MODIFICATION OF LIVER AND SERUM ENZYMES BY PARAQUAT TREATMENT IN RABBITS

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ABSTRACT

Paraquat (PQ) is known to cause progressive interstitial fibrosis in the lungs. Previous investigations have indicated that PQ acts by lipid peroxidation of the membrane. However, there are few reports on the action of PQ on hepatic enzymes. This work was carried out to investigate the modulation of various hepatic enzymes by PQ in rabbits. Paraquat was administered at a dose of 3, 6 or 12 mg/kg b. wt/day intraperitoneally to male rabbits for different periods of time. Administration of paraquat resulted in a significant decrease in plasma activities of transaminase enzymes, alkaline phosphatase and liver transketolase. No significant change was found in the activities of plasma and hepatic lactate dehydrogenase and alkaline phosphatase. A marked increase in blood glucose was noticed 48 hours after paraquat administration.

KEY WORDS

paraquat, alkaline phosphatase, lactate dehydrogenase, glutamate oxalate transaminase, glutamate pyruvate transaminase, transketolase

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INTRODUCTION

Paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride, PQ) is a broad spectrum herbicide. It is highly toxic to mammalian species and is well known to result in the formation of delayed pulmonary lesions characterized by progressive interstitial fibrosis, associated with a high degree of lethality both in animals and man /1-3/. The incidence of human poisoning, caused by deliberate or inadvertent intake of paraquat solution, makes this a substance of great toxicological interest /4/.

Studies of PQ toxicity have indicated that it acts through lipid peroxidation of the affected membrane /5/. Relatively few studies have been carried out /6-9/ to investigate the effect of PQ on enzyme levels in animals. Most studies have used mice as the susceptible model, whereas rabbits have been reported to be PQ resistant /1, 6-8, 10/. Thus, very little is known about variations in enzyme levels after the exposure of rabbits to paraquat.

In the present study various enzymes were studied in rabbits exposed to PQ using variable doses and time. The variations in enzyme levels could then be used as an index of PQ toxicity. In a previous study /7/, we showed that rabbits are not resistant to PQ, since large variations were observed in the membrane lipid moieties of PQ treated animals. These changes probably took place through lipid peroxidation, thus seriously damaging the integrity of the living cell /7/.

MATERIALS AND METHODS

The present study was conducted mainly on male rabbits of mixed breed with an average weight of 1.5-2.0 kg. Paraquat was supplied as "gramoxone" and was purchased from Imperial Chemical Industries Ltd., England. Reduced nicotinamide adenine dinucleotide, malic dehydrogenase, alpha-ketoglutarate, pyruvate, L-aspartate, DL-alanine, triose phosphate isomerase-glycerophosphate dehydrogenase mixture (TPI/GDH), ribose-5-phosphate and xylulose-5-phosphate

Abbreviations: PQ, paraquat (1,1'-dimethyl-4,4'-bipyridylium dichloride); ALP, alkaline phosphatase; LDH, lactate dehydrogenase; GOT, glutamate oxalate transaminase; GPT, glutamate pyruvate transaminase; TK, transketolase; derived LDH, total plasma LDH - myocardium LDH.

were obtained from Sigma Chemical Co., St. Louis, MO, USA. DEAE-Sephadex A-50 was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. All other chemicals were of analytical grade.

Rabbits were given intraperitoneal injections of PQ dissolved in normal saline. Rabbits were divided into four groups: the first group (31 rabbits) was given daily intraperitoneal PQ injections at a dose of 3 mg/kg/day, the second group (38 rabbits) 6 mg/kg/day, the third group (7 rabbits) 12 mg/kg/day, and the fourth group (23 rabbits) received normal saline which served as the control. The four groups of rabbits were then each divided into five subgroups and received the following regimen of injections:

Subgroup 1 received one daily dose for 1 day;
Subgroup 2 received one daily dose for 2 days;
Subgroup 3 received one daily dose for 3 days;
Subgroup 4 received one daily dose for 1 week;
Subgroup 5 received one daily dose for 2 weeks.

At the end of the treatment period, animals were anaesthetized by an intraperitoneal injection of sodium pentobarbitone. The animals were then sacrificed by cutting the carotid artery, and blood was collected in citrated tubes. The samples were centrifuged at 3000 rpm for 10 minutes. Plasma was stored in small aliquots at -20°C until used for enzyme assay.

