# CHAPTER 9

## EFFECT OF ACETYLCHOLINESTERASE INHIBITORS ON THE TRANSPORT OF GLUCOSE ACROSS HEPATIC CELL MEMBRANE AND ON HEPATIC ENZYMES UNDER *IN VITRO* CONDITIONS

Acetylcholine (ACh) induced membrane permeability changes is a common mechanism involved in most of the neural stimulus-response coupling in tissues. The permeability change induced by ACh involves movement of $Na^+$, $K^+$ and $Ca^{++}$ ions through specific ion channels. The influx of $Na^+$ and outflux of $K^+$ depolarizes the plasma membrane which culminates in the cell showing a specific response. The repolarization is by activating the sodium pump which actively transports $Na^+$ out of the cell in exchange of $K^+$, expending energy in the process. In highly metabolic tissues, like liver, when ions move into the cell, certain other substances such as glucose and amino acids may also gain entry into the cell interior. In tissues such as intestine, liver and kidney glucose and amino acids are moved across the membrane through such flow coupled transport. In the intestine and kidney the hyperconcentration of $Na^+$ induces permeability changes and $Na^+$ moves into the cell. In the ensuing active process of sending $Na^+$ out of the cell glucose is absorbed from the lumen. However, liver cells are not faced with such hypernatrimia to induce $Na^+$ influx into the cell. Here, ACh secreted by cholinergic fibres belonging to vagus might be playing a role in changing the permeability of the hepatic cell membrane and thereby inducing $Na^+$ influx. Several studies

conducted in our laboratory (See Review Pilo and Patel, 1978) clearly showed that both avian and mammalian liver cells take up more glucose in the presence of ACh. The action of ACh in avian liver cells was much more prominent than in the mammalian hepatocytes. ACh was also found to increase the activity of glycogen synthetase (Shimazu, 1983) which could also result in chemiosmotic pull of glucose across hepatic cell membrane. The action of ACh on the avian liver on glucose uptake assumes greater importance in view of the fact that insulin release from the pancreas is sluggish in response to hyperglycaemia (Hazelwood, 1973). Perhaps, in aves, ACh induced glucose uptake is more dominant than that induced by insulin. ACh released all along the sinusoidal lining in the liver could then stimulate all the hepatocytes to take up glucose. Previous in vivo studies have shown elevation of ChE activity in liver, following glucose administration on both pigeon and rat suggesting that ACh is secreted more (Pilo and Patel, 1978). The sinusoidal linings also exhibit a very significant localization of AChE which could inactivate the ACh very quickly. Inhibition of AChE then would prolong the action of ACh on the plasma-membrane due to ACh accumulation. (Milosevic, 1970; Koundinya and Rama Murthy, 1978). Acetylcholinesterase inhibitors such as monocrotophos, acothione and prostigmine have been chosen for the assessment of the biochemical effects of these on the uptake of glucose by the pigeon liver slices. Monocrotophos and acothione are organo-phosphorus compounds, highly toxic and they affect the living

system tremendously. Prostigmine is an inhibitory drug, which inhibits AChE (Lahue, 1981).

Monocrotophos possesses capacity to inhibit blood cholinesterase activity in human volunteers (Simpson et al., 1969; Verberk, 1977). The organophosphorus compounds (Monocrotophos and acothione) are potent inhibitors of cholinesterase (ChE) and the mechanism of action on ChE enzyme is fairly well understood (Aldridge and Dawson, 1952; Aldridge, 1953; 1969; Fleisher et al., 1970; Silver, 1974). The organophosphorus compounds work like a substrate analogue for acetylcholine and inhibit cholinesterase enzyme activity by forming a complex. These compounds form covalent bonds with esterase moeity.They react in a bimolecular manner. The cholinesterase enzymic proprotein gets phosphorylated thus becoming stable and hence does not remain capable of affecting usual hydrolysis. As a result of ChE inhibition, acetylcholine accumulates. It is well known by now that ChE enzyme exists in various animals in different isoenzymic forms, each isoenzyme having qualitatively different properties. The various isoenzymic forms have been extensively reviewed by Silver (1974). It is therefore logical to expect that depending upon the variation in the isoenzymic composition of the cholinesterase complex the action of compounds may vary.

Exposure to a toxic agent can cause traumatic damage to cells and can result in many bio-and physico-chemical alterations.

Abou-Donia et al. (1979) observed increased acid phosphatase activity in the serum of leptophos exposed fowls as a result of increase in brain lysosomal enzymes. He has compared the action of organophosphorus to that of Wallerian degeneration. In Wallerian degeneration, tissue energetics is known to be affected along with increase of lysosomal enzyme activities (Joseph, 1973). It is likely that organophosphorus compounds under sustained treatment might also induce such degenerative changes as well as the tissue energetics. On this assumption our experiments were extended to estimation of acid and alkaline phosphatases. $Na^+$-$K^+$-ATPase activity as well as the total protein content were also measured to study the influence of the acetylcholinesterase inhibitors on the pigeon liver slices.

