^+) -activated ATPase was found to be sensitive to DDT, with 74.6% of the enzyme's specific activity inhibited by 20 ppm DDT. The (Mg^{++})-activated ATPase was also sensitive to inhibition by DDT but not to the extent of the (Na^+, K^+) -activated ATPase. Due to previous work which showed that DDT antagonized valinomycin-induced K^+ conductance in a synthetic membrane, the effect of K^+ on DDT inhibition was examined kinetically. The K_m for K^+ in gill preparations treated with 5 ppm DDT was greater than the K_m of the control, indicating that DDT inhibition of (Na^+, K^+)-activated ATPase involves alteration of the affinity of the enzyme for K^+.

INTRODUCTION

The principle pesticide in use worldwide is DDT [2, 2-bis (p-chlorophenyl) 1, 1, 1-trichloroethane] although little is known about the biochemical mechanisms of its toxic action (O'Brien, 1967). Whereas it is generally agreed that its toxic effects are neural in vertebrates and invertebrates, DDT may affect other systems.

One system which may be especially sensitive to inhibition by DDT is the active transport system of cell membranes. Janicki and Kinter (1967) found that DDT impaired fluid absorption by eel (Anguilla rostrata) intestinal mucosa. They proposed that the extreme toxicity of DDT in teleosts involves an impairment of osmoregulation, specifically an inhibition of the active transport of cations by (Na^+, K^+)-activated ATPase, and they believe this inhibition is a general property of organochlorine pesticides. Their proposal is supported by preliminary studies which indicated that DDT affects (Mg^{++})-activated ATPase and (Na^+, K^+)-activated ATPase in the gills of winter flounder (Janicki and Kinter, 1970).

Of the vertebrates, fish are the most susceptible

to organochlorine pesticides, including DDT (O'Brien, 1967). In "minnows" the lethal dose of DDT for 50% of the population in twenty-four hours (LD_{50}) is 34 ppm in the water as compared to an oral LD_{50} for rats of 113 ppb DDT in their food (O'Brien, 1967). The susceptibility of teleosts to organochlorine pesticides may be the result of their ability to accumulate these compounds from the water or it may be an innate reaction (O'Brien, 1967), perhaps due to the disruption of osmoregulation caused by inhibition of (Na^+, K^+)-activated ATPase as suggested by Janicki and Kinter (1971).

A possible clue to the mechanism of DDT inhibition of (Na^+, K^+)-activated ATPase is offered by Hilton and O'Brien (1970) who found that DDT antagonized the valinomycin-induced transport of K^+ across a synthetic lecithin membrane. They postulate that valinomycin acts by forming a hole, 4 Å in diameter, in the membrane or by forming a complex with K^+ to transport it through the membrane. As DDT antagonized this valinomycin-induced K^+ conductance in the model system, they further suggest that this phenomenon could be related to the decrease in K^+ efflux in axons treated with DDT. If the (Na^+, K^+)-activated ATPase provides a similar ionic hole or site in a hydrophobic environment of the membrane allowing the K^+ to pass through the membrane,

then DDT may inhibit ATPase activity by interfering with this K^+ binding site.

It is premature to explain the mechanism of DDT toxicity in terms of its effect on the energy requiring "sodium-potassium pump" or K^+ transport. However, there is sufficient evidence to warrant further investigation. Therefore, an attempt was made to determine if DDT inhibited (Na^+, K^+)-activated ATPase in gill tissue preparations of the euryhaline teleost, Fundulus heteroclitus (Linnaeus). Secondly, a kinetic study was made to assess the effect of DDT on the apparent affinity of the ATPase for K^+.

LITERATURE REVIEW

As early as 1945, Lauger, et al. had demonstrated that cholesterol and membrane lipids have a high affinity for DDT, suggesting that DDT acts on the nerve membrane by complexing with the nerve cell lipids in some unknown way. Further research has attempted to define a possible DDT-membrane complex in terms of steric hindrance (Mullins, 1955 and Rogers, 1953), electronic bonding of the van der

Waals type (Gunther, 1957) and charge transfer due to DDT's high affinity for electrons (Matsumura and O'Brien, 1966)

Sternburg and Kearns (1952) offered a theory to explain the toxicity of DDT based on the presence of a toxin in DDT-treated animals. They believe that this DDT-induced toxin rather than DDT itself acts on the neurons. This theory is an extension of an older "stress" theory which stated that DDT caused death "due to exhaustion" with the exhaustion resulting from excessive activity in the nerves. This DDT-induced stress factor has been structurally identified in only one study as L-leucine (Tashiro, et al., 1972), and it is detectable only after prolonged treatment with DDT (O'Brien, 1967). With such limited evidence, this theory is not acceptable as an explanation for the presence of the primary lesion thought to be caused by membrane destablization (O'Brien, 1967).

Matsumura and Patil (1969) found that DDT inhibited (Na^+, K^+)-activated ATPase of membranes in the nerve ending fraction of rat brain tissue. On the basis of their work, they believe that the toxicity of DDT may result from it ability to inhibit the Na^+ and K^+ transport system in the nerves. Akera, et al. (1971) found that there is a

direct correlation between DDT inhibition of (Na^+, K^+)-activated ATPase activity and temperature *in vitro*. As (Na^+, K^+)-activated ATPase has a reduced activity at lower temperatures, the inhibition by DDT would apparently decrease at lower temperatures.

Koch (1969, 1970) and Cutkomp, *et al*. (1971) present evidence which appears to conflict with the preceding, finding that the primary effect of DDT was on the (Mg^{++})-activated ATPase system rather than the (Na^+, K^+)-activated ATPase system. It is not possible to explain these apparently conflicting data at this time, but it should be noted that neither Koch (1969, 1970) nor Cutkomp, *et al*. (1971) examined the tissues used in preceeding studies and the study described here.