Glutamate oxalate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activities were determined according to the methods described by Karmen /11/ and Wroblewski and La Due /12/, respectively. Lactate dehydrogenase (LDH) isoenzyme activity was measured by the method of Bergmeyer and Bernt /13/. Alkaline phosphatase (ALP) activity was measured using the method described by Bowers and McComb /14/.

Liver, lungs, heart and kidneys were collected, immediately washed with normal saline, blotted on filter paper, weighed and frozen until use. For determination of liver enzymes, one part of the liver was homogenized for 30 seconds in nine parts of cold Tris-HCl buffer (50 mM, pH 7.6). The clear supernatant was assayed for total LDH and transketolase (TK) activities /15/.

Student's t-test and paired t-test were used for statistical analysis. Significance was accepted at $p < 0.05$.

RESULTS

Out of 75 rabbits, 16 (21%) died. No deaths occurred in control rabbits and rabbits treated with 3 mg/kg/day during the study period. The deaths occurred in the rabbits treated with the higher doses of PQ (6 and 12 mg/kg/day). One hundred percent mortality rate was observed in rabbits administered PQ at a dose of 12 mg/kg//day and a 50% mortality rate in the 6 mg/kg/day dose group after six days.

The effect of intraperitoneal administration of PQ on the activities of plasma transaminases at different time intervals in shown in Figures 1 and 2. Animals treated with a dose of 3 mg/kg/day of PQ showed a considerably lower activity of GOT and GPT as compared to controls. The decrease in activity was significant at 3 days and one week, respectively. The decrease in GOT activity was 33% at one week and 37% at two weeks of PQ treatment. However, administration of PQ at a dose of 6 mg/kg/day resulted in an initial non-significant increase in the activity of both GOT and GPT at 24 and 48 hours. This was followed by a significant decrease in the activity of these enzymes at 72 hours, with a perpetual decrease at one and two weeks.

The effect of PQ on the activity of plasma alkaline phosphatase is illustrated in Figure 3. PQ treated animals at both doses caused a significant decrease in ALP activity as compared to controls. A significant decrease in the activity of this enzyme was first seen at 48 hours, followed by a further decrease at one week, with a slight increase in its activity at 72 hours. However, the activity of this enzyme returned to normal levels at two weeks. The hepatic alkaline phosphatase activity of PQ-treated animals was significantly increased in the 3 mg/kg/day group after two weeks of treatment. The animals treated with PQ at a dose level of 6 mg/kg/day did not show any significant change in activity (Table 1). Plasma activities of total lactate dehydrogenase and derived hepatic LDH are given in Tables 2 and 3. PQ treated animals at a dose of 3 mg/kg/day demonstrated a gradual but insignificant decrease in total LDH up to 3 days. This was followed by a gradual but insignificant increase after one and two weeks of PQ treatment. The activity was increased by 13% after two weeks. A similar pattern of LDH was observed in rabbits treated with 6 mg/kg/day. Hepatic derived LDH activity did not change significantly at either dose. Liver LDH activity is shown in Table 4. A gradual increase in liver LDH activity was observed in the 3 mg/kg/day PQ treated group until it reached a significant level at 3

