CHAPTER 8

EFFECT OF DEXAMETHASONE ON THE MOVEMENT OF GLUCOSE ACROSS HEPATOCYTE MEMBRANE IN *IN VITRO* CONDITIONS

Glucocorticoid plays a major role in maintaining blood glucose level and most of its actions are manifested in the liver due to the presence of large number of receptors (Ballard *et al.*, 1974). Dexamethasone di-sodium phosphate, a synthetic glucocorticoid, decreased glucose uptake by tissues, along with decreased lactate and pyruvate output and decreased glycogen, G-6-P and glycerol-3-phosphate contents in the cells (Yorks, 1967). Dexamethasone, however, did not alter either the rate of pyruvate utilization, the tissue content of ATP, ADP, AMP, and citrate or the output of glycerol. Dexamethasone also caused small reduction in the incorporation of (1-14C) glycine into adipose tissue protein and nucleic acid fractions. Glucocorticoid induced insulin resistance in man and rats both *in vivo* and *in vitro* (Conn and Fajans, 1956; Fain *et al.*, 1964; Mc Kiddi *et al.*, 1968; Malchoff *et al.*, 1982). Administration of low doses of dexamethasone to rats caused insulin resistance (Kahn *et al.*, 1978; and Olefsky, *et al.*, 1975) and a decreased insulin binding capacity of the cells (Gravin Jr. *et al.*, 1974). Corticosterone administration has also been shown to increase Na^{+}-K^{+}-ATPase activity in the sub-mandibular gland of rat (Bartolomer *et al.*,1983).

Glucocorticoids have diabetogenic effects when given alone or in combination with ACTH or bovine growth hormone. Further, Bates and Garrison (1971) have observed glucosuria in rats treated with corticosterone and dexamethasone either alone or in combination. Again glucocorticoid in excess has been shown to result in insulin resistance as well as in glucose intolerance in man and experimental animals (Conn and Fajans, 1956; Pupo et al.,1966; Mc Kiddie et al., 1968; Modigliani et al., 1970). The insulin resistance was manifested in hyperglycaemia inspite of hyperinsulinemia (Mc Kiddie et al., 1968; Modigliani, et al., 1970) or administration of exogenous insulin (Conn et al., 1956). Adipose tissue of animals treated with glucocorticoid showed decreased basal (Munk and Koritz, 1962; Munk, 1962) and insulin stimulated glucose metabolisms (Benett et al., 1972) with decreased sensitivity to insulin. The binding of insulin to its receptor was decreased in both adipose and hepatic tissues from such animals (Goldfine et al., 1973; Olefsky et al., 1975; Kahn et al., 1978). On the other hand, tissues from animals with adrenal insufficiency showed increased sensitivity to insulin and increased insulin binding (Kahn et al., 1978; Haring et al., 1980). Since glucocorticoid could counteract the action of insulin or vice versa, hyper- or hypo-glycaemic manifestation would depend on the level of these hormones in the circulating

blood. However, the action of glucocorticoid in the presence of ACh or during increased vagal stimulation has not been investigated. The present chapter deals with the action of dexamethasone alone or in combination with insulin or ACh on the movement of glucose across the pigeon liver cells.

MATERIALS AND METHODS

Adult pigeons (Columba livia), weighing 180-250 grams, maintained in laboratory conditions on balanced diet, were used for the present experiment. Animals were sacrificed after an overnight starvation. The liver was perfused with cold KRB buffer and then quickly excised. The liver was placed on ice and cut into thick slices weighing 40-50 mg and these slices were incubated in 10 ml flasks with 5 ml of KRB buffer, containing glucose and albumin. The liver slices were incubated for 90 min. at 37°C in a water bath shaker with 120 oscillations/min. The slices were incubated in media of following different categories.

(1) KRB Medium (5 ml) + Glucose (3 mg/ml) + Albumin (2 mg/ml) + Dexamethasone (.04 mg/ml)

(2) KRB Medium (5 ml) + Glucose (3 mg/ml) + Albumin (2 mg/ml) + Dexamethasone (.04 mg/ml) + insulin (1 Unit/ml)

(3) KRB Medium (5 ml) + Glucose (3 mg/ml) + Albumin (2 mg/ml) + Dexamethasone (.04 mg/ml) + ACh (15 mg/ml).