## MATERIALS AND METHODS

Adult pigeons (Columba livia) weighing 180-250 gms. maintained in laboratory conditions on balanced diet were used for the present experiments. Animals were sacrificed after 24 hours of starvation period. The liver was perfused with cold Krebs Ringer medium (KRB) and then quickly excised. The liver was placed on ice and cut into slices of 100-120 $\mu$ thickness. The slices were weighed and were placed in 10 ml flask with 5 ml of KRB. The liver slices were incubated for 90 min. at 37°C in a water bath shaker. The slices were incubated in media of following different categories.

(1) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + MCP (.001 ml/I.M.)

(2) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + MCP (.001 ml/I.M.) + Insulin (1 unit/ml)

(3) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + MCP (.001 ml/I.M.) + ACh (15 mg/ml)

(4) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Acothione (.001 ml/I.M.)

(5) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Acothione (.001 ml/I.M.) + Insulin (1 unit/ml)

(6) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Acothione (.001 ml/I.M.) + ACh (15 mg/ml)

(7) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Prostigmine (.02 mg/ml)

(8) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Prostigmine (.02 mg/ml) + Insulin (1 unit/ml)

(9) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Prostigmine (.02 mg/ml) + ACh (15 mg/ml).

The slices, before and after incubation were quickly washed with chilled KRB buffer and homogenised with distilled water and homogenate was used for enzyme and protein estimations, as per methods described in Chapter 1. Glucose was estimated in the medium before and after incubation.

## RESULTS

The data on the effect of cholinesterase inhibitors are presented in Figs. 9-1 to 9-8.( Glucose, Fig.9-1; Glycogen, Fig.9-2; Phosphorylase, Fig.9-3; AChE, Fig.9-4; Acid phosphatase, Fig.9-5; Alkaline phosphatase, Fig. 9-6; $Na^+$-$K^+$-ATPase , Fig.9-7; LDH , Fig. 9-8).

Monocrotophos (Table 9-1):

Monocrotophos alone in the incubation medium resulted in an uptake of glucose. Monocrotophos with insulin or ACh had the same effect. However, with ACh, glucose uptake was much more than with insulin. AChE activity showed a decrease when monocrotophos was in the medium whether alone or in combination with insulin or ACh. A general decrease of ATPase, alkaline phosphatase, SDH and LDH activities was also observed when liver slices were incubated with monocrotophes, either alone or in combination with insulin or ACh. However, acid phosphatase showed an increase. Phosphorylase also showed an increase in its activity.

Acothione (Table 9-2):

Like monocrotophos , acothione also affected the uptake of glucose by the liver slices from the medium. A further increase in glucose uptake was noted when acothione was present along with insulin or acetylcholine. Combined effects of acothione and ACh on glucose uptake was more than that of acothione + insulin. Phosphorylase and acid phosphatase showed an increase in their

Fig. 9-1. Effect of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on glucose uptake by pigeon liver slices under *in vitro* conditions.

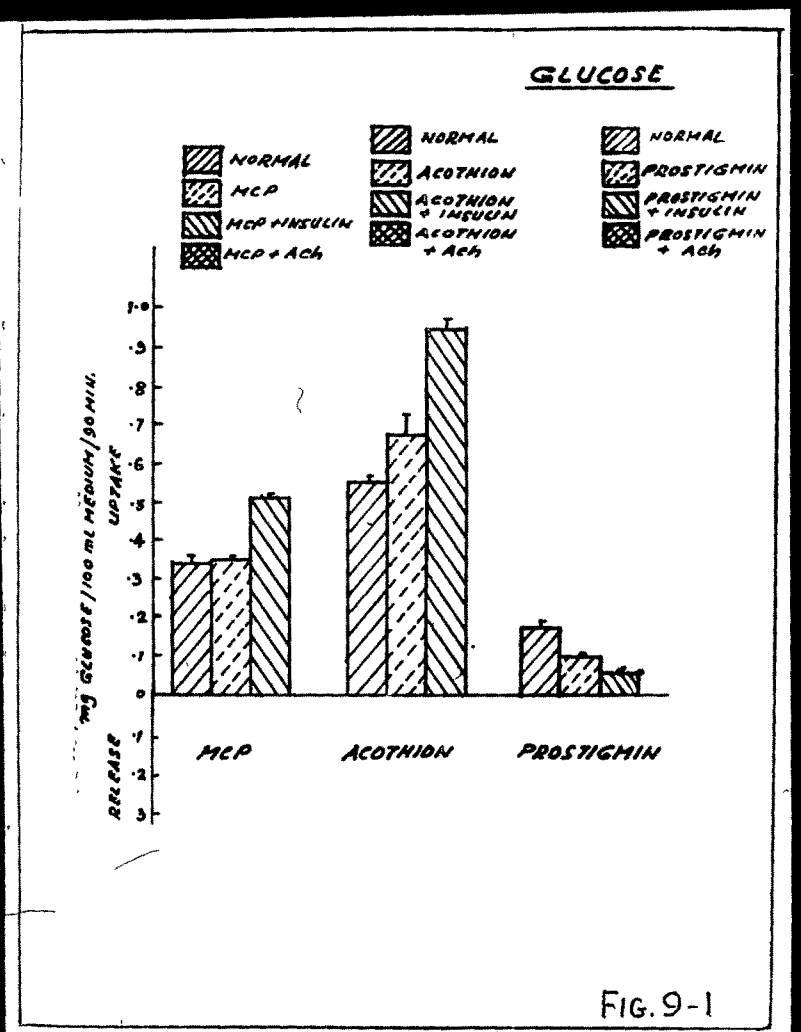
GLUCOSE
NORMAL
MCP
MCP + INSULIN
MCP + Ach
NORMAL
ACOTHION
ACOTHION + INSULIN
ACOTHION + Ach
NORMAL
PROSTIGMIN
PROSTIGMIN + INSULIN
PROSTIGMIN + Ach
RELEASE
MCP
ACOTHION
PROSTIGMIN