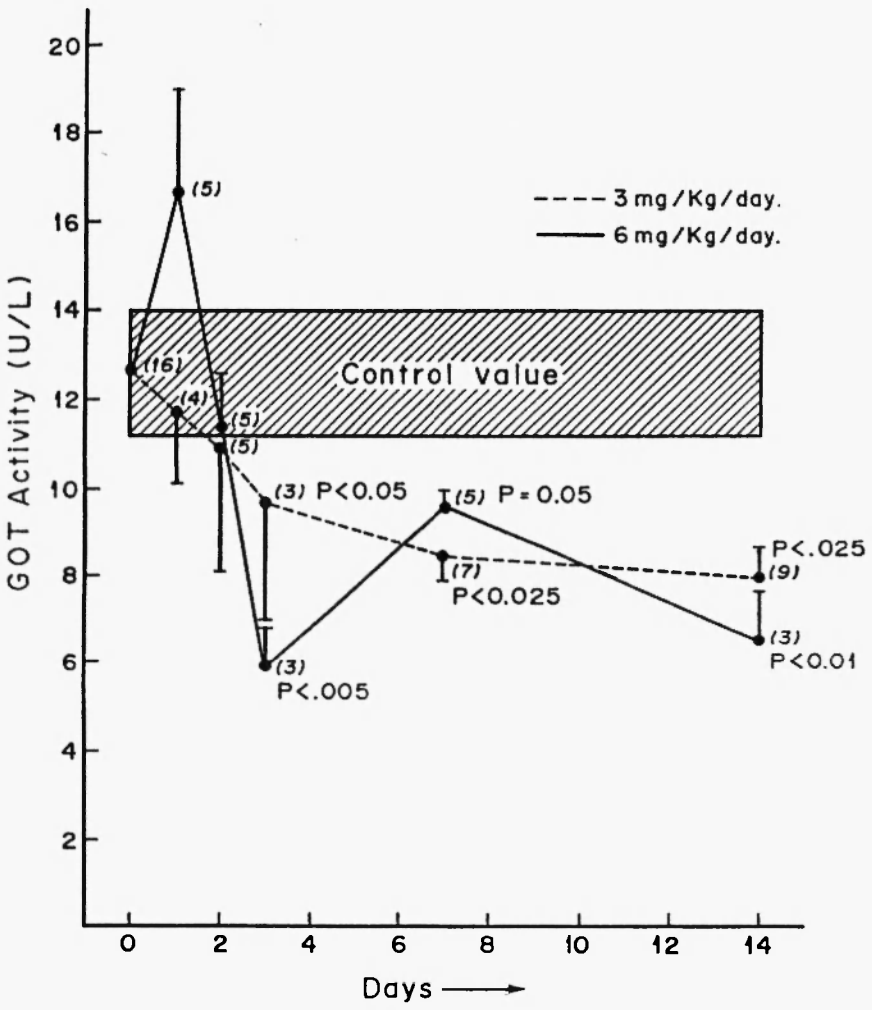

Fig. 1: Effect of paraquat on glutamate oxalate transaminase activity in plasma. Rabbits were injected intraperitoneally with 3 mg and 6 mg/kg/day of PQ. Each point represents the mean value±SE, with the number of animals in parentheses. P values on the vertical lines represent the level of statistical significance between PQ-treated rabbits and control animals. The activity is expressed as U/l.

