CHAPTER II A

STUDIES ON THE EFFECTS OF DRUGS ON MUSCLE

1. TRANQUILISERS

INTRODUCTION

Studies on effects of tranquilisers have revealed:
(i) Sedative effects on organisms and tissue metabolism
(Satoskar and Bhandarker, 1975), (ii) on activity of enzymes
and (iii) release of hormones (Beckman, 1958).
However, not much is known about their effects on metabolism
in skeletal muscle. Therefore, it was of some interest to
investigate the effects of tranquilisers (Chlorpromazine,
Chlordiazepoxide and Butobarbitone) on some enzymes and
metabolites of the pectoralis muscle of rats.

MATERIAL AND METHODS

Adult, healthy albino rats (Rattus norvegicus) weighing
between 200 to 250 gms (males) were maintained under standard
laboratory conditions (Temperature 26°C ± 2°C) on normal supply
of food (Hindustan Lever Ltd., Bombay) and water ad libitum.
The following drugs (Tranquilisers) were used:
(1) Chlorpromazine (Largactil) injections I.P., 1% W/V,
May and Baker Ltd., Bombay-78, lot No. 328.
(11) Chlordiazepoxide (Ifibrium) N.F. 10 mg/tablet, Unique
Pharmaceutical Laboratories, Bombay-18, lot No. 1687.
(iii) Butobarbitone (Soneryl) 100 mg/tablet B.P., May and Baker Ltd., Bombay-78, lot No. 1495.

Of the three drugs (i) and (ii) are known to be very soluble in water, whereas (iii) is sparingly soluble (Satoskar and Bhandarkar, 1975). Therefore, a known quantity of each drug was dissolved in water of known volume so as to obtain a concentration of 1 mg/ml. In case of (ii) and (iii), the tablets were first powdered, dissolved in water and the clear aqueous layer was used for the experiments. The animals were given an intramuscular injection of the respective drug (1 mg/animal) daily for 7 and 15 days continuously and then sacrificed. Each group consisted of 12 animals. The intramuscular injection is the most appropriate mode of administration of each of these drugs, since a subcutaneous injection may cause local irritation and intravenous administration might prove fatal due to a sudden fall in blood pressure (Satoskar and Bhandarkar, 1975). To a group of animals (six rats), combined treatment of chlorpromazine (CPZ) and ascorbic acid (100 mg/animal/day) was given for 7 days. After the respective treatments, the animals were sacrificed by decapitation specifically, in order to avoid any side effects of Nembutal or ether anaesthesia. The analyses of various enzymes and metabolites were carried out on pectoralis major muscle of normal (control) and drug
treated animals as detailed in Chapter I A. Six replicates were done for each treatment and parameter. The results were analyzed statistically using the student's 't' test. 

(i) Succinate dehydrogenase (Kun and Abood, 1949); for histochemical localization of SDH (George and Talesara, 1961)

(ii) Alkaline and acid phosphatases (Belfield and Goldberg, 1971)

(iii) Phosphorylase (Fiske and Subbarow, 1925 and Cori et al., 1943)

(iv) Fructose (Foreman et al., 1973)

(v) Glycogen (Seifter et al., 1950)

(vi) Protein (Gornall et al., 1949)

(vii) Free ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complex (AA-MM) (Chinoy et al., 1974)

(viii) Ascorbic acid free radical (AA-FR) special peroxidase (Chinoy, 1973)

(ix) Citric acid (Ettinger et al., 1952)

The tissue was homogenized in 5 ml of 0.5 N perchloric acid and kept in refrigerator for 2-3 hours. The solution was neutralised with 2.5 ml of 1 N KOH and allowed to remain for 3 hours at 20°C and thereafter centrifuged. Four ml of the supernatant was used for the assay to which was added 1 ml of 9 N H₂SO₄. The tubes were cooled below 15°C in an ice
bath and then 0.5 ml of KBr and 1.5 ml of 30% KMnO₄ were added. The solutions were mixed, left for ten minutes in an ice bath and then decolourised by dropwise addition of 1% H₂O₂. Then 7 ml of n-Heptane was added, the tubes were shaken and the two layers were allowed to separate. 5 ml of the top layer was transferred to another tube containing 2 ml of 30% KOH and 4 ml of pyridine, the solutions were shaken and boiled in a water bath at 80°C for 4 minutes. After cooling the optical density (Y) of the middle layer was read at 520 nm. The levels of citric acid were calculated by employing the regression formula:

\[ X = 21.206 Y + 5.529.3 \]

Where, \( Y \) = O.D. of the unknown sample and
\( X \) = Concentration.

The concentration was expressed as mg/100 mg fresh tissue weight.

RESULTS

1. Succinate dehydrogenase (SDH):

Succinate dehydrogenase activity decreased in comparison to controls by all three drugs after 7 and 15 days treatment except that a slight increase was obtained by 15 days of butobarbitone (Soneryl) administration. The decrease was significant (\( P < 0.001 \)) by chlordiazepoxide (Librium)
treatment. Combined CP2 + ascorbic acid treatment caused a decrease in SDH activity as compared to controls and drug treatment (Fig. 3). The localization pattern of SDH was the same as in normal i.e., more in type I and intermediate fibers than type II (Figs. 1, 2) but the overall intensity was less as observed from the longer incubation period.

2. Alkaline phosphatase:

The alkaline phosphatase activity was increased by all the three drugs by 7 and 15 days treatment except 7 days of CPZ treatment. The increase was significant (P < 0.02) by butobarbitone (15 days). The enzyme activity increased beyond control levels by CPZ + ascorbic acid administration for 7 days (Fig. 3).

3. Acid phosphatase:

The results were the same as for alkaline phosphatase and a very significant (P < 0.001) increase was observed in pectoral muscle of animals treated with CPZ + ascorbic acid for 7 days (Fig. 3).

4. Phosphorylase:

As compared with normal controls, the phosphorylase activity increased by all three drugs. The increase was significant (P < 0.001) by CPZ and butobarbitone treatments (Fig. 3).
5. **Fructose:**

The levels of fructose decreased by butobarbitone treatment of 7 and 15 days \( P < 0.001, < 0.01 \) as well as 15 days of CPZ and chlordiazepoxide administration. However an increase \( P < 0.001 \) was noted by 7 days of treatments by the latter two drugs (Fig. 3).