A (Na^+, K^+)-activated ATPase is found in nearly all cell membranes, and its function is to transport Na^+ and K^+ across the membranes (Skou, 1965). The activity of the (Na^+, K^+)-activated ATPase is high in marine teleost gill tissue where it plays an important role in the transport of Na^+ across the epithelial membranes and, therefore, in the adjustment of these animals to a marine environment (Jampol and Epstein, 1970 and Katz and Epstein, 1968). This "salt pump" is defined by Post (1968) as"a membrane transport

system that is stoichiometrically coupled to the breakdown of an intermediate." The (Na^+, K^+)-activated ATPase transports three Na^+out of the cell and two K^+into the cell and splits the terminal phosphate bond in adenosine triphosphate (ATP) in the presence of Mg^{++} (Post, 1968).

Skou (1965) characterizes the (Na^+, K^+)-activated ATPase as follows:

(1) It is located in the cell membrane.

(2) A site with a high affinity for Na^+ on the inside is coupled with a site with a high affinity for K^+ on the outside of the intact membrane.

(3) An organized lipoprotein complex is necessary for the specificity of Na^+ and K^+.

(4) Cardiac glycosides such as ouabain inhibit ATPase activity.

A variety of membrane preparations and approaches have been used to explore the mechanism of the ATPase reaction. However, little is known at this time concerning that mechanism (Towle, 1970). The first step in the reaction is known to involve a phosphorylated intermediate that requires Mg^{++} and Na^+ with ATP supplying the phosphate. Then K^+ stimulates the breakdown of the phosphorylated intermediate,

releasing orthophosphate (P_i). Whereas the requirement for Na^+ is absolute (Post, 1968); other monovalent cations can substitute for K^+ (Skou, 1965). Ouabain and other cardiac glycosides inhibit (Na^+, K^+)-activated ATPase, perhaps by stabilizing the phosphorylated enzyme complex (Towle, 1970). It is believed that the phosphate group in the intermediate is attached to the carboxyl group of an amino acid in the protein, presumably a glutamyl residue, and the mechanism might involve a shift of this phosphate along different amino acid residues to a site where K^+ activates the hydrolysis of the phosphate (Robinson, 1971).

The kinetic evidence indicates that there are two sites for Na^+ and K^+ inside and outside the cell membrane, respectively, with different affinities for each (Somogyi, 1968), and these sites are separate from the active site of the enzyme (Skou, 1965). The Na^+ site can be competitively inhibited by high concentrations of K^+ (Skou, 1965). It is thought that ATP, Na^+ and K^+ cause a conformational change in the enzyme, providing a means for the possible rotation of the active center (Somogyi, 1968 and Koyal, et al., 1971). In this way K^+ moves into the cell and Na^+ moves out (Somogyi, 1968).

In trying to determine the nature of the

(Na^+, K^+)-activated ATPase enzyme system, it has been found that an organized phospholipid structure is essential for activity (Skou, 1965). However, it is not yet clear whether this phospholipid is an integral part of the ATPase system (Towle, 1970). As DDT is extremely apolar (O'Brien, 1967), the phospholipid associated with the ATPase system or other membrane lipids would provide a hydrophobic site for DDT attachment.

METHODS and MATERIALS

Adult female mummichogs, F. heteroclitus, were collected on January 1, 1972, from Mobjack Bay at Severn, Virginia. They were maintained at 18° to 20° C in filtered water, 24 ppt salinity, obtained at the collecting site.

A modified procedure of Janicki and Kinter (1971) was used to measure the (Na^+, K^+)-activated ATPase and (Mg^{++}) activated ATPase activities in mummichog gill preparations. Each test was done in triplicate, and each concentration of DDT was used at least three times.

After decapitation, the gills were excised and

the tissue removed from the cartilaginous arches. The gill tissue of two to four fish was homogenized in a Potter-Elvehjem homogenizer with a smooth Teflon pestle in a mixture of 0.25 M sucrose, 0.005 M disodium ethylenediamine tetraacetic acid (EDTA), and 0.30 M imidazole-HCL (pH 7.4), making a 2% (w/v) homogenate.

The homogenate was centrifuged at 10,000 x g at 0° C for 15 minutes to remove unhomogenized material, nuclei and mitochondria. The supernatant containing membrane frgaments, microsomes, and the "soluble" portion was used as the enzyme source.

Reagent grade DDT was dissolved in dimethyl formamide (DMF) for the enzyme assay so that the incubation mixture would contain DMF in a final concentration of less than 5%. According to Janicki and Kinter (1971), and confirmed in the present study, this concentration of DMF had no effect on the (Na^+, K^+)-activated ATPase and inhibited only 20% of the (Mg^{++})-activated ATPase. The concentrations of DDT in the incubation mixture ranged from 1 ppm (2.8×10^{-6} M) to 50 ppm (1.4×10^{-4} M).

The ATPase assay contained in the final volume 20.0 mM imidazole-HCl (pH 7.0); either 120 mM NaCl and 1.0 mM ouabain, or 20 mM KCl and 100 mM NaCl; 0.10 ml of the enzyme

preparation; and DDT or DMF. This mixture was incubated at 25° C 30 minutes to allow the DDT to permeate the preparation. Then the enzyme reaction was initiated by adding 0.2 ml of a solution containing in final concentration 50 mM $MgCl_2$ and 50 mM disodium ATP, neutralized with imidazole. The final volume of the incubation mixture was 2.0 ml.

After 30 minutes at 25 C, the reaction was terminated with the addition of 2.0 ml of ice-cold 10% (w/v) trichloroacetic acid to precipitate the protein. The tubes were placed in an ice bath for at least ten minutes and the precipitate was then removed by centrifugation at 10,000 x g, 0 C for ten minutes.

As Janicki and Kinter (1971) found that DDT interfered with the Fiske-SubbaRow (1925) test, for phosphorus, toluene (3.0 ml) was mixed with the supernatant to remove the DDT. The toluene layer was separated from the aqueous layer with low speed centrifugation on a clinical centrifuge, followed by aspiration, leaving the lower layer for the phosphorus determination (Fiske and SubbaRow, 1925).

The amount of protein present in the enzyme preparation was determined using the method of Lowry, et al. (1951). The specific activity of the enzyme was expressed as the umoles of Pi released from ATP per mg protein per min.