After the incubation, the slices were quickly washed with chilled KRB Medium and were digested in KOH for glycogen estimation. The glycogen was estimated in fresh slices as well as in slices after incubation. The glucose concentration was determined in the medium before and after incubation and the differences were calculated. Methods followed for glycogen and glucose estimations are described in Chapter 1. At the end of incubation, the liver slices were processed for estimations of Na^+-K^+-ATPase, SDH, LDH, Acid and Alkaline phosphatases, phosphorylase and AChE as per methods given in Chapter 1.

RESULTS

When the liver slices were incubated in a medium containing glucose and dexamethasone, there was no glucose uptake. On the contrary, there was a release of glucose into the medium. When dexamethasone was present in the medium together with insulin or ACh, uptake of glucose into the liver cells took place (Table 8-1, Fig. 8-1).

Na^+-K^+- ATPase activity showed no significant change when the medium contained only dexamethasone. However, Na^+-K^+-ATPase activity showed a significant increase when dexamethasone was present in combination with insulin or ACh. Possibly, the increase was manifested by the action of insulin or ACh. Similarly phosphorylase showed significant increase only when dexamethasone was present with either insulin or ACh (Table 8-2; Fig. 8-2).

Alkaline phosphatase in the liver slices at the end of incubation period showed a significant increase when dexamethasone was present in the medium. There was no further increase in the

Fig. 8-1. Effect of dexamethasone, alone or in combination with insulin or acetylcholine (ACh) on glucose uptake, and glycogen content in pigeon liver slices under *in vitro* conditions.

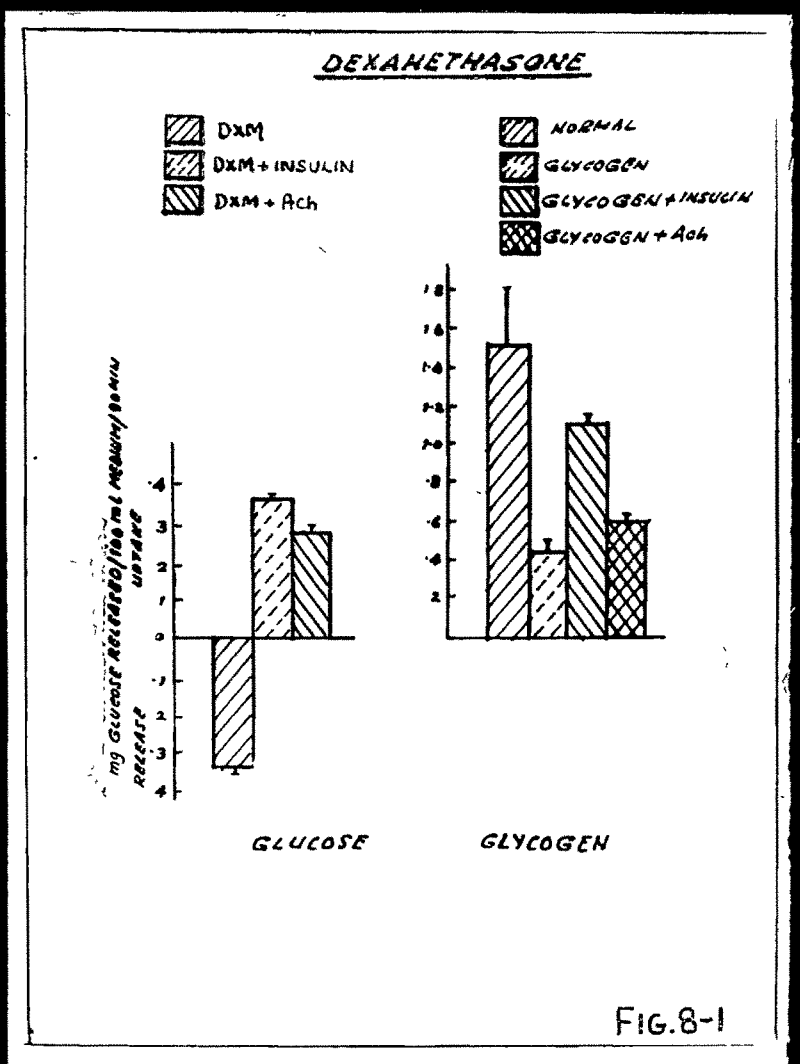
DEXAMETHASONE
DxM
DxM + INSULIN
DxM + Ach
NORMAL
GLYCOGEN
GLYCOGEN + INSULIN
GLYCOGEN + Ach
GLUCOSE
GLYCOGEN

FIG. 8-1

Fig. 8-2. Effect of dexamethasone, alone or in combination with insulin or acetylcholine (ACh) on Na^+-K^+-ATPase and acetylcholinesterase (AChE) activities in pigeon liver slices under <u>in vitro</u> conditions.