FIG. 9-1

Fig. 9-2. Effect of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on glycogen content in pigeon liver slices under <u>in vitro</u> conditions.

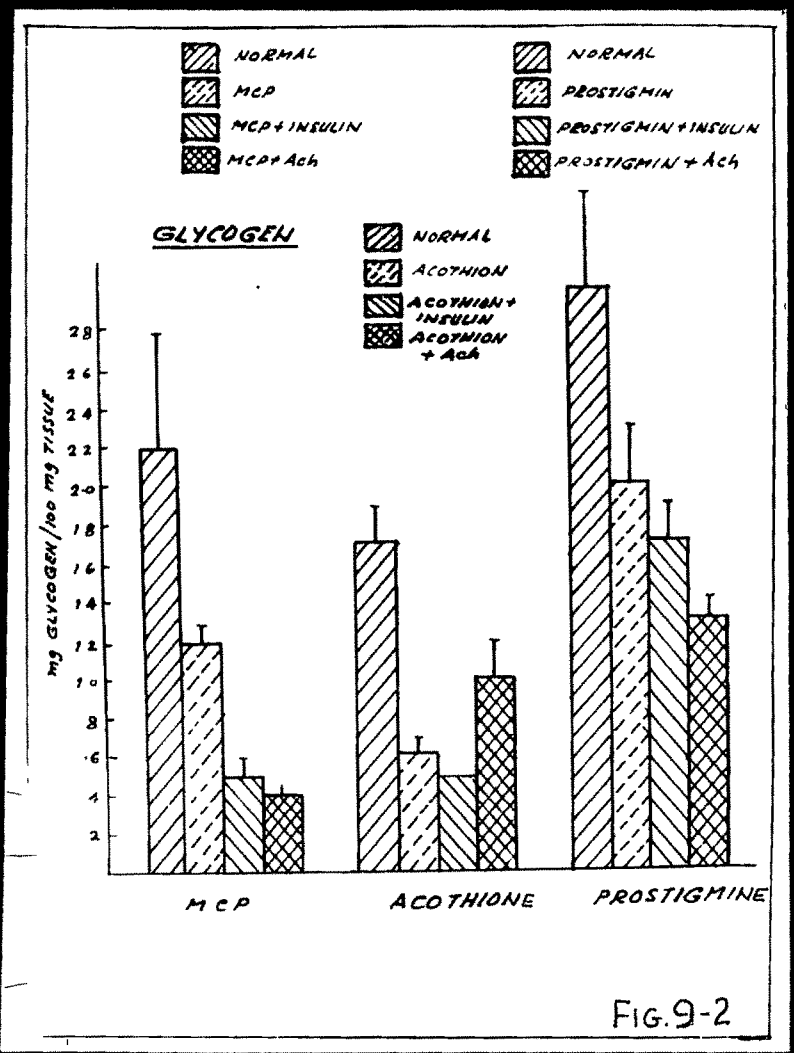
NORMAL
MCP
MCP + INSULIN
MCP + Ach
NORMAL
PROSTIGMIN
PROSTIGMIN + INSULIN
PROSTIGMIN + Ach
GLYCOGEN
NORMAL
ACOTHION
ACOTHION + INSULIN
ACOTHION + Ach
mg GLYCOGEN/100 mg TISSUE
28
26
24
22
20
18
16
14
12
10
8
6
4
2
MCP
ACOTHIONE
PROSTIGMINE

FIG. 9-2

Fig. 9-3. Effec t of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on phosphorylase activity in pigeon liver slices under in vitro conditions.

Fig. 9-4. Effect of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on acetylcholinesterase (AChE) activity in pigeon liver slices under in vitro conditions.