6. **Glycogen:**

The levels of glycogen increased on the whole as compared to normal control animals throughout the experimental period by treatment with all the three drugs. The most significant increase \( P < 0.001 \) was observed by chlordiazepoxide (7 and 15 days) and butobarbitone (15 days) treatments \( P < 0.001 \). However 7 days of treatment with the latter drug caused a decline in the levels of glycogen (Fig. 3).

7. **Citric acid:**

An inconsistent increase of decrease was observed in the concentration of citric acid by all the three drugs (Fig. 3).

8. **Protein:**

An increase in protein contents was observed by butobarbitone (7 and 15 days, \( P < 0.01, < 0.001 \) and CPZ (15 days, \( P < 0.01 \) administrations whereas by chlordiazepoxide, there was not much change than in the normal control. By combined treatment of CPZ + ascorbic acid for 7 days, an
increase in protein was observed as compared to normal muscle (Fig. 3).

9. Ascorbic acid (AA):

The drugs caused an increase (chlordiazepoxide, 7 and 15 days and CPZ, 15 days) in the levels of free ascorbic acid (AA) as compared to normal. However, by administration of butobarbitone and CPZ for 7 days, the levels were more or less same as in control. On the whole, ascorbigen (ASG) levels were either increased (CPZ and butobarbitone) or were the same as in control muscle. No significant changes were observed in the rate of utilization of ascorbic acid (AAU) by treatment with the three drugs except that 15 days CPZ treatment brought about an increase ($P < 0.05$) and 7 days of treatment, a decrease ($P < 0.001$) in AAU. The rate of ascorbic acid macromolecule complex (AA-MM) was significantly decreased ($P < 0.001$, $< 0.01$) by the three drugs, with the exception of 15 days chlordiazepoxide administration (Fig. 4).

10. AA-FR special peroxidase:

No significant changes were observed in the activity of AA-FR special peroxidase by the three drugs (Fig. 4).
DISCUSSION

From the results it is evident that with a general increase in phosphorylase and glycogen levels and correspondingly an inconsistent decrease or increase in fructose and citric acid, the rate of glycolysis in muscle reveals a departure from the normal pattern. The effects of tranquillizers on carbohydrate metabolism of muscle could be closely compared to those brought about by analgesic drugs (Chapter II B). Similarly, the inconsistent changes in the concentration of succinate dehydrogenase and protein as well as the significant increase in the activities of alkaline and acid phosphatases observed (Figs. 3), are comparable to similar results noted for non-narcotic analgesics (Chapter II B). The increase in activity of acid and alkaline phosphatases could be attributed to the mode of action of these drugs in revealing the normally masked sites of phosphatase activity (De Waart et al., 1966). Therefore the foregoing data suggests a general impairment in oxidative metabolism and therefore probably the contractile properties of the muscle in drug treated animals. As the tranquillizers and/or anaesthetic drugs like chlorpromazine, Phenobarbital and halothane tend to block neuromuscular transmission by depression of end plate response to acetylcholine (Bourne, 1973), a study on the effects of chlorpromazine and similar
drugs on cholinesterase activity and neuromuscular junctions in muscles of drug treated rats is discussed in Chapter VIII.

Administration of a variety of drugs including barbiturates and CPZ lead to increased synthesis and utilization of L-ascorbic acid in rats and guinea pigs (Longnecker et al., 1940; Conney et al., 1961; McKernan and Teague, 1973). This enhanced synthesis and utilization of the vitamin is correlated with an increased formation of histamine by the drugs (Gupta et al., 1970; Subramanian et al., 1974) and is responsible for detoxification or biological inactivation of histamine in vivo (Subramanian et al., 1973; Nandi et al., 1974). Histamine is known to constrict most large blood vessels and dilate small vessels (Rocha E, Silva, 1955; Quastel and Hackett, 1973) and may alter the blood supply to the muscle. It has also been reported that vitamin C deficient guinea pigs were more sensitive to drugs and were unable to metabolize the drug efficiently (Garattini and Shore, 1962). The results of this study showed an increase in alkaline and acid phosphatases in muscle of rats treated with CPZ + ascorbic acid administration along with the increase in levels of ascorbic acid and bound ascorbigen by drug treatment. These data suggest that (i) the drugs probably favor enhanced synthesis of vitamin and support the observations of Longenecker et al., (1940) and McKernan and Teague (1973). The
vitamin is utilised via the special peroxidase pathway for the maintenance of muscular activity and (ii) a possible beneficial role of ascorbic acid in drug treated animals in view of the involvement of ascorbic acid in metabolic turnover of muscles (Chapter II B).

**SUMMARY**

A biochemical study on pectoralis major muscle of rats treated with different tranquilisers (Chlorpromazine, chlordiazepoxide and butobarbitone) reveals their influence on carbohydrate metabolism, oxidative pathways and activities of phosphatases. The normal physiological functions of muscles were affected. A beneficial role of ascorbic acid in maintaining muscle metabolism in drug treated animals is suggested.
REFERENCES


3. Chapter I A. Studies on adaptive modifications in vertebrate skeletal muscle. 1. Evidences for the occurrence of structural and metabolic evolutionary trends in vertebrate skeletal muscle.

4. Chapter II B. Studies on the effects of drugs on muscle. II. Non-Narcotic Analgesics.


Figs. 1 and 2.

Showing: the localization of SDH activity in the normal and chlorpromazine treated rat pectoral muscle. x.375.

I = Type I (Red fibers)
II = Type II (White fibers)
Int. = Intermediate fibers
Fig. 3.

Showing the concentrations of SDH, alkaline and acid phosphatases, protein, fructose, glycogen, phosphorylase and citric acid in rat pectoral muscle

1 = Normal rat
2 = Chlorpromazine treated, 7 and 15 days
3 = Chlorpromazine + ascorbic acid treated, 7 days
4 = Chlordiazepoxide, 7 and 15 days
5 = Butobarbitone, 7 and 15 days
Fig. 4.

Showing the levels of ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU), ascorbic acid-macromolecule complex (AA-MM complex) and ascorbic acid-free radical (AA-FR) special peroxidase activity in rat pectoral muscle.