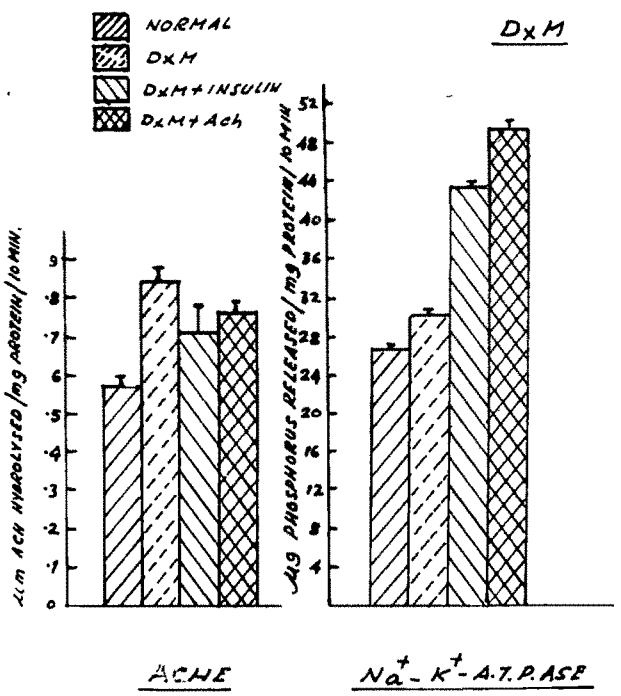
NORMAL
DxM
DxM + INSULIN
DxM + Ach
DxM
µm ACh HYDROLYSED/mg PROTEIN/10 MIN.
µg PHOSPHORUS RELEASED/mg PROTEIN/10 MIN
ACHE
Na+ - K+ - A.T.P.ASE

FIG. 8-2

Fig. 8-3. Effect of dexamethasone, alone or in combination with insulin or acetylcholine (ACh) on acid phosphatase (Ac Pase) and alkaline phosphatase (Alk PO_4ase) activities in pigeon liver slices under <u>in vitro</u> conditions.

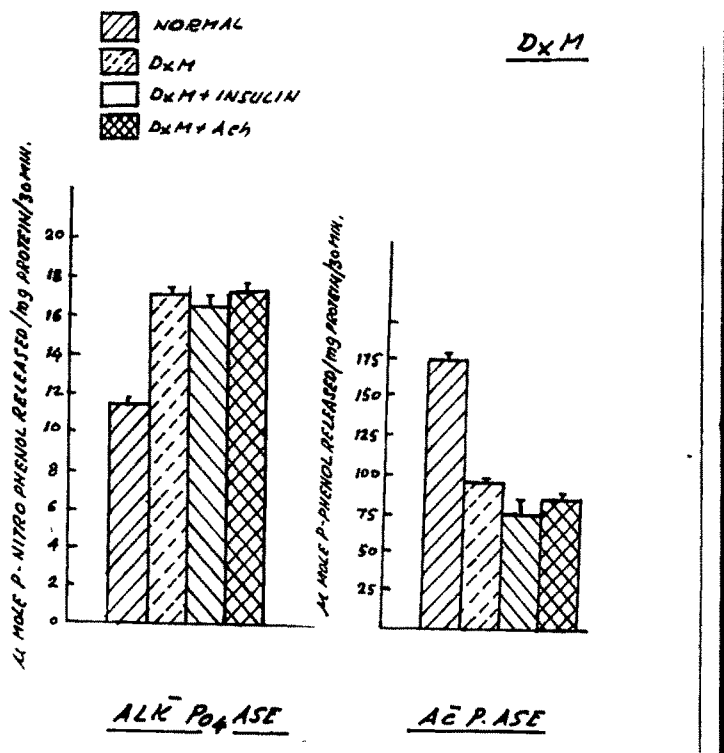
NORMAL
DxM
DxM + INSULIN
DxM + Ach
DxM
μ MOLE P-NITRO PHENOL RELEASED/mg PROTEIN/30 MIN.
0
2
4
6
8
10
12
14
16
18
20
μ MOLE P-PHENOL RELEASED/mg PROTEIN/30 MIN.
25
50
75
100
125
150
175
ALK PO4 ASE
ACP. ASE

FIG. 8-3

Fig. 8-4. Effect of dexamethasone, alone or in combination with insulin or acetylcholine (ACh) on lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH) in pigeon liver slices under *in vitro* conditions.

