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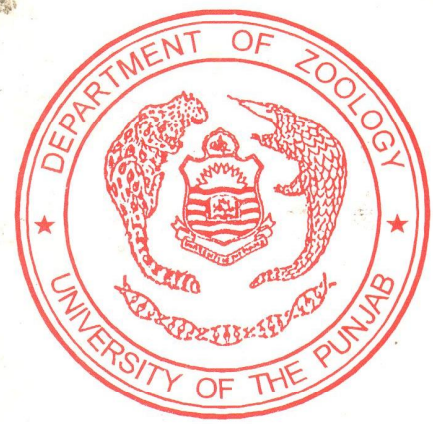


DEPARTMENT OF ZOOLOGY

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# FISHES OF THE RIVER GOMAL AND ITS TRIBUTARIES IN PAKISTAN

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**Abstract**: Gomal is an important river of Pakistan with the river Zhob as its main tributary. The survey of Gomal drainage system (rivers Gomal, Zhob and Wana stream) was done between 1988 and 1994 in various seasons. The fishes were collected using all kinds of prevalent methods. Thirty two species belonging to 23 genera, 9 families, 5 orders and 2 superorders were collected. There are important food fishes and are consumed locally: *Tor Putitora* (Hamilton), *Naziritor zhobensis* (Mirza), *Cirrhinus mrigala* (Hamilton), *Schizothorax plagiostomus* Heckel, *Schizocypris brucei* Regan, *Labeo dero* (Hamilton), *Labeo dyocheilus pakistanicus* Mirza and Awan. Some other species including *Barilius pakistanicus* Mirza and Sadiq, *Barilius vagra* (Hamilton), *Barilius bicirrhatus* (McClelland), *Botia birdi* Choudhuri, *Schistura arifi* Mirza and Banarescu, *Schistura punjabensis* (Hora) and *Schistura pakistanica* (Mirza and Banarescu) are beautiful ornamental fishes. *Garra wanae* Regan, *Schistura arifi* Mirza and Banarescu and *Schistura pakistanica* (Mirza & Banarescu) are endemic to the Gomal drainage system. Zhob valley can be regarded as a paradise of mahasheers because two species of mahasheers *viz. Tor putitora* (Hamilton) and *Naziritor zhobensis* (Mirza) are found here in abundance and provide good game to the anglers.

**Key words**: Fresh water fishes, Gomal system, NWFP, Pakistan.

## INTRODUCTION

The river Gomal originates from Afghanistan and enters into Pakistan through Gomal Pass. It passes towards east along the border of Balochistan and N.W.F. Province of Pakistan. At Khajuri Kach, it receives the river Zhob and then enters the Indus plain in Dera Ismail Khan district. After dividing into various branches, it joins the river Indus south of D.I. Khan during floods. Between Gul Kach and Khajuri Kach, it receives the Wana Toi from Wana.

The river Zhob is one of the largest rivers of Balochistan. It originates from the Kund mountains in between Muslimbagh and Kan Mehtarzai. It flows eastward and then takes a turn towards north near Gwal Haiderzai. After receiving several tributaries from the Toba Kakar Range in the north and the hill ranges between the Zhob and Loralai districts in the south, it ultimately falls into the river Gomal near Khajuri Kach. Its length between its source and the confluence with the Gomal is about 240 miles (384 km).

The fish fauna of the river Zhob was not discussed by Day (1880) and Zugmayer (1913). The first paper on the fish fauna of this river was by Mirza (1966), who recorded *Schizocypris brucei* Regan from this river.

Mirza (1967) described a new mahasheer from this river and named it as *Tor zhobensis*. This species now is known as *Naziritor zhobensis* (Mirza).

Mirza, Banarescu and Nalbant (1969) described *Noemacheilus pakistanicus* from

Kum Karez of Hindubagh (now Muslimbagh). This species is now known as *Schistura pakistanica* (Mirza and Banarescu).

Mirza (1969) discussed the systematics and zoogeography of the fishes of the genus *Cyprinion* Heckel and recorded *Cyprinion watsoni* (Day) from the river Zhob.

Mirza and Naik (1969) published the first comprehensive report on the fishes of Zhob district. They recorded 10 species including a new species, *viz.*, *Glyptothorax naziri*.

Mirza and Angvi (1972) revised the fish fauna of the Zhob district adding two more species, *viz. Noemacheilus rhadineus* Regan and *Mastacembelus armatus* (Lacepede). The record of *N. rhadineus* Regan, however, was based on a large specimen of *Schistura pakistanica* (Mirza and Banarescu).