1 = Normal rat
2 = Chlorpromazine treated, 7 and 15 days
3 = Chlordiazepoxide, 7 and 15 days
4 = Butobarbitone, 7 and 15 days
CHAPTER II B

STUDIES ON THE EFFECTS OF DRUGS ON MUSCLE

2. NON-NARCOTIC ANALGESICS

INTRODUCTION

Non-narcotic analgesic drugs chiefly salicylates are known to have manifold effects on the metabolism of carbohydrates, proteins and fats in animals (Garattini et al., 1969; Satoskar and Bhandarkar, 1975). Although several reports are available on the effects of these drugs on various tissues, yet there is a paucity of data with specific reference to changes brought about by them in skeletal muscle metabolism. Since tranquillisers affect pectoral muscle activity (Chapter II A), it was thought worthwhile to investigate the effects of three non-narcotic analgesics on pectoral muscle of albino rats.

MATERIAL AND METHODS

Healthy, adult male albino rats (Rattus norvegicus) weighing between 200-300 gms were used for the experiments. They were maintained in air conditioned animal house (Temperature 26° ± 2°C) and 14 hours day light) on standard diet (Hindustan Lever Ltd, Bombay) and water ad libitum.
The drugs used were:

(a) Acetylsalicylic acid (Aspirin) Nicholas of India Ltd, Bombay-71.

(b) Analgin (Analgin 0.5 gms/tablet) USSR, Everest Chemical Industries, 27 Vasant Nagar, Ahmedabad.

(c) Metamizole, Hoechst Pharmaceuticals Ltd, Backbay Reclamation, Bombay-400020.

As these drugs are known to be soluble in water, a known quantity of each drug was dissolved in distilled water of known volume so as to obtain a concentration of 800 µg/ml, which was found to be the minimum effective dose. In case of (b) and (c) the tablets were powdered, dissolved in water and the clear top aqueous layer was used for the experiments. A total of twelve animals in each group were given an intramuscular injection of the respective drug (800 µg/ml/rat) daily for 7 and 15 days and then sacrificed on day 8 and 16 respectively by cervical dislocation specifically in order to avoid any side effects of nembutal or ether anaesthesia. To a group of six rats, combined treatment of acetylsalicylic acid + ascorbic acid (100 mg/animal/day, fed orally) for 7 days was given and on day 8th the rats were autopsied. The biochemical estimations on the following enzymes and metabolites were carried out on the pectoralis major muscle of normal and the drug treated rats as per methods described in Chapters I A and II A.
(i) Succinate dehydrogenase (Kun and Abood, 1949) and the histochemical localization of SDH (George and Talesara, 1961).

(iii) Alkaline and acid phosphatases (Bellfield and Goldberg, 1971).

(iii) Phosphorylase (Fiske and Subbarow, 1925 and Cori et al., 1943).

(iv) Fructose (Foremann et al., 1973).

(v) Glycogen (Seifter et al., 1950).

(vi) Protein (Gornall et al., 1949).

(vii) Citric acid (Ettinger et al., 1952).

(viii) Free ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complex (AA-MM) (Chinoy et al., 1974).

(ix) Ascorbic acid free radical forming (AA-FR) special peroxidase (Chinoy, 1973).

A minimum of six replicates were done for each treatment and parameter and the results were statistically analysed using the student's 't' test.

RESULTS

The results are presented in figures 1 to 4.

1. Succinate dehydrogenase (SDH):

As compared to controls, the enzymic activity was reduced by 7 days of analgin and metamizole treatments and
throughout by acetylsalicylic acid administration. The decrease was more significant by acetylsalicylic acid (15 days, \( P < 0.02 \)) and by metamizole (7 days, \( P < 0.01 \)). On the other hand, SDH activity was increased by 15 days of analgin and metamizole treatments. In rats treated with acetylsalicylic acid + ascorbic acid (100 mg/day/animal) for 7 days, the enzyme activity was significantly \((P < 0.001)\) reduced in comparison to normal and 7 days acetylsalicylic acid treated rats (Fig. 3).

Histochemical localization pattern of SDH was the same as in normal, but the overall intensity was less as was observed from the longer incubation period in comparison with the incubation period of normal rat muscle (Figs. 1, 2).

2. **Alkaline phosphatase:**

On the whole, the activity of alkaline phosphatase increased by all three analgesics except that a decrease was observed by acetylsalicylic acid (15 days). The enzyme activity was increased much beyond the control values in rats treated with acetylsalicylic acid and ascorbic acid for 7 days (Fig. 3).

3. **Acid phosphatase:**

The acid phosphatase activity was significantly elevated by all the drugs. The increase was most pronounced \((P < 0.01)\) by metamizole and aspirin (15 days). The combined
administration of acetylsalicylic acid and ascorbic acid for 7 days resulted in an increase ($P < 0.2$) in enzyme activity (Fig. 3).

4. Phosphorylase:

Although the enzymic activity was enhanced by all the drugs, a more significant ($P < 0.001$) increase was found after 15 days of analgesic drug treatment than at 7 days (Fig. 3).

5. Fructose:

Fructose levels decreased by all the drugs. The reduction was more significant by acetylsalicylic acid and analgin ($P < 0.001$) than by metamizole and more at 15 days of each treatment (Fig. 3).

6. Glycogen:

On the whole, glycogen levels were increased by all the drugs but more significantly ($P < 0.001$) by metamizole $<$ analgin $<$ acetylsalicylic acid and also at 7 days than 15 days of the respective treatment (Fig. 3).

7. Protein:

Acetylsalicylic acid administration resulted in a slight decrease in protein concentration as compared to control levels, otherwise it was enhanced by the other drugs. The levels of protein increased significantly ($P < 0.01$) by combined administration of acetylsaalicylic acid and ascorbic
acid for 7 days (Fig. 3).

8. **Citric acid:**

A significant reduction in the levels of citric acid was observed in acetylsalicylic acid ($P < 0.001$) and analgin ($P < 0.001$, $< 0.01$) treated rats whereas it was significantly ($P < 0.001$) increased by metamizole treatment (Fig. 3).