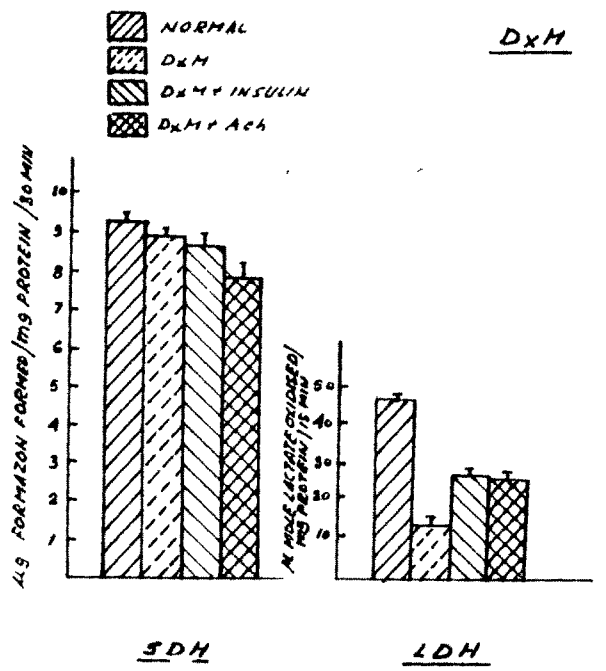
NORMAL
DxM
DxM + INSULIN
DxM + Ach
DxM
µg FORMAZON FORMED/mg PROTEIN /30 MIN
µ MOLE LACTATE CLEARED/ mg PROTEIN/15 MIN
SDH
LDH

FIG. 8-4

Table 8-1

Effect of dexamethasone, alone or in combination with insulin or acetylcholine on the uptake or release of glucose by pigeon liver slices under *in vitro* conditions. (Mean ± SEM)

Additives	Glucose		Glycogen Depletion (2)
	Uptake (1)	Release (1)	
Dexamethasone	-	0.3330 ±0.0224	1.0903 ±0.2311
Dexamethasone + Insulin	0.3602 ±0.0132	-	0.3783 ±0.0434
Dexamethasone + ACh	0.2773 ±0.0247	-	0.8319 ±0.2605

(1) Mg glucose taken up or released by 100 mg liver.

(2) Mg glycogen depletion/100 mg liver.

Table 8-2

Effect of dexamethasone alone or in combination with insulin or acetylcholine, on the enzyme activities in the pigeon liver slices under *in vitro* conditions.

Enzymes	Control(1) (Tissue)	Dexamethasone	Dexamethasone + Insulin	Dexamethasone + ACh
Na^+-K^+-ATPase µg phosphorus released/mg protein/ 10 minutes	26.70 ± 0.51	30.00 NS ± 3.30	43.15*** ± 3.43	49.86**** ± 1.84
AChE µm ACh hydrolysed/mg protein/ 10 minutes	0.57 NS ±0.03	0.84*** ±0.04	0.71 NS ±0.07	0.76*** ±0.03
Acid Phosphatase µm P-nitrophenol released/100 mg protein/30 minutes	175.77 ± 2.18	94.36**** ± 1.84	75.31**** ± 9.41	85.92**** ± 2.75
Alkaline Phosphatase µm P-nitrophenol released/ 100 mg protein/ 30 minutes	11.38 ± 0.49	19.09**** ± 0.76	18.36**** ± 0.72	19.26**** ± 0.58
LDH µm lactate oxidised/mg protein/ 15 minutes	46.92 ± 3.15	14.67**** ± 1.24	27.96**** ± 1.82	26.21**** ± 2.29
SDH µg formazan formed/mg protein /30 minutes	9.22 ±0.32	8.81 NS ±0.29	8.68 NS ±0.34	7.82* ±0.45
Phosphorylase µg phosphorus released/mg protein/ 10 minutes	237.99 ± 10.64	263.31 NS ± 17.14	363.98**** ± 10.80	294.19*** ± 9.91

(1) Enzyme values of fresh slices not subjected to incubation.