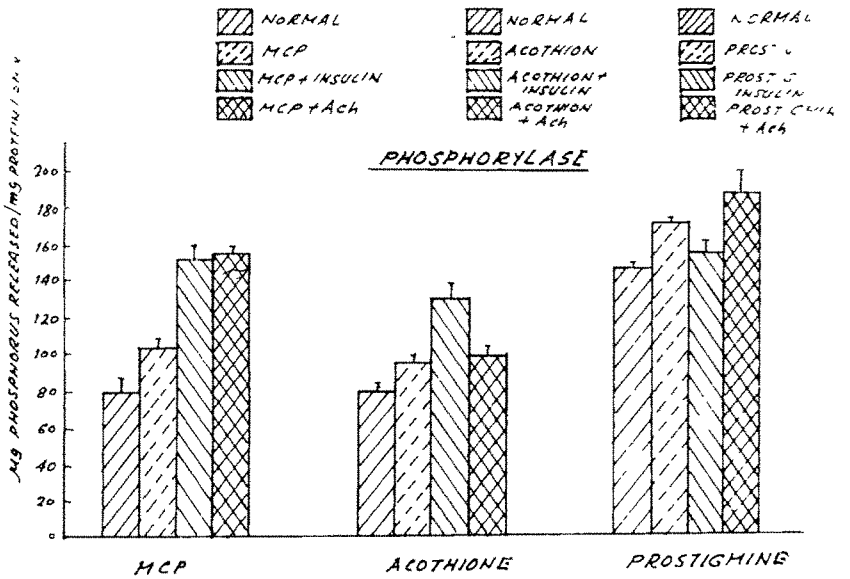
NORMAL
MCP
MCP + INSULIN
MCP + Ach
NORMAL
ACOTHION
ACOTHION + INSULIN
ACOTHION + Ach
PHOSPHORYLASE
MCP
ACOTHIONE
PROSTIGMINE

FIG. 9-3

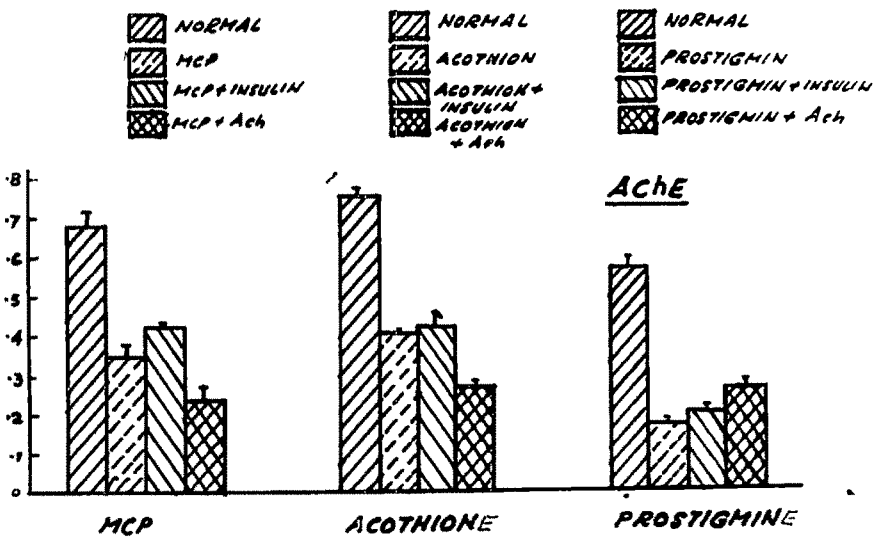
NORMAL
MCP
MCP + INSULIN
MCP + Ach
NORMAL
ACOTHION
ACOTHION + INSULIN
ACOTHION + Ach
NORMAL
PROSTIGMIN
PROSTIGMIN + INSULIN
PROSTIGMIN + Ach
AChE
MCP
ACOTHIONE
PROSTIGMINE

FIG 9-4

Fig. 9-5. Effect of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on acid phosphatase activity in pigeon liver slices under *in vitro* conditions.

Fig. 9-6. Effect of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on alkaline phosphatase activity in pigeon liver slices under *in vitro* conditions.

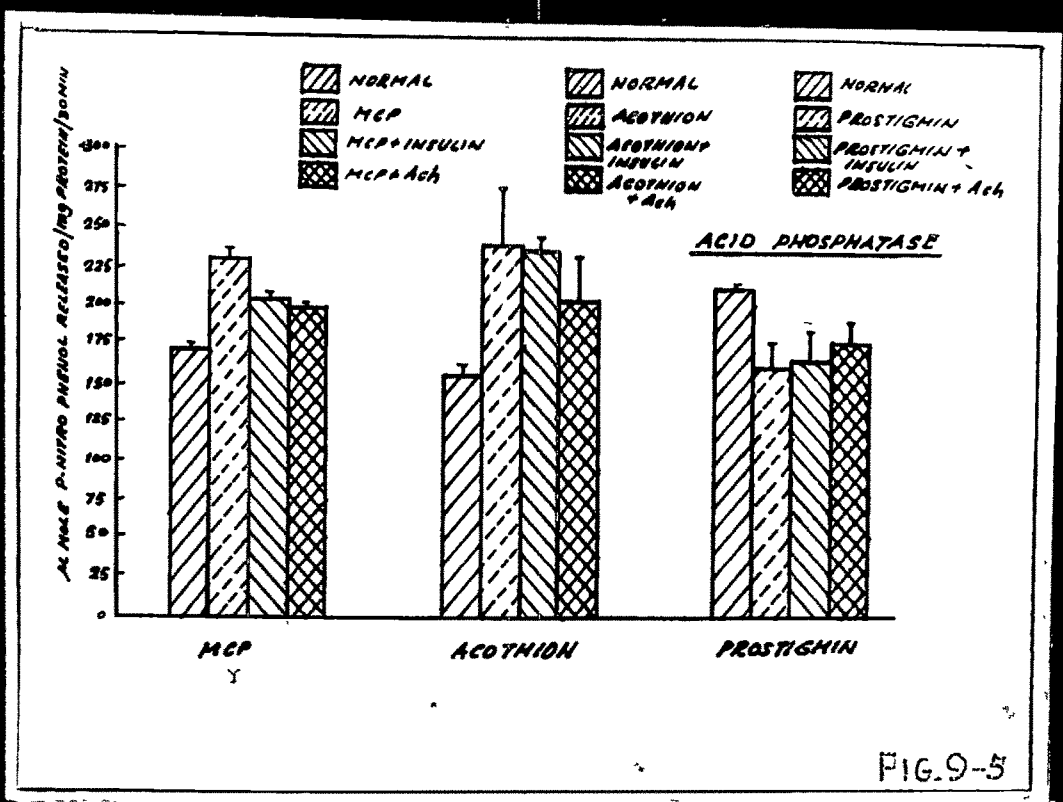
NORMAL
MCP
MCP+INSULIN
MCP+Ach
NORMAL
ACOTHION
ACOTHION+ INSULIN
ACOTHION + Ach
NORMAL
PROSTIGMIN
PROSTIGMIN + INSULIN
PROSTIGMIN + Ach
ACID PHOSPHATASE
MCP
ACOTHION
PROSTIGMIN