The (Na^+, K^+)-activitated ATPase activity was expressed as the difference between the total enzyme activity in the absence of ouabain and the (Mg^{++})-activated, ouabain-insensitive ATPase activity.

A statistical analysis was made of the variance among means of the DDT-treated samples and the DMF-treated controls, using the Newman-Keuls test of ordered means (Weiner, 1971).

The K_m or enzyme affinity constant for K^+ was determined for the (Na^+, K^+)-activated ATPase in the presence of DMF or 5 ppm DDT, varying the concentration of KCl. Michaelis-Menten and Lineweaver-Burke determinations of the apparent K_m for K^+ were made.

RESULTS and DISCUSSION

The specific activity of (Na^+, K^+)-activated ATPase in the six gill preparations used as controls in the DDT inhibition studies ranged from 0.019 to 0.063 umoles P_i/mg/min and the mean was 0.0388 umoles P_i/mg/min (Table 1). In the assays of all these preparations, the K^+ concentration was 20 mM as suggested by Janicki and Kinter (1971). At

30 mM K^+, the concentration later determined as the optimum, the specific activity of two preparations used for the study of K^+ effectswere 0.047 and 0.041 umoles P_i/mg/min.

It is difficult to compare specific activity values measured here with those found in the literature as different tissue preparations and enzyme assays were used. In this work and the work of others (Epstein, et al., 1969, and Jampol and Epstein, 1970) the specific activity values were probably underestimates of the true concentration of (Na^+, K^+)-activated ATPase in the gill epithelium. This is due to the high amount of presumably non-enzymatic, supportive tissue found there (Jampol and Epstein, 1970).

Jampol and Epstein (1970) found that the specific activity of (Na^+, K^+)-activated ATPase in heteroclitus gill homogenates averaged 0.0165 umoles P_i/mg/min whereas Epstein, et al. (1969) had found the specific activity in the (Na^+, K^+)-activated ATPase in their heteroclitus preparations ranged from 0.09 to 0.24 umoles P_i/mg/min. Slight variations in technique from one experimental group to another, as well as variations which might result from individual differences in the fish, could have caused the scatter in the specific activity values reported here and in the literature.

The DDT inhibition of both (Mg^{++})-activated ATPase and (Na^+, K^+)-activated ATPase was expressed as the mean percent inhibition of the specific activity measured in the presence of DMF (Table 2, Fig. 1 & 2). An analysis of the variance of the mean percent inhibition of (Mg^{++})-activated ATPase in the presence of increasing concentrations indicated that there was a significant difference among the means at the .05 confidence level (Table 3). A Newman-Keuls test of ordered means indicated that 50 ppm was significantly different from all the other treatments and the inhibition at 10 ppm differed significantly from 1 ppm at the .05 confidence level (Table 4).

An identical analysis was made on the (Na^+, K^+)-activated ATPase mean percent inhibition in the presence of increasing concentrations of DDT. There were significant differences among the means (Table 5) and the Newman-Keuls test showed that the mean percent inhibition at 20 ppm and 50 ppm differed significantly from all the other means at the .05 confidence level (Table 6). The mean percents of inhibition of 2.5 ppm, 5 ppm and 10 ppm also were significantly different from that of 1 ppm at the .05 confidence level.

The DDT inhibited the (Mg^{++})-activated ATPase

less than the (Na^+, K^+)-activated ATPase. The maximum inhibition of the (Mg^{++})-activated portion was 46% at 50 ppm DDT, whereas the maximum inhibition of the (Na^+, K^+)-activated portion was 74.6% at 20 ppm DDT. In eel intestinal mucosa, 50% inhibition of (Na^+, K^+)-activated ATPase was reached at 15 ppm DDT and the maximum inhibition of 70% was reached at 250 ppm DDT (Janicki and Kinter, 1971). In the present study, 45.5% inhibition was reached at 2.5 ppm DDT, indicating that the (Na^+, K^+)-activated ATPase in the heteroclitus gill is much more sensitive to inhibition by DDT than eel intestinal mucosa (Janicki and Kinter, 1971). Since (Na^+, K^+)-activated ATPases from different tissues exhibit quantitative differences in their enzymatic properties (Skou, 1965), it is reasonable to assume that there could be both quantitative and qualitative tissue and species differences in inhibition by DDT. The limits of DDT solubility in 5% DMF prevented determining whether 100% inhibition of (Na^+, K^+)-activated ATPase could be induced with higher concentrations of DDT. The incubation mixture clouded with the addition of 50 ppm DDT, an indication that not all the DDT was dissolved and available to the enzyme. This could account for the lack of increase in percent inhibition between 20 and 50 ppm DDT.

The mean specific activities of the DMF-treated controls and the DDT-treated samples were compared (Table 7, Figure 3). Analysis of the variance of these means indicated that there was significant difference among the means at the .05 confidence level (Table 8). The Newman-Keuls test of ordered means showed that there was significant difference between the control and all the DDT-treated groups and significant difference between the mean specific activity at 50 ppm and 1 ppm at the .05 confidence level (Table 9). DDT also acts to decrease the variance within a sample.

A kinetic study of the effect of different K^+ concentrations on DDT inhibition of (Na^+, K^+)-activated ATPase indicated that there is competitive inhibition by DDT with respect to K^+ (Figures 4 and 5). In the Michaelis-Menten plot, the apparent K_m for K^+ of (Na^+, K^+)-activated ATPase treated with DMF was 2.5 mM and the apparent K_m of the DDT-treated sample was 20.0 mM K^+ (Figure 2). The V_{max} values were the same for both the DMF and the DDT-treated enzymes, indicating the inhibitory effect of DDT could be fully reversed by increasing the K^+ concentration. The competitive inhibition observed here differs from non-competitive inhibition in which the V_{max} would be lowered

and could not be restored (Lehninger, 1970).