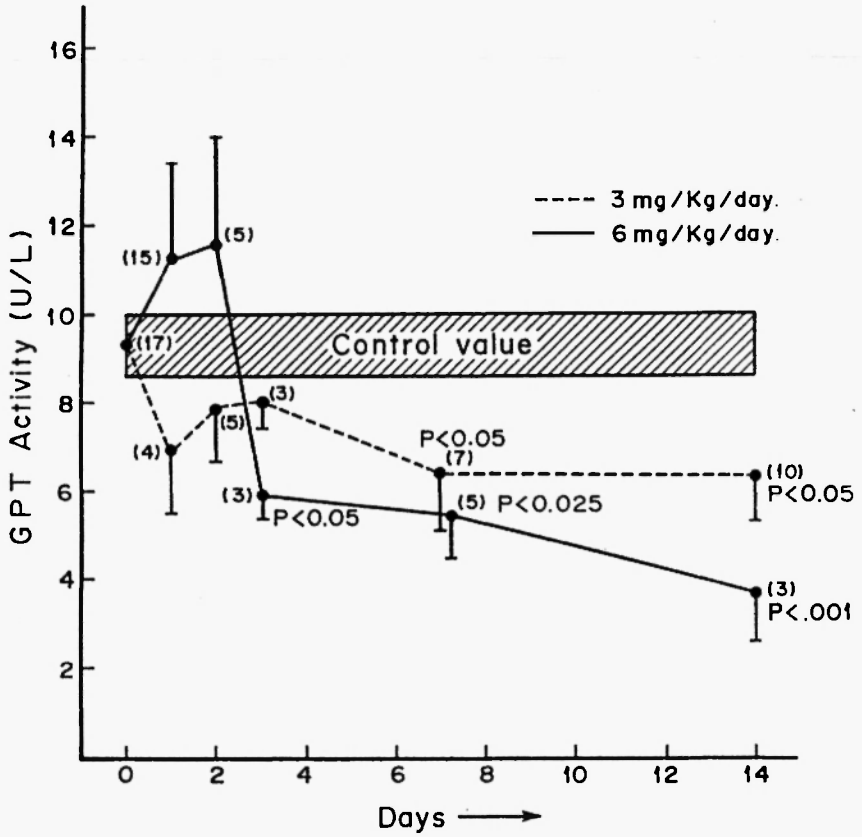

Fig. 2: Effect of paraquat on glutamate pyruvate transaminase activity in plasma. Rabbits were injected intraperitoneally with 3 mg and 6 mg/kg day of PQ. Each point represents the mean value±SE, with number of animals in parentheses. P values on the vertical lines represent the level of statistical significance between PQ-treated rabbits and control animals. The activity is expressed as U/l.

days. This increase was followed by a perpetual decline in activity at one and two weeks. There were no significant changes in the liver LDH activity in the group treated with PQ at a dose level of 6 mg/kg/day. The liver TK activity in the PQ treated group was decreased as compared to controls, reaching statistical significance at both 3 and 7 days after treatment (Fig. 4).

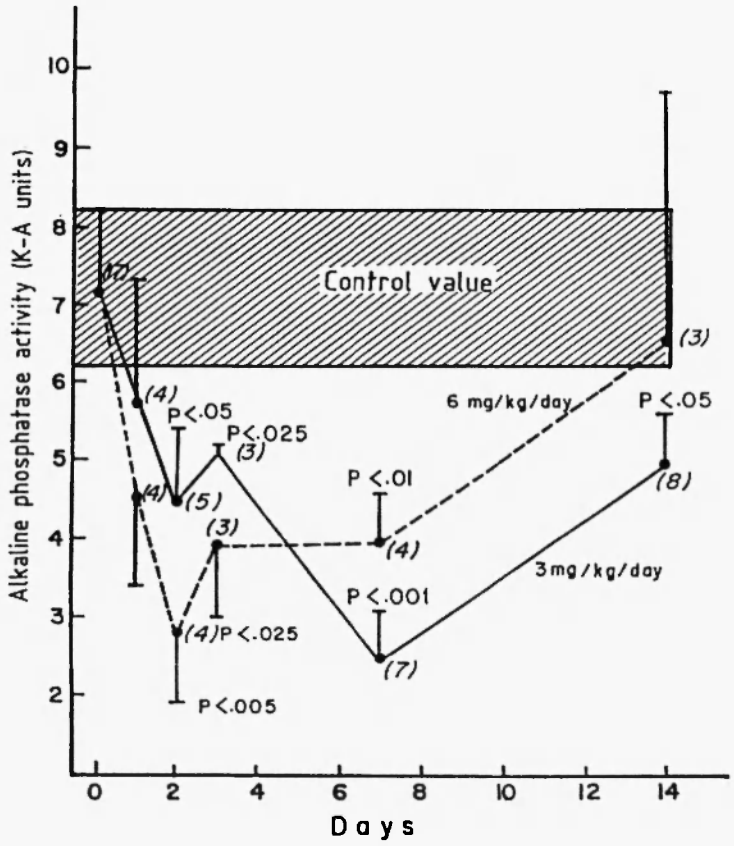

Fig. 3: Effect of paraquat on alkaline phosphatase activity in plasma. PQ was administered intraperitoneally at 3 mg and 6 mg/kg/day. Each point represents the mean value±SE, with number of animals in parentheses. P values on the vertical lines represent the level of statistical significance between PQ-treated rabbits and control animals. The activity is expressed as K-A units.

The decrease in ALP activity in the present study is in agreement with observations of other workers /7/. The increase of ALP activity to its normal level after two weeks of PQ treatment of rabbits could be due to the adaptation of animals to PQ toxicity during this period. Again, the decrease in ALP activity suggests either an inhibition of the enzyme or decreased synthesis due to PQ toxicity.

TABLE 1
Effect of PQ on hepatic ALP activity

	PQ treatment, mg/kg/day	
	3 mg	6 mg
Time	ALP mmole/g liver/min	
Control	4.4±1.25 (6)	4.4±1.25 (6)
1 day	4.6±1.19 (5)	2.6±0.57 (5)
2 days	3.2±1.00 (5)	4.2±1.38 (5)
3 days	6.6±0.69 (3)	13.2±5.55 (3)
2 weeks	10.7±1.34*(4)	6.2±0.93 (4)

Results are expressed as mean±SE.
The numbers in parentheses indicate the number of animals in each group.
*Significant at $p<0.05$.