FIG. 9-5

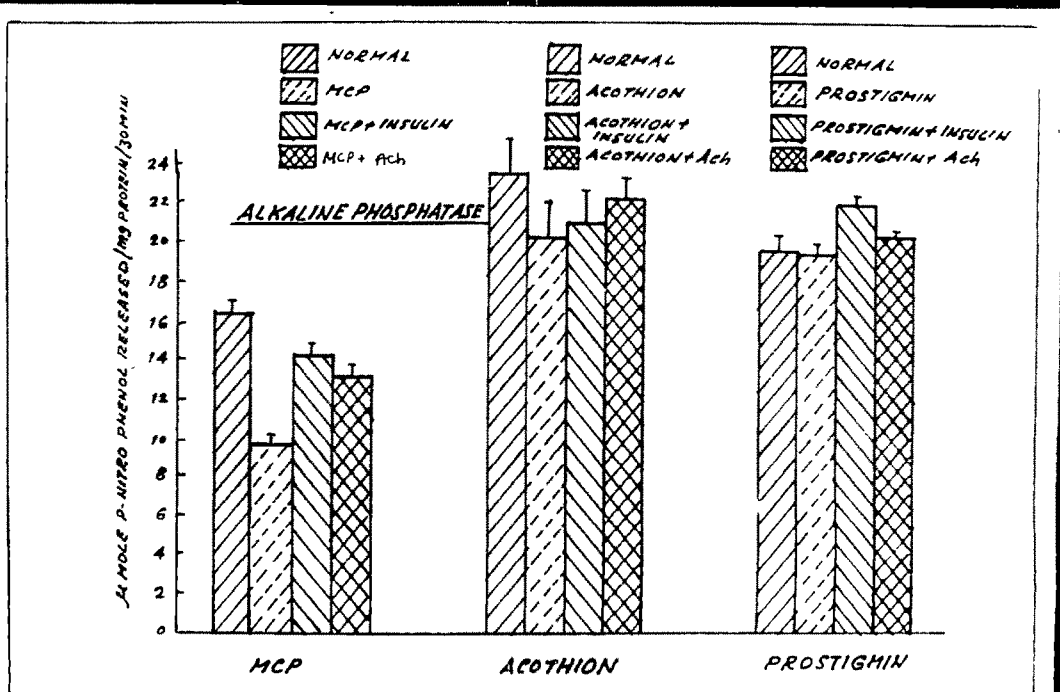
NORMAL
MCP
MCP+INSULIN
MCP+ Ach
NORMAL
ACOTHION
ACOTHION+ INSULIN
ACOTHION+Ach
NORMAL
PROSTIGMIN
PROSTIGMIN+INSULIN
PROSTIGMIN+ Ach
ALKALINE PHOSPHATASE
MCP
ACOTHION
PROSTIGMIN

FIG. 9-6

Fig. 9-7. Effect of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on $Na^+$-$K^+$-ATPase activity in pigeon liver slices under <u>in vitro</u> conditions.

Fig. 9-8. Effect of acetylcholinesterase inhibitors alone or in combination with insulin or acetylcholine (ACh) on lactate dehydrogenase (LDH) activity in pigeon liver slices under <u>in vitro</u> conditions.