A Lineweaver-Burk plot of the data indicated that the apparent K_m was 3.0 mM K^+, for the DMF-treated (Na^+, K^+)-activated ATPase and 25 mM K^+ for the DDT-treated enzyme. Although a slight increase in the V_{max} was noted for DDT-treated (Na^+, K^+)-activated ATPase, this difference cannot be evaluated in this study. To make further conclusions concerning the effect of DDT on the V_{max} of the reaction it would be desirable to have values for the specific activity of a DDT-treated enzyme at concentrations of KCl between 40 mM and 70 mM.

The evidence for competition between DDT and K^+ is clear. The apparent rise in the K_m as shown by the Michaelis-Menten and Lineweaver-Burk and the unchanged V_{max} plots indicate that DDT and K^+ are in competition, perhaps for a site on or near the active center of the (Na^+, K^+)-activated ATPase enzyme. Yamasaki and Narahashi (1959) found that the action of DDT in the crural nerve of the American cockroach is associated with the passive efflux of K^+. They suggested that DDT in some way inhibited this passive efflux of K^+ as high concentrations of K^+ reduced the effects of DDT (Yamasaki and Narahashi, 1960). The work of Hilton and O'Brien (1970) with the valinomycin-induced

transfer of K^+ across a synthetic membrane showed that DDT antagonized this carrier system. Even if valinomycin in this case acts to form a passageway through the membrane, thus transporting K^+ across the membrane, the mechanism of DDT could involve steric and or ionic interference with K^+ bonding.

One of the most striking characteristics of the DDT molecule is the $-CCl_3$ group which has a high electron affinity (O'Brien, 1967). Therefore, interference of K^+ or any cation transport by DDT could reasonably involve such an ionic mechanism. Likewise, the bulk of the $-CCl_3$ group could easily by steric interference affect both active, mediated cation transport by (Na^+, K^+)-activated ATPase and the passive transport as seen in the K^+ efflux in nerve tissue (Yamasaki and Narahashi, 1959 and Narahashi and Haas, 1967).

Another aspect of DDT action that is particularly relevant here, though not explored experimentally, is its affinity for membrane components (O'Brien, 1967). As ATPase is associated with membrane fragments and its activity depends upon the presence of an organized phospholipid structure (Skou, 1965), DDT by some mechanism could interfere with or alter this ATPase-membrane-phospholipid complex.

Though the data presented here do suggest that there is competitive inhibition of (Na^+, K^+)-activated ATPase by DDT in terms of K^+, the exact nature of this competition is unknown. DDT could compete with K^+ at the K^+ site on the enzyme. Possibly, the competition results from the attraction of K^+ to the $-CCl_3$ portion of the DDT molecule. in effect reducing the K^+available to activate the de-phosphorylation of the intermediate formed in the ATPase reaction. Definitive judgements are limited somewhat by the lack of knowledge concerning the mechanism of DDT toxicity in other systems and the mechanism of the (Na^+, K^+)-activated ATPase enzyme. Further studies on the effect of DDT in relation to Na^+, the substrate ATP, and the phospholipid in the membrane fragments would possibly indicate whether DDT affects the (Na^+, K^+)-activated ATPase enzyme in other ways not related to K^+ transport.

In summary, the (Na^+, K^+)-activated ATPase in the gill tissue of heteroclitus was highly sensitive to inhibition by DDT, more so than the (Mg^{++})-activated ATPase. Kinetic evidence indicates that this inhibition, at least in part, involves competitive inhibition by DDT in relation to K^+.

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Table 1. Specific activity of (Mg^{++})-activated ATPase and (Na^+, K^+)-activated ATPase in gill preparations of Fundulus heteroclitus treated with DMF.

Sample	Specific activity - umoles p_i/mg/min. (Mg^{++})-ATPase	(Na^+, K^+)-ATPase
1*	0.084	0.063
2*	0.144	0.034
3*	0.093	0.046
4*	0.097	0.033
5*	0.110	0.038
6*	0.097	0.019
mean	0.104	mean 0.0388
K_m prep.** A		0.047
B		0.041

* 20 mM K^+

** 30 mM K^+

Table 2. Mean percent inhibition of (Na^+, K^+)-activated ATPase activity and (Mg^{++})-activated ATPase activity by DDT.

ppm DDT	(Mg^{++})-ATPase umoles P_i/mg/min			(Na^+, K^+)-ATPase umoles P_i/mg/min		
	mean	SD	SE	mean	SD	SE
1	3.33	4.04	2.33	27.30	5.13	2.96
2.5	9.50	2.89	1.45	45.50	10.72	5.36
5	12.60	5.13	2.96	45.30	13.61	7.87
10	20.30	1.53	0.88	53.30	4.16	2.40
20	18.00	8.72	5.04	74.60	2.31	1.33
50	46.00	11.30	6.53	74.00	3.00	1.73

Table 3. Analysis of Variance - Mean percent inhibition of Mg^{++})-ATPase activity by DDT.

Source	SS	df	MS	F
Treatments	3387.53	5	677.51	16.78*
Error	525.00	13	40.38	

* significance at the .05 level $F_{.95}$ (5, 13) = 3.03

Table 4. Newman-Keuls Test of Ordered Means - Mean percent inhibition of (Mg^{++})-ATPase activity by DDT.

ppm DDT		ppm DDT 1	2.5	5	20	10	50
	means	3.33	9.5	12.6	18.0	20.3	46.0
1	3.33	--				16.97*	42.67*
2.5	9.5		--				36.50*
5	12.6			--			33.50*
20	18.0				--		27.20*
10	20.3					--	25.70*
50	46.0						--

* Significance at the .05 level

Table 5. Analysis of Variance - Mean percent inhibition of (Na^+, K^+)-ATPase activity by DDT.

Source	SS	df	MF	F
Treatments	5109.28	5	1021.9	15.972*
Error	831.60	13	63.9	

* significance at .05 level

$F_{.95}$ (5, 13) = 3.03

Table 6. Newman-Keuls Test of Ordered Means - Mean percent inhibition of (Na^+,K^+)-ATPase activity by DDT.

		ppm DDT					
		1	5	2.5	10	50	20
ppm DDT	means	27.3	45.3	45.5	53.3	74.0	74.6
1	27.3	-	18.0*	18.2*	26.0*	47.7*	43.7*
5	45.3		-			28.7*	29.3*
2.5	45.5			-		27.5*	29.1*
10	53.3				-	20.4*	21.3*
50	74.4					-	
20	74.6						-

* significance to .05 level

Table 7. The mean specific activity of (Na^+,K^+)-activated ATPase treated with DMF and DDT.