9. **Ascorbic acid:**

The levels of free ascorbic acid (AA) increased by acetylsalicylic acid treatment but it remained almost similar to those of the normal controls by 7 and 15 days of analgin and metamizole treatments. Ascorbigen (ASG) concentration increased by acetylsalicylic acid (7 and 15 days), analgin (15 days) and metamizole (7 days) treatments, while its level was nearly the same as in untreated rats by administration of analgin (7 days) and metamizole (15 days). Ascorbic acid utilization rate (AAU) was not much altered by 15 days of the drug treatment in comparison with untreated rats. A slight increase was obtained by acetylsalicylic acid and a decrease by analgin and metamizole treatments respectively for 7 days. The levels of ascorbic acid macromolecule (AA-MM) complex decreased by all the three drugs except by 15 days treatment with acetylsalicylic acid. The decrease was significant ($P < 0.01$) in muscles of 7 days acetylsalicylic acid, metamizole as well as 15 days analgin and metamizole treated rats (Fig. 4).
10. **AA-FR special peroxidase:**

The activity of AA-FR special peroxidase was decreased by analgin (7 and 15 days) treatment, whereas it increased by acetylsalicylic acid and metamizole (7 days) and significantly (P < 0.001) by the latter treatment (Fig. 4).

**DISCUSSION**

Of all the analgesic drugs investigated in the present study, acetylsalicylic acid (Aspirin) was found to be the most potent in exerting its influence on muscle metabolism. Satoskar and Bhandarkar (1975) and Garattini *et al.*, (1969) have indicated that salicylates exert their action on carbohydrate metabolism in rats by causing a glucose depletion effect, enhanced peripheral glucose utilization and inhibition of gluconeogenesis. In the present study, the increase in levels of glycogen and phosphorylase and a consistent decrease in the concentration of fructose as compared to controls, by all three analgesics, suggests an increased rate of glycogenolysis (Cahill *et al.*, 1957) as well as enhanced synthesis and utilization of glycogen in skeletal muscle fibers (Dubowitz and Pearse, 1960), since phosphorylase is the main catalytic force leading to phosphorylative degradation and glycolysis (Stetten and Stetten, 1960). But the data of the present study also reveals a slowing down in
the rate of glycolysis due essentially to the decrease in fructose levels and reduction of aerobic glycolysis by HMP shunt pathway (Satoskar and Bhandarkar, 1975). Thus it is evident that analgesic drugs and tranquilisers (Chapter II A) cause serious alterations in carbohydrate metabolism and alter the glycolytic machinery for obtaining energy needed for normal muscle function. That aspirin inhibits prostaglandins in several tissues which in turn affect the activity of cyclic adenosine monophosphate (C-AMP), is known (Hinman, 1972). The increase in phosphorylase obtained in this study could be thus attributed to the above observations. Therapeutic doses of salicylates cause a pronounced and progressive increase in the consumption of O₂ (Cochran, 1954) primarily by the skeletal muscle (Tenney and Miller, 1955). Similarly, Mehlman et al. (1972) showed increased respiratory rates in aspirin fed rats. They also demonstrated that ADP/oxygen ratio was decreased and mitochondrial ATPase activity was unchanged. A marked uncoupling of oxidative phosphorylation also occurs by aspirin (Smith, 1966). A greater decrease in activity of succinate dehydrogenase (SDH), an oxidative mitochondrial enzyme known to be present in higher amounts in type I fibers of skeletal muscle (George and Berger, 1966; Ariano et al., 1973; Chapter I A) by aspirin than by Novalgin or analgin suggests that the former is a more potent analgesic as compared to the other two drugs. Furthermore, it
is likely that the decrease in SDH levels could probably be attributed to structural and functional changes in the mitochondria by analgesics. Ultrastructural changes in the inner membranes of mitochondria of adrenocortical cells in codeine addicted rats have been demonstrated by Yano et al. (1971). They observed deformities in the membranes of mitochondria and its less dense matrix.

The activities of alkaline and acid phosphatases were on the whole increased as compared to normal, by all analgesics studied (Fig. 3). This is probably due to the fact that drugs of the aspirin group do not stabilize lysosomes (Garattini et al., 1969), or else due to the binding of the drugs with the active sites of the enzymes.

Salicylates stimulate protein catabolism (Beckman, 1958), so that a decrease in its concentration was obtained especially by treatment with aspirin. However, the increase in protein by analgin and novalgin might be due to their comparatively less potent analgesic effect.

The effects of the analgesics on ascorbic acid turnover were similar to those of tranquilisers (Chapter II A). The analgesics, particularly aspirin brought about an increased mobilization of ascorbic acid from the bound ascorbigen and enhanced its utilization in muscle. The greater mobilization of the vitamin is probably a response of the tissue to withstand the imposed stress and may be the result
of increased synthesis (Samuels et al., 1940; Ritz et al., 1940; Longenecker et al., 1940; Conney et al., 1961). Ascorbic acid probably is beneficial for the recovery of enzymes like alkaline and acid phosphatases but its dose used or the extent of treatment imparted was not adequate for the recovery of SDH activity. Similarly, the importance of supplementary ascorbic acid administration to individuals receiving aspirin therapy of more than a week's duration has been indicated (Loh et al., 1973), since aspirin alters the metabolic availability of ascorbic acid.

SUMMARY

The effects of three non-narcotic analgesic drugs on the metabolism of pectoralis major muscle of rats were studied. Aspirin was the most potent drug in causing disturbances in carbohydrate metabolism and the mitochondrial oxidative pathway resulting in alterations in muscle function. The overall increase in alkaline and acid phosphatases, together with decrease in protein particularly by aspirin, suggests that analgesics are not capable of stabilizing lysosomes and enhanced proteolytic activity prevails in muscle. The increased mobilization of ascorbic acid from the bound state with its enhanced utilization is a physiological response to withstand the stress condition. The results are discussed in the light of recent findings.
REFERENCES


4. Chapter I A. Studies on adaptive modifications in vertebrate skeletal muscle. 1. Evidences for the occurrence of structural and metabolic evolutionary trends in vertebrate skeletal muscles.

5. Chapter II A. Studies on the effects of drugs on muscle. I. Tranquilisers.


Figs. 1 and 2.

Showing the localization of SDH staining in the normal and acetyl salicylic acid treated rat pectoral muscle. X375.