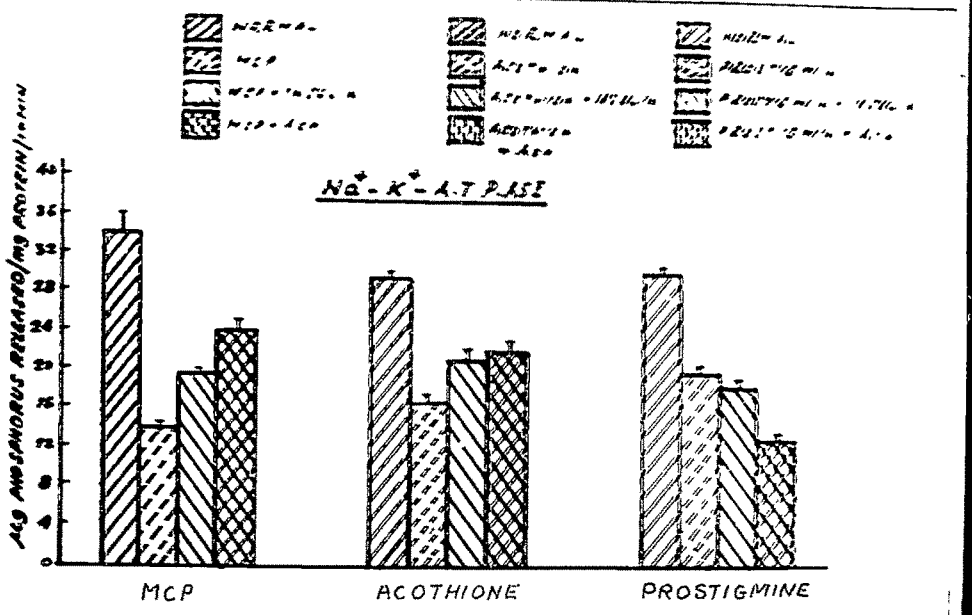
Na⁺-K⁺-A.T PASE
MCP
ACOTHIONE
PROSTIGMINE

FIG.9-7

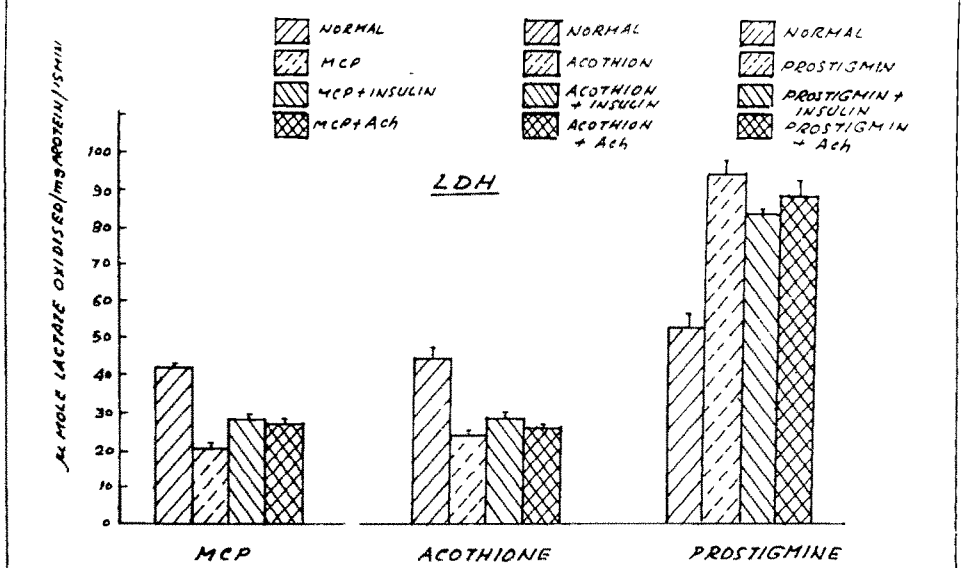
NORMAL
MCP
MCP+INSULIN
MCP+Ach
NORMAL
ACOTHION
ACOTHION + INSULIN
ACOTHION + Ach
NORMAL
PROSTIGMIN
PROSTIGMIN + INSULIN
PROSTIGMIN + Ach
LDH
MCP
ACOTHIONE
PROSTIGMINE

FIG.9-8

Table 9-1

Effect of cholinesterase inhibitors, monocrotophos, acothione and prostigmine alone or in combination with insulin or acetylcholine, on the uptake or release of glucose by pigeon liver slices under *in vitro* conditions (Mean ± S.E.M).

| Additives | Glucose | | Glycogen Depletion (2) |
|---|---|---|---|
| | Uptake (1) | Release (1) | |
| Monocrotophos | 0.3433 ±0.0242 | - - | 0.9726* ±0.4373 |
| Monocrotophos + Insulin | 0.3592* ±0.0118 | - - | 1.7549* ±0.4527 |
| Monocrotophos + ACh | 0.5186* ±0.0068 | - - | 1.8015* ±0.5698 |
| Acothione | 0.5593 ±0.0276 | - | 1.0984 ±0.1696 |
| Acothione + Insulin | 0.6844 ±0.0510 | - | 1.2151 ±0.2626 |
| Acothione + ACh | 0.9553* ±0.0300 | - | 0.7222 ±0.1208 |
| Prostigmine | 0.1869 ±0.0046 | - | 0.9929 ±0.2026 |
| Prostigmine + Insulin | 0.1088 NS ±0.0045 | - | 1.2556 ±0.3101 |
| Prostigmine + ACh | 0.06814**** ±0.0030 | - | 1.6640 ±0.3851 |

(1) Mg glucose taken up or released by 100 mg liver. (2) Mg glycogen depletion/100 mg liver