TABLE 2
Effect of PQ on the total plasma and hepatic derived LDH activities

	PQ treatment, 3 mg/kg/day	
	Total plasma	Hepatic derived
Time	LDH, U/l	
Control	113.0±11.6 (17)	91.0±12.4 (16)
1 day	102.1±10.6 (5)	76.0+7.7 (5)
2 days	79.9±7.8* (5)	60.7+3.6 (5)
3 days	75.5±10.9 (3)	69.9±13.3
1 week	94.3±19.1 (7)	80.1±18.4 (7)
2 weeks	127.4+12.1 (10)	106.6±13.0 (10)

Results are expressed as mean±SE
The numbers in parentheses indicate the number of animals in each group.
*Significant at $p<0.05$.

The observed increase in the activity of various enzymes, such as GOT, GPT, ALP and LDH, at 3 days by PQ treatment suggests that the 3 days are probably the initial line of defence of these animals to combat PQ toxicity. The consistent decrease in the levels of all the enzymes studied in these experiments indicates a weakening resistance of the animals for about one week and then a probable adaptation of the animals to PQ toxicity at about two weeks, thus returning the enzyme levels back to normal.

TABLE 3
Effect of PQ on the total plasma and hepatic derived LDH activities

	PQ treatment, 6 mg/kg/day	
	Total plasma	Hepatic derived
Time	LDH, U/l	
Control	113.0±11.6 (17)	91.0±12.4 (16)
1 day	100.0±14.0 (5)	76.0±7.7 (5)
2 days	74.4±14.9 (5)	58.1±17.3 (5)
3 days	83.0±11.8 (3)	69.2±9.9 (3)
1 week	161.0±58.8 (5)	93.9±35.7 (5)
2 weeks	111.3±28.0 (3)	88.4±26.0 (3)

Results are expressed as mean±SE.
The numbers in parentheses indicate the number of animals in each group.

TABLE 4
Effect of PQ on hepatic LDH activity

	PQ treatment, mg/kg/day	
	3 mg	6 mg
Time	LDH μmole/g liver/min	
Control	105.7±11.3 (16)	105.7±11.3 (16)
1 day	107.2±3.0 (3)	102.4±20.8 (5)
2 days	115.4±11.7 (5)	94.4±13.6 (4)
3 days	189.8±9.0* (3)	152.6±10.8 (3)
1 week	87.0±14.9 (8)	96.4±18.4 (6)
2 weeks	86.1±9.2 (7)	84.0±16.6 (3)

Results are expressed as mean±SE.
The numbers in parentheses indicate the number of animals in each group.
*Significant at $p < 0.05$.

Liver LDH decreased insignificantly after two weeks of PQ treatment. A decrease in LDH activity in liver after a long period of treatment has also been observed in other types of herbicide intoxication /16/. In plasma, the activity level of LDH showed an increase after two weeks. A similar increase occurs in a wide variety of disorders, such as myocardial infarction, acute hepatic disease, pulmonary infarction and a variety of muscle diseases. Interestingly, at 14 days the activity returned to normal levels.