I = Type I (Red fiber)
II = Type II (White fiber)
Int. = Intermediate fiber
Fig. 3.

Showing the concentrations of SDH, alkaline and acid phosphatases, protein, fructose, glycogen, phosphorylase and citric acid of rat pectoral muscle.

1 = Normal rat muscle
2 = Acetyl salicylic acid treated, 7 and 15 days
3 = Acetyl salicylic acid treated + ascorbic acid 7 days
4 = Analgin treated, 7 and 15 days
5 = Metamizole treated, 7 and 15 days
Fig. 4.

Showing the levels of ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU), ascorbic acid macromolecule complex (AA-MM complex) and ascorbic acid-free radical (AA-FR) special peroxidase activity in rat pectoral muscle.

1 = Normal rat
2 = Acetyl salicylic acid treated, 7 and 15 days
3 = Analgin treated, 7 and 15 days
4 = Metamizole treated, 7 and 15 days
CHAPTER II C

STUDIES ON THE EFFECTS OF DRUGS ON MUSCLE

3. NARCOTIC ANALGESICS

INTRODUCTION

Narcotics interact with cholinergic mechanisms both in the periphery and the central nervous systems (Levitt, 1975). The binding of morphine to neural tissue occurs primarily at the synapse and most probably at the post-synaptic membrane influencing synaptic transmission at the receptor site (Pert et al., 1974). Morphine impairs the release of acetylcholine (ACH) at cholinergic sites in the guinea pig ileum (Schaumann, 1957), autonomic ganglia (Pelikan, 1960) and at the neuromuscular junctions (Pinsky and Fredrickson, 1971; Bell and Rees, 1974).

Morphine depresses the respiratory centre and decreases the metabolic rate resulting in a slight fall in body temperature with reduction of muscular activity and peripheral vasodilation (Satoskar and Bhandarkar, 1975; Levitt, 1975). Another narcotic viz., heroin (diacetyl morphine) is converted to monoacetyl morphine and then to morphine in the brain (Levitt, 1975). Bhakthan (1973) observed that in both type of muscle fibers of Vastus Lateralis muscle of thigh in heroin
addicted male and female subjects, the mitochondrial cristae and matrices were disrupted and several lysosome-like bodies were found. Several glycogen granules were also observed in the type II fibers of this muscle. Therefore, it was thought worthwhile to investigate the effects of narcotic analgesics on muscle metabolism of albino rats in the light of the above data.

MATERIAL AND METHODS

Adult, healthy rats weighing between 200-300 gm were used for the present investigation. They were maintained on standard diets (Hindustan Lever Ltd., Bombay) and water ad libitum, in an air-conditioned animal house (Temperature, 26 ± 2°C and 14 day light hours).

The narcotic analgesics used were:

(i) Morphine—morphine sulphate Injection I.P., 15 mg/ml, Alembic Chemical Works Co. Ltd., Alembic Road, Baroda-3, mfg. No. G/4, Lot No. 6372.

(ii) Opium—Obtained in pure form from Government Opium and Hemp Drug Depot, Ahmedabad.

A known quantity of the drug (i) was diluted with glass distilled water of known volume so as to obtain a concentration of 1.5 mg/ml. In case of (ii) the opium flakes were powdered, dissolved in water and the clear aqueous layer was used for the experiments. The animals were divided into 3 groups, each consisting of 12 animals. They were given
an intramuscular injection of the respective drug (1.5 mg/ml/rat) daily for 7 days and then sacrificed on day 8 by cervical dislocation.

Biochemical investigations of the following enzymes and metabolites were carried out on the pectoralis major muscle of the normal and drug treated animals as detailed in Chapters I A and II A.

1. Succinate dehydrogenase (Kun and Abood, 1949) and the histochemical localization (George and Tallsara, 1961)
2. Alkaline and acid phosphatases (Belfield and Goldberg, 1971)
3. Phosphorylase (Fiske and Subbarow, 1925; Cori et al., 1943)
4. Fructose (Foreman et al., 1973).
5. Glycogen (Seifter et al., 1950).
6. Protein (Cornall et al., 1949).
7. Free ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU) and ascorbic acid macromolecule complex (AA-MM) (Chinoy et al., 1974).
9. Citric acid (Ettinger et al., 1952).

To a group of 12 animals, combined treatment of ascorbic acid (100 mg/day/rat) and morphine (1.5 mg/ml/day) was given daily for 7 days and then subsequently the animals were sacrificed on 8th day.
A minimum of six replicates were done for each parameter and the results were statistically analysed using the student's 't' test.

RESULTS

The results are presented in Figs. 1 and 2 and Tables I.

1. **Succinate dehydrogenase (SDH):**
   
   SDH activity was decreased by both morphine and opium administrations but the enzyme completely recovered to normal level by the combined treatment of morphine and ascorbic acid (Table I).

   The intensity of histochemical localization of SDH was less, as the activity was observed after a longer period of incubation than in the control (Figs. 1 & 2).

2. **Alkaline and acid phosphatases:**
   
   The activity of both phosphatases were increased by the two drugs and significantly \((P \leq 0.001)\) by morphine administration. On the other hand, by combined administration of morphine and ascorbic acid, the enzymic activities recovered to normal levels (Table I).

3. **Phosphorylase:**
   
   Phosphorylase was significantly increased \((P \leq 0.001)\) by 7 days of administration of both the drugs in comparison with the normal (Table I).
4. Fructose:

The levels of fructose also increased by both opium and morphine. The increase was more by opium treatment (Table I).

5. Glycogen:

A significant (P < 0.001) increase in glycogen concentration was observed by both the drugs as compared to normal control (Table I).

6. Citric acid:

Citric acid concentrations were also increased by the two drugs in comparison to normal control (Table I).

7. Protein:

In comparison to normal control, the levels of protein were decreased by opium and morphine treatments. However, the combined administration of morphine and ascorbic acid resulted in a significant increase (P < 0.001) in protein (Table I).

8. Ascorbic acid:

Free ascorbic acid (AA) levels were elevated by both the drugs (Table II). Ascorbigen (ASG) concentration was not much affected by opium and morphine (Table II). There was no change in rate of AAU with opium administration as compared to controls while a slight enhancement was observed by morphine (Table II). The rate of ascorbic acid macromolecule was significantly (P < 0.001) reduced by both drugs as compared to normal control (Table II).