* $P < 0.05$ ** $P < 0.02$ *** $P < 0.01$ **** $P < 0.001$

Table 9-2

Effect of monocrotophos, alone or in combination with insulin or acetylcholine, on the enzyme activities in the pigeon liver slices under in vitro conditions. (Mean ± S.E.M)

| Enzymes | Control+ (Tissue) | Monocrotophos | Monocrotophos+ Insulin | Monocrotophos + ACh |
|---|---|---|---|---|
| $Na^+$-$K^+$-ATPase µg phosphorus released/Mg protein/10 min. | 34.2240 ± 2.5650 | 14.0470* ± 0.9896 | 19.6602* ± 0.4353 | 24.3772* ± 1.2403 |
| AChE µm Ach hydrolysed/mg protein/ 10 minutes | 0.6840 ±0.0473 | 0.3504* ±0.0328 | 0.4211* ±0.0160 | 0.2390* ±0.0444 |
| Acid Phosphatase µm P-nitrophenol released/100 mg protein/30 minutes | 171.4363 ± 7.1722 | 232.2801* ± 7.6428 | 206.2365* ± 5.3385 | 191.8508 NS ± 4.5808 |
| Alkaline Phosphatase. µm P-nitrophenol released/ 100 mg protein/ 30 minutes | 16.5381 ± 0.6225 | 9.7411 NS ±0.5190 | 14.2270 NS ± 0.6997 | 13.0830 NS ± 0.7708 |
| LDH µm lactate oxidised/ mg protein/ 15 minutes | 42.7848 ± 1.4614 | 21.0405*** ± 0.8604 | 28.0365* ± 0.8601 | 27.4801 NS ± 1.0497 |
| SDH µg fomazan formed/ mg protein/30 minutes | 10.2377 ± 0.3473 | 5.8370**** ±0.6976 | 8.4007** ±0.5538 | 9.1682 NS ±0.5627 |
| Phosphorylase µg phosphorus released/mg protein/ 10 minutes | 80.3946 ± 8.8785 | 103.9687 NS ± 5.0728 | 102.5060 NS ± 6.7124 | 104.5752 NS ± 3.3075 |

(+) Enzyme values in the fresh liver slices not subjected to incubation.
NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$, **** $P < 0.00$

Table 9-3

Effect of acothione, alone or in combination with insulin or acetylcholine, on the enzyme activities in the pigeon liver slices under *in vitro* conditions (Mean ± S.E.M)

| Enzymes | Control(1) (Tissue) | Acothione | Acothione +Insulin | Acothione + ACh |
|---|---|---|---|---|
| $Na^+$-$K^+$-ATPase µg phosphorus released/mg protein/ 10 minutes | 29.6305 ± 0.4778 | 16.8363** ± 1.0770 | 21.2622 ± 1.0733 | 22.6576 ± 1.2960 |
| AChE µm ACh hydrolysed/mg protein/ 10 minutes | 0.7401 ±0.0494 | 0.3792* ±0.0337 | 0.4171* ±0.0513 | 0.2549** ±0.0258 |
| Acid Phosphatase µm P-nitrophenol released/100 mg protein/30 minutes | 156.2681 ± 10.5748 | 246.6198** ± 28.7989 | 238.6776* ± 8.4513 | 203.2963* ± 30.2982 |
| Alkaline Phosphatase um P-nitrophenol released/ 100 mg protein/ 30 minutes | 23.4093 ± 2.0944 | 20.2047 ± 1.7339 | 20.9121 ± 1.8784 | 22.2488 ± 1.2680 |
| LDH µm lactate oxidised/mg protein/ 15 minutes | 45.4365 ± 3.8131 | 24.8623* ± 1.6928 | 29.9403* ± 1.9147 | 26.8567* ± 1.2517 |
| SDH µg formazan formed/mg protein/ 30 minutes | 9.2330 ±0.5022 | 6.3551*** ±0.2389 | 7.7892 ±0.3593 | 7.8580 ±0.4168 |
| Phosphorylase µg phosphorus released/mg protein/10 minutes | 79.8809 ± 4.1955 | 94.0992 ± 3.7417 | 128.0094 ± 10.6409 | 97.6543 ± 7.9786 |

(1) Enzyme values in the fresh liver slices not subjected to incubation.

NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$,

**** $P < 0.001$.

Table 9-4

Effect of prostigmine, alone or in combination with insulin or acetylcholine, on the enzyme activities in the pigeon liver slices under *in vitro* conditions. (Mean ± S.E.M)

| Enzymes | Control(1) (Tissue) | Prostigmine | Prostigmine + Insulin | Prostigmine + ACh |
|---|---|---|---|---|
| $Na^+$-$K^+$-ATPase µg phosphorus released/ mg protein/ 10 minutes | 29.9220 ± 0.4406 | 19.5195** ± 0.7311 | 18.5087** ± 0.6648 | 12.9858**** ± 0.6786 |
| AChE µm ACh hydrolysed/mg protein/ 10 minutes | 0.5619 ±0.0337 | 0.1618** ±0.0105 | 0.1994* ±0.0207 | 0.2597* ±0.0355 |
| Acid phosphatase µm P-nitrophenol released/100 mg protein/30 minutes | 209.9666 ± 5.2382 | 159.3191* ± 15.9843 | 164.9845* ± 19.5637 | 172.7981* ± 16.5195 |
| Alkaline Phosphatase µm P-nitrophenol released/ 100 mg protein/ 30 minutes | 19.6661 ± 0.8049 | 19.4880 ± 0.6156 | 21.8463 ± 0.7938 | 20.3450 ± 0.3817 |
| LDH µm lactate oxidised/mg protein/ 15 minutes | 53.0170 ± 4.0361 | 95.4141* ± 4.2483 | 84.9537* ± 1.2070 | 89.8445* ± 5.4352 |
| SDH µg formazan formed/ mg protein/ 30 minutes | 7.2650 ±0.3620 | 3.7453* ±0.3736 | 6.8768 ±0.1883 | 8.0021 ±0.8595 |
| Phosphorylase µg phosphorus released/mg protein/ 10 minutes | 145.6178 ± 3.7114 | 169.6982 ± 3.8675 | 152.5810 ± 8.2790 | 185.2083 ± 12.6561 |