Sample	umoles P_i/mg/min		
	mean	SD	SE
DMF (control)	0.0388	0.013	0.0053
1 ppm DDT	0.0220	0.003	0.0015
2.5 ppm DDT	0.0164	0.006	0.0027
5 ppm DDT	0.0135	0.005	0.0025
10 ppm DDT	0.0167	0.005	0.0029
20 ppm DDT	0.0080	0.002	0.0010
50 ppm DDT	0.0053	0.0005	0.0003

Table 8. Analysis of Variance - Mean specific activities of DMF-treated controls and DDT-treated experimental groups.

Source	SS	fd	MS	F
Treatments	0.00359	6	0.00060	9.26*
Error	0.00142	22	0.00006	

* Significance at .05 level $F_{.95}$ (2,6) = 2.55

Table 9. Newman-Keuls Test of Ordered Means - Mean specific activities of DMF-treated controls and DDT-treated experimental groups of (Na^+,K^+)-ATPase.

		ppm DDT						
		50	20	5	2.5	10	1	0
ppm DDT	means	.0053	.0080	.0135	.0164	.0167	.0220	.0388
50	.0053	-					.0167*	.0335*
20	.0080		-					.0280*
5	.0135			-				.0253*
2.5	.0164				-			.0224*
10	.0167					-		.0221*
1	.0220						-	.0168*
0	.0388							-

* Significance at the .05 level

Figure 1. Mean percent inhibition of the specific activity of (Mg^{++})-activated ATPase by DDT. Point represents the mean and the line represents $\pm$ 1 S.E.

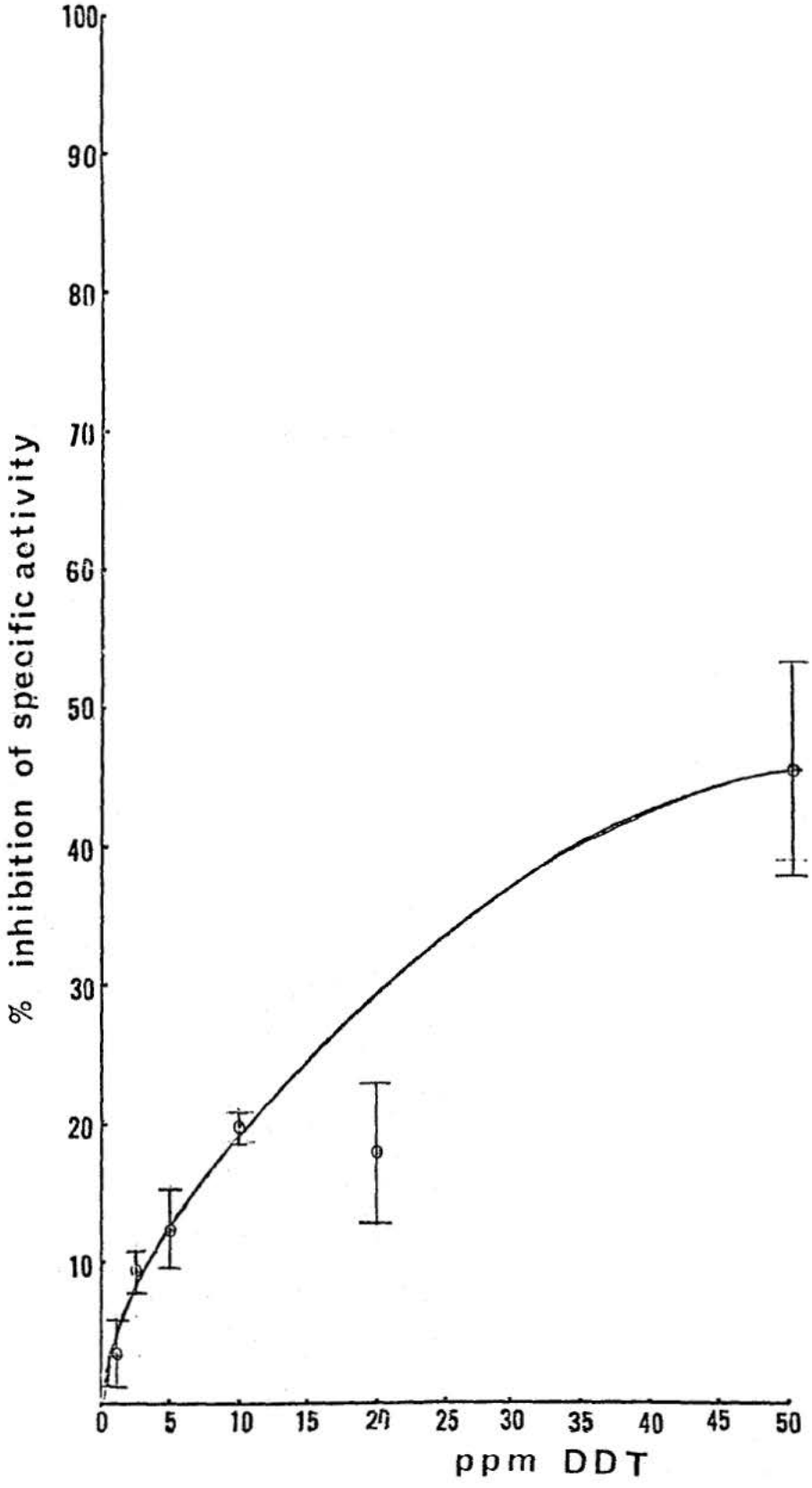

% inhibition of specific activity
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
50
ppm DDT

Figure 2. Mean percent inhibition of the specific activity of (Na^+,K^+)-activated ATPase by DDT. The point represents the mean and the line represents $\pm$ 1 S.E.