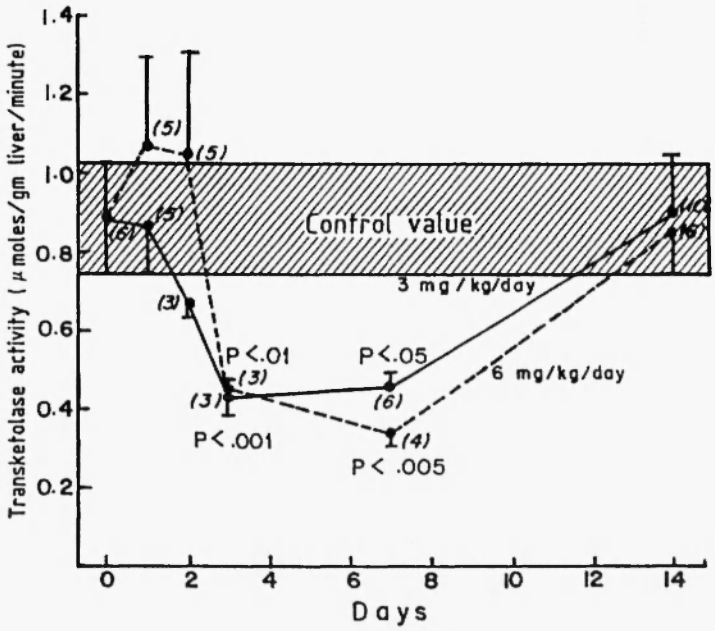

Fig. 4: Effect of paraquat on transketolase activity in liver. Rabbits were administered PQ intraperitoneally at 2 different doses of 3 mg and 6 mg/kg/day. Each point represents the mean value±SE, with number of animals in parentheses with the level of significance at the vertical line. The activity is expressed as μmoles/g liver/minute.

TABLE 5
Effect of PQ on glucose concentration

	PQ treatment, mg/kg/day	
	3 mg	6 mg
Time	Glucose concentration mg/dl	
Control	122.5±8.0 (13)	122.5±8.0 (13)
1 day	117.6±11.4 (5)	120.8±7.4 (5)
2 days	119.8±10.8 (5)	116.2±3.6 (5)
3 days	172.3±1.2* (3)	159.0±3.4 (3)
1 week	160.4±14.4* (7)	249.0±28.0 (3)
2 weeks	167.5±16.7* (6)	106.7±10.6 (3)

Results are expressed as mean±SE.
The numbers in parentheses indicate the number of animals in each group.
*Significant at $p < 0.05$.

The plasma sugar concentrations of rabbits treated with PQ i.p. are listed in Table 5. PQ treated animals at a dose of 3 mg/kg/day demonstrated a gradual increase in glucose concentration with a significant increase at 3, 7 and 14 days. The animals treated with 6 mg/kg/day PQ showed an increase in plasma sugar concentration at 3 and 7 days, but this increase was not statistically significant.

DISCUSSION

Out of 75 rabbits at risk, 16 (21%) died, a result comparable with that observed by Butler and Kleinerman /17/. Hair loss, drainage around nose and eyes and diarrhea observed in this study support the observations of Clark *et al.* /1/, who reported similar symptoms of paraquat toxicity in their study on rats and rabbits.

The activities of transaminase enzymes were measured as indicators of liver cell injury and hepatotoxicity. With any liver injury, an increased level of GPT should be observed. By contrast, the plasma levels of these enzymes had decreased significantly in PQ treated animals. These results are in agreement with the results of Vuksa *et al.* /8/, who reported a decrease in the activity of these enzymes after PQ administration in the drinking water of rats for 30 days. Our histological studies on PQ treated rabbits have not shown considerable damage to liver cells, and hence leakage of these enzymes to plasma is not likely to occur (Afzal *et al.*, manuscript in preparation).

A significant decrease in TK activity of PQ treated rabbits at 3 and 7 days may be due to the inhibition of some of the enzymes of the pentose phosphate pathway which may be speculated as causing a decrease in the levels of cytoplasmic NADPH. So far no reports have been published about the direct effect of PQ on TK activity.

The increased level of plasma glucose concentration associated with PQ treatment is in agreement with the results of Giri *et al.* /6/, who observed an increase in glucose concentration due to decreased insulin levels caused by the effect of PQ on the beta cells of the islets of Langerhans.

In summary, the present investigation suggests that PQ toxicity causes a decrease in the activity of transaminases, alkaline phosphatase, transketolase and liver LDH, either by inhibition of the enzyme or by decreased synthesis. The dose efficacy of 3 mg and 6 mg of PQ in rabbits was found to be the same, which might result from poor

absorption of PQ at higher concentrations. Poor absorption of PQ at higher concentrations has been reported by Barabas *et al.* /18/ and Vuksa *et al.* /8/, but more work needs to be carried out on this point.

ACKNOWLEDGEMENTS

The technical assistance in the preparation of this manuscript rendered by Ms. J. Erickson, Mr. W. Robert Morrow and Mr. P.E. Jordan is greatly appreciated. We appreciate the scholarly input in the preparation of this manuscript by Dr. C.J. DiFonzo. The authors are thankful to the Research Management Unit, Kuwait University, for a project grant no. SBO-21.

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