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

*** $P < 0.01$, **** $P < 0.001$

activity of the enzyme when dexamethasone was present in the medium along with insulin or ACh. Acid phosphatase, on the contrary showed a significant decrease in the presence of dexamethasone, and this decrease in activity remained so in spite of the presence of insulin or ACh (Table 8-2;Fig.8-3).

Both SDH and LDH showed a reduced activity in the liver slices when dexamethasone was present alone in the medium. Insulin or ACh did not prevent the dexamethasone induced inhibition of enzyme activity (Table 8-2; Fig. 8-4).

A more or less significant increase was observed in the activity of AChE whether dexamethasone was present alone or in combination with insulin or ACh (Table 8-2; Fig. 8-2).

Glycogen content showed a decrease irrespective of the combination of additives employed (Table 8-1; Fig. 8-1).

DISCUSSION

The ability of dexamethasone to inhibit glucose uptake is believed to be through its inhibition of hexokinase. The decrease in the rate of glucose transport could be also due to depression of carrier synthesis or reversible conversion of carrier to an inactive apo-carrier. The ability of insulin to reverse rapidly the dexamethasone induced inhibition of glucose uptake was tested to distinguish between the two possibilities (Yorke, 1967). There is an evidence that glucose transport in adipose tissue can be

mediated by carrier mechanism, similar to that postulated for this process in erythrocytes and muscle. The maximum velocity of a carrier mediated transport system is determined by the concentration of active carrier in the membrane and the rate of translocation of the substrate-carrier complex through the membrane. The concentration of active carrier could be regulated either by changes in its rates of de-novo synthesis or break down or by the relative rate of its conversion to and from an inactive precursor. A decrease in the concentration of active carrier causes decrease in the maximum velocity. However, the rapid and complete reversal by insulin of the dexamethasone induced inhibition of glucose uptake would disagree with a change in total available carrier concentration (active carrier + inactive precursor). Thus it seems more likely that these hormones exert their influence on glucose transport in adipose tissue by altering the rate of interconversion of active carrier and its precursor or by altering the mobility of glucose-carrier complex.

Adipose tissue taken from adrenalectomized rats treated with dexamethasone released fatty acids at faster rates in vitro, than the tissue from unoperated controls (Fain, 1962). The accelerated release of fatty acids was associated with an inhibition of glucose uptake (Fain et al., 1963). The effect of dexamethasone on fatty acid release and reduction of glucose uptake was suppressed by the addition of a small amount of insulin (Cahill et al., 1960). When dexamethasone and insulin were present together the effect of insulin dominated and only

glucose uptake resulted (Fain et al., 1963).

Dexamethasone is known to produce some adaptive changes in the plasma membrane and to cause insulin resistance which could result in an inhibition of glucose uptake. Previous reports indicate that insulin resistance produced in vivo by glucocorticoid administration is accompanied by decreased insulin binding (Olefsky, 1975; Kahn et al., 1978; De Pirro et al., 1981) and post receptor alteration according to Begum et al. (1984).

In the present experiment it was seen that dexamethasone did not stimulate glucose uptake, but when insulin was present together with dexamethasone in the medium, glucose uptake by liver cells occurred. ACh also stimulated glucose uptake even in the presence of dexamethasone. Insulin represses the stimulating effect of glucocorticoid on several key enzymes involved in hepatic gluconeogenesis. Glucocorticoid treated liver slices accumulate G-6-P which is derived from gluconeogenesis (Steiner, 1969). In this context, the presently observed release of glucose from liver slices into the medium in presence of dexamethasone could be due to the glucose derived from the accumulated G-6-P.