(1) Enzyme values in the fresh liver slices not subjected to incubation.

NS - Not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$

**** $P < 0.001$.

activities when acothione was present in the medium whether alone or in combination with insulin or ACh. ATPase, alkaline phosphatase, SDH and LDH all showed a decrease (as was the case with monocrotophos) irrespective of the fact that acothione was present alone or in combination with insulin or ACh. Acothione had profound influence over AChE as was evident from the observation that AChE was inhibited even in the presence of ACh or insulin in the medium.

Prostigmine (Table 9-3):

Prostigmine also induced glucose uptake although not as much as that observed with organphosphorus compounds. Prostigmine however, was as effective as monocrotophos and acothione in decreasing the activity of AChE in the liver slices, when present alone or in combination with insulin or ACh. Prostigmine decreased the activity of ATPase significantly in the liver slices while alkaline phosphatase showed no significant variation from the normal level. Acid phosphatase showed a slight but significant decrease, which was contrary to what was observed with monocrotophos or acothione. While SDH showed a decrease, LDH and phosphorylase exhibited an increase in their activities.

Glycogen content of liver slices incubated with monocrotophos, acothione or prostigmine, in presence or absence of insulin or ACh, showed a decrease as was observed in all in vitro experiments indicating more or less a non-enzymic reduction.

Prostigmine + ACh non-significantly increased the LDH activity.

## DISCUSSION

Several investigators have given lists of anti-cholinesterase agents (Holmstedt, 1951; Health, 1961; OBrien, 1967; Beddoe et al., 1971; Lullman et al., 1971). The interaction between the substrate and ChE enzyme moeity involves production of an ac l ted form of enzyme, and through rapid hydrolysis of the enzyme-substrate complex reusable enzyme can be reformed. Anti-cholinesterase compounds bind with aniomic or esteratic site of enzyme and interfere with orderly interaction between the enzyme and the substrate (Silver, 1974). The difference in the degree of cholinesterase inhibition in different tissues by organophosphorus compounds has been reported by Sharma et al. (1973) Koundinya and Ramamurthy (1978) Quari and Ahmed (1979) Dikshith et al. (1980). It has been reported by Bajgar (1972) that the rate of penetration of a charged inhibitor through blood brain barrier in the central nervous system may vary from area to area. He observed that O-ethyl-s-(2-dimethylaminoethyl) methyl phosphothioate, (changed at physiological pH) inhibits acetyl cholinesterase activity at different rates in different parts of the brain in vivo. It has been shown that brain gets least affected compared to liver and muscle which can be explained on account of blood-brain barrier which does not permit the penetration of organophosphorus compounds. Diisopropylflurophosphate is known to inhibit 98 % acetyl cholinesterase activity in abductor muscle

of large mouth Bass (Schneider and Weber, 1975). According to phosphorylation theory, the inhibitor phophorylates the enzyme. Some workers have pointed out that the organophosphorus compounds which are inhibitors contain an anhydride like structure and Aldridge (1953 a,b) has demonstrated amongst a series of analogues that the more unstable they are to hydrolyse the more efficient they are as inhibitors. When the acetlycholinesterase enzyme was inhibited by monocrotophos, acothione and by prostigmine, acetylcholine accumulated and as a result glucose uptake was stimulated. With insulin these compounds did not exhibit an uptake more than that was observed when they were present in the medium alone. The increased glucose uptake shown by monocrotophos and acothione could also be attributed to the fact that acid phosphatase, an enzyme believed to be involved in the uptake of glucose by liver cells, was shown to be increased in the slices. There was no increased metabolic activities in the cells, as evident from the decreased ATPase, SDH and LDH activities in the slices in presence of monocrotophos or acothione, which could account for the increased uptake of glucose. Thus, glucose entered the hepatic cells mainly due to the permeability changes of the hepatocyte membrane with the resulting flow coupled transport.

Prostigmine although could inhibit acetylcholinesterase, as effectively as organophosphorus compounds such as monocrotophos and acothione, failed to increase the uptake of glucose as much as them even in the presence of acetylcholine. This is probably due

to the decreased acid phosphatase activity observed in the liver slices when incubated with prostigmine alone or in combination with acetylcholine. The significant increased activity of phosphorylase may also contribute to the fact that prostigmine did not elicit as much glucose uptake by the liver slices as that was affected by the organophosphorus compounds.