9. AA-FR special peroxidase: The activity of AA-FR special
peroxidase was increased, and was almost to the same extent by both opium and morphine in comparison to untreated normal controls (Table II).

DISCUSSION

The results are presented in Tables I and II from which it is clear that the effects of narcotic analgesics on SDH, alkaline and acid phosphatase were closely similar to those obtained by tranquilizers and non-narcotic analgesics (Chapters II A, B). A greater increase in the activity of acid phosphatase was observed in morphine treated rats in comparison to controls. An accumulation of several lysosome like bodies in the subsarcolemmal and perinuclear regions of muscle fibers was observed in heroin addicts (Bhakthan, 1973). Heroin which is diacetyl morphine, is known to be converted to monoacetyl morphine in the body. Thus the results of this biochemical study corroborate with the EM observations of Bhakthan (1973). The changes in carbohydrate metabolism were similar to those obtained by tranquilizers and non-narcotic analgesic drugs except that fructose concentration was increased especially by opium, but not so much by morphine as it is known to inhibit principally the non-phosphorylated pathway of fructose metabolism (Dombrosky et al., 1973), while the increase could be due to the increased anaerobic glycolysis induced by morphine (Clouet, 1971). Changes in carbohydrate metabolism
of muscle by morphine have also been reported by Wong and Walsh (1969). A greater deposition of glycogen in type II fibers and a concomitant increase in intramyofibrillar lipid globules in type I fibers of vastus lateralis muscle of the thigh in heroin addicts were observed by Bhakthan (1973). As opiate drugs inhibit the oxygen uptake, depress respiration (Clouet, 1971) and bring about disruption of mitochondrial cristae and matrices in both type I and II fibers in muscle of heroin addicts (Bhakthan, 1973), the rate of oxidative metabolism might decrease resulting in reduced activity of SDH as observed in this study. The decline in protein levels in muscle especially by opium could be attributed to the inhibition of its synthesis by this drug (Clouet, 1971), although Bhakthan (1973) observed insignificant differences in myofibrils of normal muscle in comparison to those of non-addicts.

The ascorbic acid metabolic pattern in narcotic drug treated muscle was found to be similar to the one obtained by tranquilisers and non-narcotic analgesics (Chapters II A, B). The increased mobilization and utilization of the vitamin is a protective mechanism for the detoxification of drug-induced histamine (Satoskar and Bhandarkar, 1975; Subramanian et al., 1974; Nandi et al., 1974), which is known to contract most large blood vessels and dilate small arteries and venules in muscle (Rocha E. Silva, 1955; Quastel and Hackett,
The beneficial effects of ascorbic acid are manifested by the recovery of SDH and alkaline phosphatase activities and by the significant increase in acid phosphatase as a consequence of combined administration of morphine + ascorbic acid to rats for 7 days. The protective action of ascorbic acid against strychnine induced convulsion in albino mice has been reported (Yegnanarayan et al., 1973). Similarly, Zannoni et al., (1972) have observed an overall reversal of the overall drug oxidation enzyme activities in liver of Phenobarbital treated, vitamin C deficient guinea pigs which were administered ascorbic acid in vivo. These results are significant, since androgen-dependent enzymes (involved in ascorbic acid biosynthesis) in liver microsomes were impaired in morphine treated male rats (Wolstenholme and Porter, 1968), thereby indicating that the drug induces a greater mobilization of the bound vitamin rather than its synthesis, thus accounting for the increase in free ascorbic acid levels and the concomitant decline in the rate of formation of ascorbic acid macro-molecule complex (Table II). Similarly, morphine treated rats and cats were found to have higher amounts of ascorbic acid in their adrenals than control animals in response to the stress of drug treatment (Clouet, 1971; Borrell and Borrell, 1975; Borrell, et al., 1975). Furthermore, the results of the present study also reveal that the opiate narcotic-induced alterations in muscle metabolism are transient and reversible by ascorbic acid.
SUMMARY

The repercussions of treatment of rats with three narcotic analgesic drugs on their pectoral muscle function were investigated. The changes in carbohydrate metabolism and ascorbic acid turnover were almost similar to those manifested by the tranquilisers and non-narcotic analgesic drugs. Ascorbic acid played a beneficial role in the recovery of some oxidative and hydrolytic enzyme activities in drug treated animals. The opiate narcotic induced alterations in muscle metabolism were transient and reversible by ascorbic acid.


5. Chapter I A. Studies on adaptive modifications in vertebrate skeletal muscle. 1. Evidences for the occurrence of structural and metabolic evolutionary trends in vertebrate skeletal muscles.

6. Chapter II A. Studies on the effects of drugs on muscle. I. Tranquilisers.

7. Chapter II B. Studies on the effects of drugs on muscle. II. Non-Narcotic Analgesics.


### Table I

Showing the levels of SDH, alkaline and acid phosphatases, protein, phosphorylase, fructose, glycogen and citric acid in the pectoral major muscle of Opium, Morphine and Morphine + ascorbic acid treated rats

<table>
<thead>
<tr>
<th>Enzyme/Metabolites</th>
<th>Normal</th>
<th>Opium</th>
<th>Morphine</th>
<th>Morphine + ascorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate dehydrogenase</td>
<td>16.82 ± 2.30</td>
<td>10.81 ± 0.63</td>
<td>10.75 ± 0.58</td>
<td>16.35 ± 0.01</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1.65 ± 0.70</td>
<td>2.83 ± 0.28</td>
<td>13.53 ± 2.44</td>
<td>4.96 ± 0.70</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>7.22 ± 0.86</td>
<td>11.05 ± 1.14</td>
<td>29.30 ± 1.89</td>
<td>10.63 ± 0.70</td>
</tr>
<tr>
<td>Protein</td>
<td>29.90 ± 2.90</td>
<td>22.60 ± 1.20</td>
<td>27.50 ± 1.80</td>
<td>69.80 ± 0.43</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>0.012 ± 0.002</td>
<td>0.22 ± 0.01</td>
<td>0.57 ± 0.07</td>
<td>-</td>
</tr>
<tr>
<td>Fructose</td>
<td>114.83 ± 6.70</td>
<td>141.20 ± 7.00</td>
<td>120.00 ± 8.18</td>
<td>-</td>
</tr>
<tr>
<td>Glycogen</td>
<td>54.79 ± 1.06</td>
<td>171.10 ± 0.48</td>
<td>279.80 ± 5.00</td>
<td>-</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.65 ± 0.08</td>
<td>0.84 ± 0.10</td>
<td>0.92 ± 0.14</td>
<td>-</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Table II