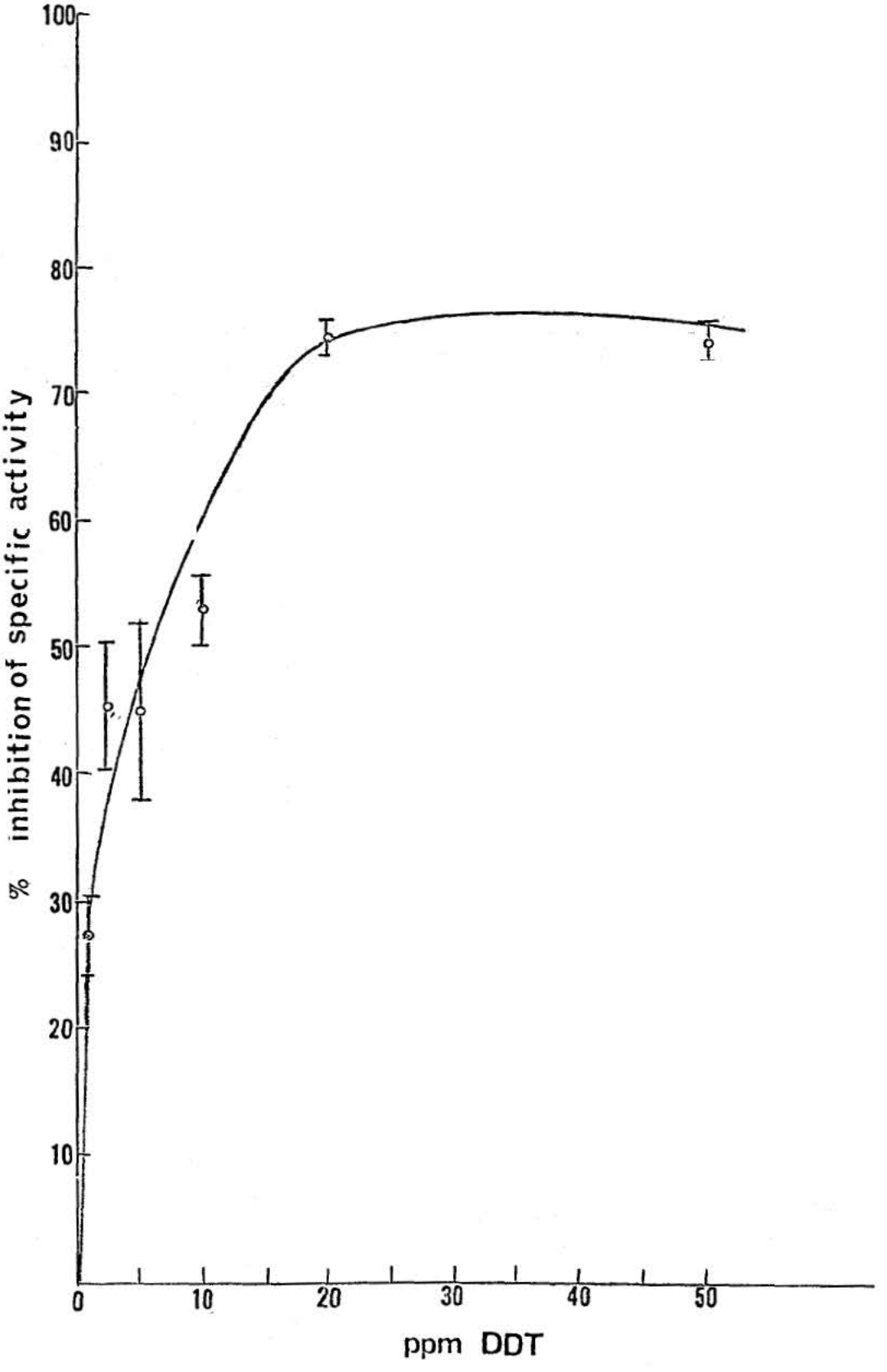

100
90
80
70
60
50
40
30
20
10
% inhibition of specific activity
0
10
20
30
40
50
ppm DDT

Figure 3. Mean specific activities of DMF-treated control and DDT-treated (Na^+,K^+)-activated ATPase. The point represents the mean and the line represents $\pm$ 1 S.E.