The most interesting observation is that dexamethasone increased the activity of alkaline phosphatase in the liver slices. This increased activity of the enzyme was not counteracted by insulin or ACh. Alkaline phosphatase is active

in liver of birds that consume large amount of protein and fat (insectivorous or raptorials). In other words, alkaline phosphatase is mainly involved in glucose release from the liver. The action of dexamethasone on the release of glucose could be thus through its stimulatory effect on alkaline phosphatase. However, insulin or ACh failed to suppress the stimulation of alkaline phosphatase by dexamethasone. Acid phosphatase on other hand decreased when the slices were incubated in [illegible] medium containing dexamethasone and this action was again not countered by insulin or ACh. Acid phosphatase is very active in liver of birds such as pigeon which consume large amount of carbohydrate rich food. Various other observations in our laboratory lead to the belief that acid phosphatase is involved in carbohydrate assimilation. Insulin or ACh when present alone in the medium effected a very high rate of glucose uptake by liver slices (Chapter 2). But, when these were present along with dexamethasone the uptake was very much reduced.

Glucocorticoid excess and glucocorticoid deficiency *in vivo* result in characteristic changes in the binding of insulin to its receptors and in the degree of insulin's biological actions (Conn *et al.*, 1950; Munck, 1962; Munck *et al.*, 1962; Wajchenberg *et al.*, 1964; Pupo *et al.*, 1966; Mc Kiddie *et al.*, 1968; Modigliani *et al.*, 1970; Bennet *et al.*, 1972; Goldfine, 1973; Olefsky *et al.*, 1975; Kahn *et al.*, 1978; Haring *et al.*, 1980). In glucocorticoid excess, insulin binding is inhibited secondary

to a decrease in the affinity of the receptor for insulin (Goldfine et al., 1973; Kahn, 1978); and insulin stimulated glucose metabolism as well as basal glucose metabolism are also decreased (Munck et al., 1962; Munck, 1962; Bennett et al., 1972). Incubation of rat adipose tissues with dexamethasone for short duration decreased basal glucose metabolism (Le Boeuf, 1962; Yorks, 1967; Fain, 1964; Czech and Fain, 1972; Olefsky, 1975). Incubation of tissues for long period in medium containing dexamethasone reduced the ability of insulin to stimulate glucose uptake (Goldfine et al., 1973; Olefsky, et al., 1975; Kahn et al., 1978). There are also reasons to believe that individual tissues may respond differently to the same hormone. Thus, glucocorticoids have been shown to decrease insulin binding capacity in fat and liver cells and human erythrocytes (Yasuda, 1980) while there was increased binding to human monocytes and lymphocytes (Beck-Nielson, 1980). Similar differences in glucocorticoid-induced changes in insulin binding capacity of receptors may also occur in in vitro situations. Several studies demonstrated that glucocorticoid hormone produces insulin resistance by directly affecting both the insulin receptor and post receptor steps. The failure to produced maximal response to insulin, despite the presence of spare receptors, implicates inhibition of post receptor steps. Kahn (1978) preferred designating this as insulin 'unresponsiveness' in contrast to changes in sensitivity of receptors to insulin. Thus, the decreased affinity of

receptors for insulin in glucocorticoid treated cells was possibly also accompanied by modifications of post receptor steps. Probably, glucocorticoid hormones decrease the efficiency of coupling of the hormone-receptor complex to biological responses. Recently, evidence has been obtained from in vivo studies that showed a decreased effectiveness of insulin bound to its receptor in hypercortisolemia (Rizza et al., 1981). Changes in both receptor and post receptor steps have also been implicated in the insulin resistance of obesity and hyperinsulinemia (Czech, 1977; Koltarman, 1980; Marshal and Olefsky, 1980). The post receptor steps affected by dexamethasone may involve inactivation of several glycolytic enzymes and activation of gluconeogenic enzymes. Precisely, this was what occurred in the slices incubated in medium containing dexamethasone. Activities of enzymes such as acid phosphatase, SDH and LDH were found to decrease while alkaline phosphatase showed an increase in activity in the slices incubated in medium containing dexamethasone. When insulin or ACh was present in the medium together with dexamethasone, glucose uptake was observed inspite of the fact that the activity levels of most of the enzymes studied were similar to what was seen in slices incubated with dexamethasone alone. In other words, both insulin and ACh could, to some extent, suppress the receptor changes induced by dexamethasone but not the post receptor steps. However, one must bear in mind that,

the type of interaction between insulin or ACh and dexamethasone explained herein is vaild only for the concentration of these substances and the length of incubation period employed in this study. A variation in the concentration and/or incubation time too may affect the type of interactions.