Showing the levels of ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAU), ascorbic acid-macromolecule (AA-MM) complex and AA-FR special peroxidase activity of the pectoral major muscle of Opium, and Morphine treated rats

<table>
<thead>
<tr>
<th>Enzymes/Metabolites</th>
<th>Normal</th>
<th>Opium</th>
<th>Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid (AA)</td>
<td>1.09 ± 0.11</td>
<td>1.26 ± 0.19</td>
<td>1.69 ± 0.09</td>
</tr>
<tr>
<td>Ascorbigen (ASG)</td>
<td>0.14 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Ascorbic acid utilization (AAU)</td>
<td>10.82 ± 1.05</td>
<td>10.4 ± 0.55</td>
<td>12.99 ± 0.57</td>
</tr>
<tr>
<td>AA-MM complex</td>
<td>1.90 ± 0.24</td>
<td>0.28 ± 0.008</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>AA-FR special peroxidase</td>
<td>3.01 ± 0.14</td>
<td>4.25 ± 0.41</td>
<td>4.30 ± 0.23</td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Figs. 1 and 2.

Showing the localization of SDH activity in normal and Morphine treated at pectoral muscle.

Fig. 1 x375 ; Fig. 2 x510

I = Type I (Red fiber)
II = Type II (White fiber)
Int. = Intermediate fiber
CHAPTER II D

STUDIES ON THE EFFECTS OF DRUGS ON MUSCLE

4. CHOLINERGIC DRUGS

INTRODUCTION

Nicotine is a naturally occurring alkaloid from the tobacco plant. It penetrates the blood-brain barrier and has important central effects while the peripheral effects are due to its stimulant action upon the autonomic ganglia, the adrenal medulla and the neuromuscular junctions (Levitt, 1975). However, nicotine acts as a stimulant at these sites only when administered in very small doses and functions as a depolarizing agent at the post synaptic membrane (Levitt, 1975). Nicotine also stimulates respiration and raises the blood pressure. Thus nicotine manifests a combination of sympathetic and parasympathetic effects.

The effects of three categories of drugs (Tranquilisers, non-narcotic analgesics and narcotic analgesics have shown changes in muscle metabolism (Chapter II A, B, C). As nicotine produces a transient depolarization of the motor end-plate resulting in a stimulation of skeletal muscles and twitchings, (Satoskar and Bhandarkar, 1975), an investigation on the metabolism of skeletal muscle in drug treated rats was undertaken. Moreover, since the beneficial effects of
ascorbic acid on muscle metabolic turnover in various drug treated rats have been highlighted in Chapters II A, B, C, the present study was undertaken to investigate the effects of tobacco and nicotine individually and the combined treatment of tobacco + ascorbic acid on pectoral muscle function of albino rats.

**MATERIAL AND METHODS**

Adult, healthy, albino rats (*Rattus norvegicus*) weighing between 200-300 gm were used for the present experiments. They were maintained in an airconditioned animal house (Temperature 26° ± 2°C and 14 day light hours) on standard diet (Hindustan Lever Ltd., Bombay) and water *ad libitum*.

The cholinergic drugs used were (i) Tobacco : (Wills filtered), (ii) Nicotine : Pure form, obtained from L.M. College of Pharmacy, Ahmedabad.

A known quantity of the drug (ii) was diluted with glass distilled water of known volume so as to obtain a concentration of 1.5 mg/ml. Tobacco was powdered and dissolved in glass distilled water and aliquots of the clean aqueous solutions were used for the experiments. Twelve animals were distributed in each group and they were given an intramuscular injection of the respective drug (1.5 mg/ml/rat) daily for 7 days and then sacrificed on day 8 by cervical dislocation specifically in order to avoid any side
effects of nembutal or ether anaesthesia. To a group of 6 rats, combined treatment of tobacco + ascorbic acid (100 mg/animal/day, fed orally) for 7 days was given and on day 8th they were autopsied. Biochemical estimations on pectoralis major muscle of normal and drug treated rats were carried out according to the details given in Chapter IA. (1) SDH (Kun and Abood, 1949), (2) Alkaline and acid phosphatases (Belfield and Goldberg, 1971), (3) Phosphorylase (Cori et al., 1943), (4) Fructose (Foreman et al., 1973), (5) Glycogen (Seifter et al., 1950), (6) Citric acid (Ettinger et al., 1952), (7) Protein (Gornall et al., 1949), (8) Free ascorbic acid, ascorbigen, ascorbic acid utilization and ascorbic acid macromolecule complex (Chinoy et al., 1974), (9) AA-FR special peroxidase (Chinoy, 1973). A minimum of six replicates were done for each parameter and the results were statistically analysed using the student's 't' test.

RESULTS

The results are presented in Tables I and II.

1. Succinate dehydrogenase (SDH):

A slight increase in SDH concentration was observed by 7 days of tobacco treatment, but the enzyme decreased with combined administration of tobacco + ascorbic acid (Table I).
2. Alkaline phosphatase and acid phosphatase:

The activities of both alkaline and acid phosphatases were increased by the two drugs (Significantly, $P < 0.001$ of acid phosphatase). On the other hand, with combined administration of tobacco and ascorbic acid for 7 days, the alkaline phosphatase increased but acid phosphatase recovered to nearly normal values (Table I).

3. Phosphorylase:

Phosphorylase activity was significantly increased ($P < 0.001$) by 7 days of treatment with tobacco in comparison to the normal (Table I).

4. Fructose:

The levels of fructose decreased by tobacco treatment as compared to normal controls (Table I).

5. Glycogen:

A significant increase ($P < 0.001$) in glycogen concentration was observed with tobacco administration (Table I).