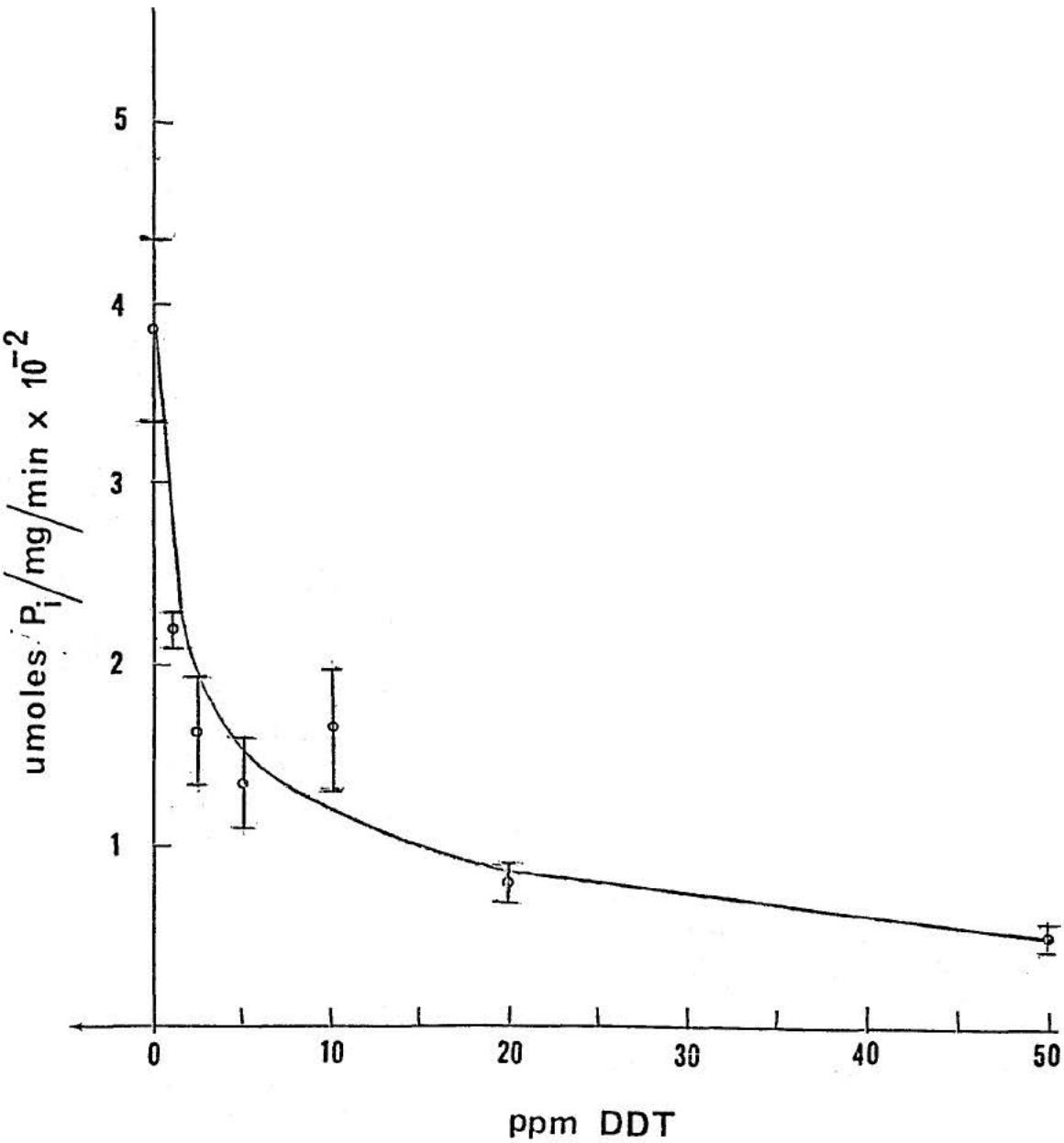

umoles P_i/mg/min x 10^{-2}
5
4
3
2
1
0
10
20
30
40
50
ppm DDT

Figure 4. Michaelis-Menten plot showing the effect of varying K^+ on specific activity of (Na^+,K^+)-activated ATPase by 5 ppm DDT. Points represent average of duplicates.

DMF K_m = 3.5 mM K^+

5 ppm DDT K_m = 20 mM K^+.