6. Citric acid:

Citric acid concentrations were increased by the drug administration as compared to untreated control rats (Table I).

7. Protein:

Insignificant changes in the levels of protein were observed by tobacco and nicotine treatments. However, the
combined administration of tobacco and ascorbic acid resulted in a significant \( P < 0.001 \) increase in protein levels (Table I).

8. Ascorbic acid:

The levels of free ascorbic acid (AA) and ascorbigen (ASG) were increased significantly by tobacco treatment \( P < 0.001 \). Ascorbic acid utilization (AAU) was not much affected. The rate of ascorbic acid macromolecule complex (AA-MM) was reduced by tobacco as compared to in normal control muscle (Table II).

9. AA-FR special peroxidase:

The AA-FR special peroxidase activity was decreased by drug administration in comparison to untreated normal control (Table II).

**DISCUSSION**

The increase in alkaline and acid phosphatases as observed by cholinergic drugs was similar to those brought about by tranquilizers, non-narcotic analgesics and narcotic analgesics (Chapters II A, B, C), but the activity of SDH was enhanced in contrast to the other drugs. As nicotine was used in a low dose the increase was greater by it (nicotine) and may be attributed to its stimulatory action of neuromuscular junctions resulting in increased muscular activity.
The alterations in carbohydrate metabolism resembled those produced by tranquillisers and non-narcotic analgesics, so that fructose decreased while glycogen, phosphorylase and citric acid were more than in control muscle. These results suggest a decrease in anaerobic glycolysis (Clouet, 1971; Levitt, 1975), but accompanied by insignificant changes in protein levels.

Alterations in ascorbic acid metabolism brought about by nicotine and tobacco were almost similar to those manifested by tranquillisers and non-narcotic analgesics except that the activity of special peroxidase was decreased in the present study. The increase in levels of free ascorbic acid and ascorbigen suggest the retention of ascorbic acid in muscle by the drugs in support of the work of Keith and Pelletier (1973). They have also reported elevation in stored amounts of ascorbic acid in liver, kidney, brain and adrenals in nicotine-injected animals. The greater tissue retention of ascorbic acid in drug treated animals and smokers (Pelletier, 1968, 1970), is a protective mechanism (Sprince et al., 1975) in response to the stress of nicotine or tobacco treatment to maintain the metabolic activity of the muscle. This hypothesis is substantiated by the fact that protein concentrations and alkaline phosphatase activity were significantly increased by combined tobacco + ascorbic acid
administration in vivo and a recovery in the activity of acid phosphatase was observed. However, SDH activity was not restored to normal level. Zannoni et al., (1972) have also shown a restoration in activity of drug oxidizing enzymes in livers of phenobarbital treated, vitamin C deficient guinea pigs administered with ascorbic acid in vivo.

SUMMARY

The effects of tobacco and nicotine (Cholinergic drugs/Neuromuscular stimulants) were studied on activity of rat pectoral muscle. Carbohydrate metabolism was altered as by tranquillisers and non-narcotic analgesic drugs. The changes in ascorbic acid turnover suggest its retention in muscle as a protective mechanism in response to the stress of drug treatment for maintaining muscle activity.
REFERENCES

1. Chapter I A. Studies on adaptive modifications in vertebrate skeletal muscle. I. Evidences for the occurrence of structural and metabolic evolutionary trends in vertebrate skeletal muscles.

2. Chapter II A. Studies on the effects of drugs on muscle. I. Tranquilisers.

3. Chapter II B. Studies on the effects of drugs on muscle. II. Non-Narcotic Analgesics.


Table I

Showing the levels of SDH, alkaline and acid phosphatases, protein, phosphorylase, fructose, glycogen, and citric acid in the pectoral muscle of normal, Tobacco, Tobacco + ascorbic acid and Nicotine treated rats.

<table>
<thead>
<tr>
<th>Enzymes / metabolites</th>
<th>Normal</th>
<th>Tobacco</th>
<th>Tobacco + Ascorbic acid</th>
<th>Nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate dehydrogenase</td>
<td>16.32 ± 2.30</td>
<td>17.84 ± 2.47</td>
<td>2.52 ± 0.17</td>
<td>19.50 ± 2.30</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1.65 ± 0.70</td>
<td>2.05 ± 0.17</td>
<td>4.67 ± 0.48</td>
<td>3.97 ± 0.32</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>7.22 ± 0.86</td>
<td>20.34 ± 0.85</td>
<td>5.24 ± 1.20</td>
<td>12.54 ± 1.20</td>
</tr>
<tr>
<td>Protein</td>
<td>29.90 ± 2.90</td>
<td>28.50 ± 1.60</td>
<td>45.50 ± 0.19</td>
<td>29.50 ± 1.85</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>0.012 ± 0.002</td>
<td>1.17 ± 0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>114.83 ± 6.70</td>
<td>65.89 ± 0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen</td>
<td>54.79 ± 1.06</td>
<td>130.50 ± 6.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.55 ± 0.08</td>
<td>0.88 ± 0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values are mean ± S.E.
Showing the levels of ascorbic acid (AA), ascorbigen (ASG), the rate of ascorbic acid utilization (AAD), ascorbic acid macromolecule (AA-MM) complex, and AA-FR special peroxidase in the pectoral muscle of normal and Tobacco treated rats.

<table>
<thead>
<tr>
<th>Enzymes/Metabolites</th>
<th>Normal</th>
<th>Tobacco</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid (AA)</td>
<td>1.09 ± 0.11</td>
<td>1.32 ± 0.19</td>
</tr>
<tr>
<td>Ascorbigen (ASG)</td>
<td>0.4 ± 0.02</td>
<td>0.48 ± 0.10</td>
</tr>
<tr>
<td>Ascorbic acid utilization (AAD)</td>
<td>10.92 ± 1.05</td>
<td>10.5 ± 1.23</td>
</tr>
<tr>
<td>Ascorbic acid macromolecule (AA-MM) complex</td>
<td>0.9 ± 0.24</td>
<td>0.75 ± 0.50</td>
</tr>
<tr>
<td>AA-FR special peroxidase</td>
<td>3.01 ± 0.14</td>
<td>2.20 ± 0.13</td>
</tr>
</tbody>
</table>

*The values are mean ± S.E.*