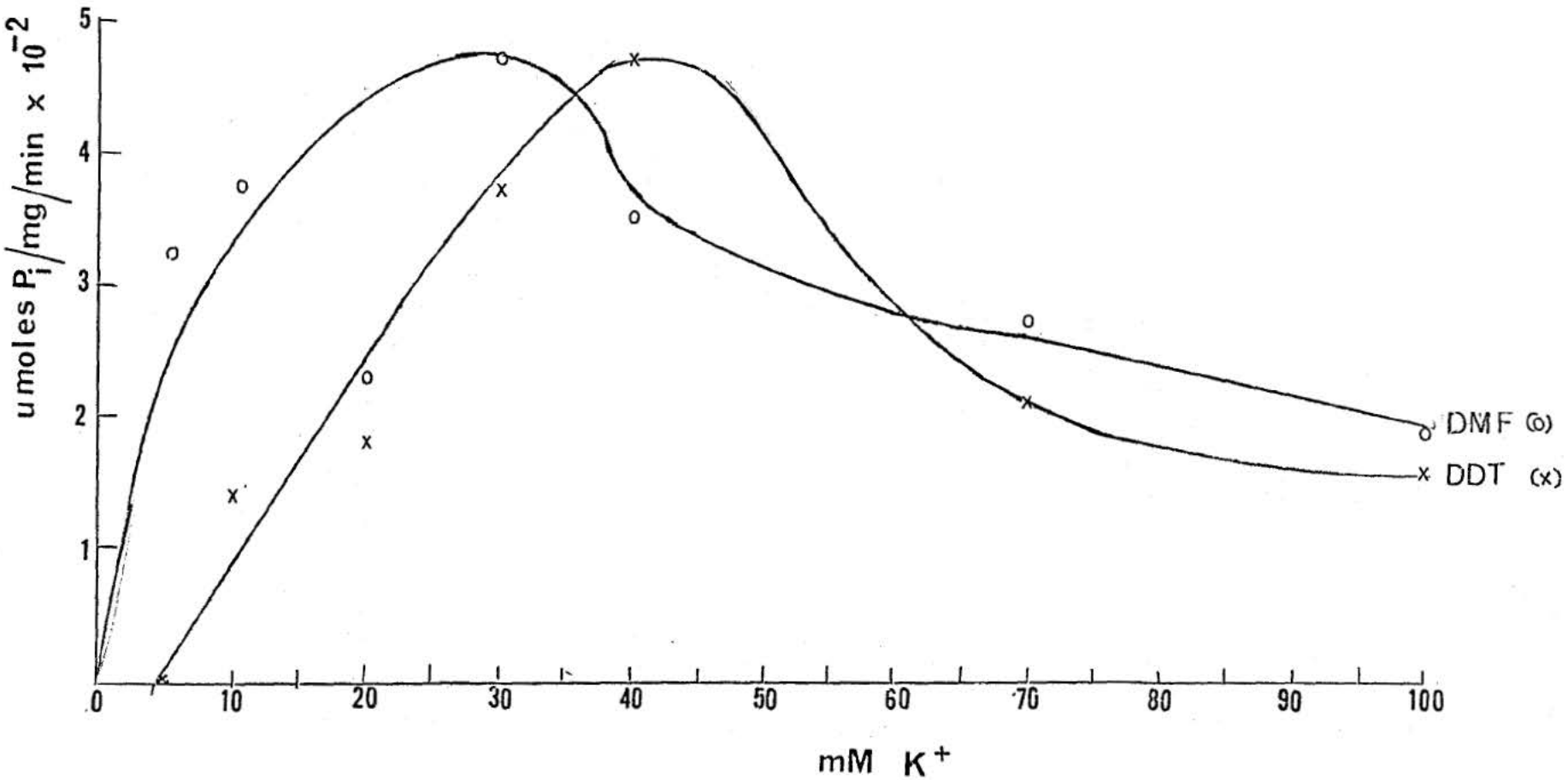
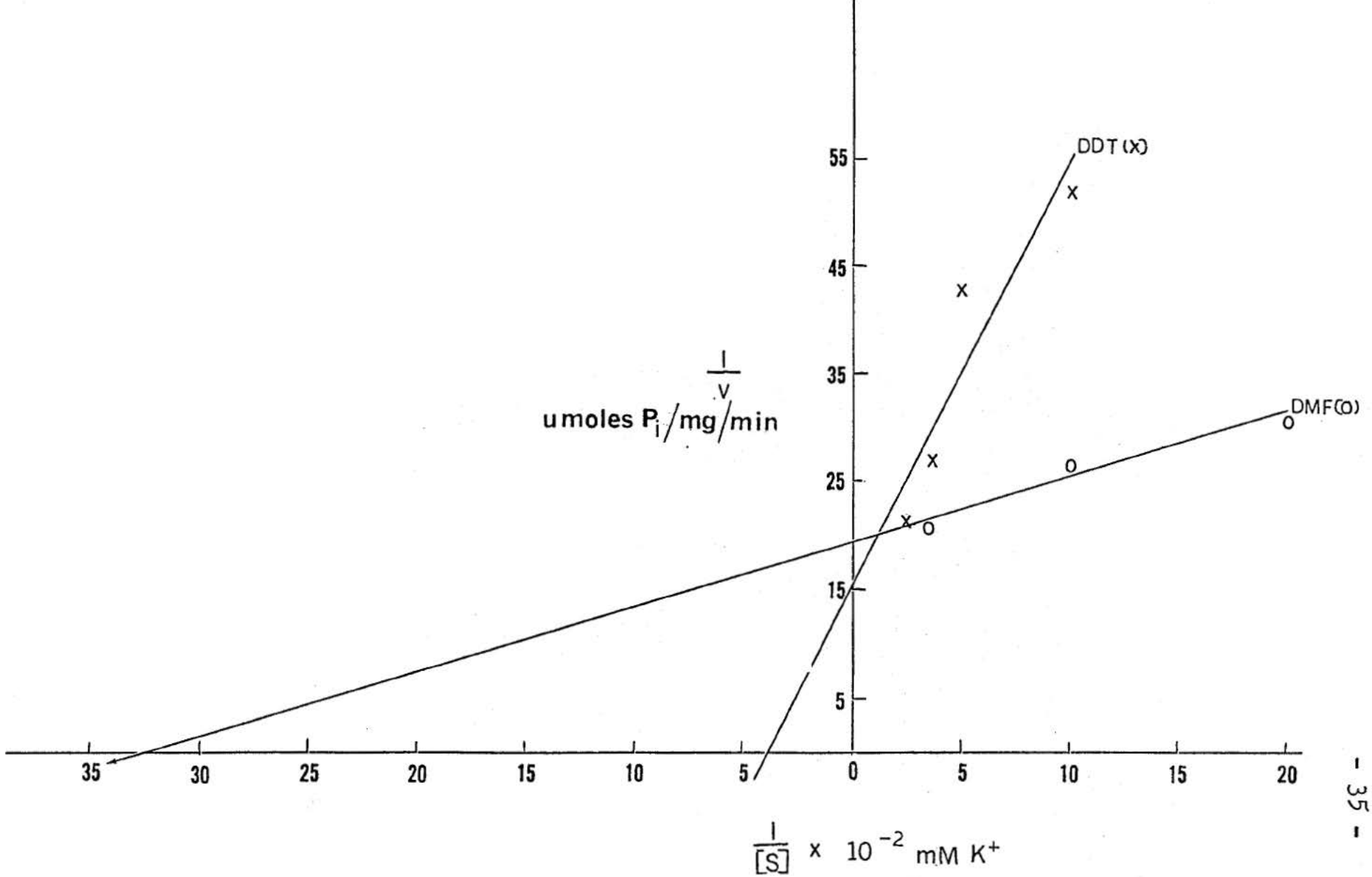

u moles P_i/mg/min x 10^{-2}
5
4
3
2
1
0
10
20
30
40
50
60
70
80
90
100
mM K^+
DMF (o)
DDT (x)

Figure 5. Lineweaver-Burk plot showing the effect of varying K^+ on the specific activity of (Na^+,K^+)-activated ATPase by 5 pp, DDT. Lines derived by least squares method and points represent an average of duplicates.

DMF K_m = 3 mM K^+

DDT K_m = 25 mM K^+


DDT (x)
DMF (o)
$\frac{1}{V}$
umoles P_i/mg/min
55
45
35
25
15
5
35
30
25
20
15
10
5
0
5
10
15
20
$\frac{1}{[S]}$ x 10^{-2} mM K^+

VITA

Nancy Rhea McCutcheon Slate was born in Norfolk, Virginia, April 20, 1944. She attended Richmond Public Schools, and was graduated from George Wythe High School in June, 1962. She received a Bachelor of Arts degree from Randolph-Macon Woman's College in June, 1966, with a major in Biology. After graduation, she taught Biology and General Science in the Richmond Public Schools for three years and in the Indianapolis schools for one year. She has been a teacher in the Mathematics-Science Center's summer program for four years. In September, 1970, she entered the University of Richmond Graduate School and while there became a member of the Association of Southeastern Biologists and Beta Beta Beta Honorary Biological Society. During her two years at the University, she has held a Williams Fellowship. She has accepted a position to teach at Trinity Episcopal High School in Richmond in September, 1972. She completed the requirements for the Master of Arts Degree from the University of Richmond in August, 1972. She is married to William K. Slate, II.