Mirza (1972 and 1974) discussed the systematics and zoogeography of the freshwater fishes of Balochistan including the Zhob valley.

Mirza (1975) published a monographic paper on the freshwater fishes and zoogeography of Pakistan discussing the distribution of fishes in the river Zhob in addition to the other rivers of Pakistan.

Mirza (1980) revised the systematics and zoogeography of the freshwater fishes of Pakistan.

Coad (1981) in his checklist of the fishes of Afghanistan also dealt with the fishes of the Zhob river.

Mirza, Nalbant and Banarescu (1981) revised the fishes of the genus *Schistura* in Pakistan, and described *Schistura arifi* from the river Zhob.

Mirza (1989 and 1995) described the distribution of the freshwater fishes of Pakistan and adjoining areas.

Mirza, Javed and Tariq (1994) published a preliminary list on the fishes of the river Zhob based on the present collection.

The fish fauna of the Wana Toi was dealt with by Regan (1914), who described one new genus *Schizocypris* and two new species *viz.*, *Schizocypris brucei* and *Discognathus wanae*.

## SYSTEMATIC ACCOUNT

The fishes of the river Gomal and its tributaries belong to the class *Teleostomi*, subclass *Actinopterygii* and infraclass *Teleostei*. So the account of fishes given in this paper starts from superorder. In addition to the fishes collected by us, the fishes recorded by Regan (1914) from Wana Toi and Mirza and Naik (1969) from the river Zhob have also been included. There are 32 species, belonging to 23 genera, 9 families,

5 orders and 2 superorders of the teleostean fishes.

| | |
|---|---|
| SUPERORDER: | OSTARIOPHYSI |
| ORDER: | CYPRINIFORMES |
| FAMILY: | CYPRINIDAE |

1. *Barilius modestus* (Day)

There are a few specimens of this species collected from the river Zhob near Khajuri Kach, which are without vertical bars. This species remains small in size and hence is of little economic importance from the fisheries point of view.

2. *Barilius pakistanicus* Mirza and Sadiq

This species is very common and is found almost throughout the river. It is clearly identified by the black bars extending from the back to the lateral line. The number of bars is variable from 3 to 9.

Since this species is also small in size, hence is of little economic importance. It is a beautiful fish and can be used for ornamental purposes.

3. *Barilius vagra* (Hamilton)

This species is found along with *Barilius pakistanicus* from which it can be distinguished by the short vertical bars not reaching the lateral line. This is also a small fish and of no economic importance but can be used for ornamental purposes.

4. *Barilius bicirrhatus* (McClelland)

This fish was previously described from Jalalabad in Afghanistan by McClelland in 1842. Now it is collected from river Gomal at Khajuri Kach in South Waziristan Agency. It does not grow to a large size so is of little economic importance but may be kept as an ornamental fish.

5. *Cirrhinus mrigala* (Hamilton)

This species is very rare in this river as only two specimens were collected from Mughal Kot. The larger specimen is 32.5 cm in length and 359 gm in weight.

6. *Crossocheilus diplocheilus* (Heckel)

This species is quite common in the rivers Gomal and Zhob. It was collected from the river Zhob near Zhob city, Mir Ali Khel, Viala, Mina Bazar etc. From river Gomal it was collected from Pir Kach, Basti Kowr and near Tank city. It was collected from Wana stream near Mughal Khel village and New Dubkot.

7. *Cyprinion watsoni* (Day)

This species is one of the commonest fishes of the Gomal river and its tributaries. A

large number of specimens were collected from various localities of the rivers Gomal, Zhob and Wana stream.

This species also remains small in size and hence is of little importance as food.

8. *Garra gotyla* (Gray)

This species is quite common in the river Zhob. Several specimens of this species were collected from Badain Zai, Brunj Kili, Musafirpur, Zhob city, Mir Ali Khel, Viala and Khajuri Kach. It is not recorded from Wana stream.

This species remains small in size and hence is of little economic importance.

9. *Garra wanae* (Regan)

This fish is endemic to South Waziristan Aagency (Wana). It is only found between village Dubkot and Wana Toi. *Garra wanae* is mostly found along the sides of the stream where water is slow and transparent. It is of no economic importance because its flesh is sour and its size remains small.

10. *Labeo dero* (Hamilton)

This species is also quite common in the river Zhob. It was collected from Zhob City, Viala, Badainzai, Mina Bazar, Brunj Kili, Mir Ali Khel and Khajuri Kach. It was not captured from the upper reaches of Gomal, nor it was found in Wana stream.

It grows to large size and is consumed as food. The longest specimen collected from this river is 30.9cm in length. This specimen was collected from Khajuri Kach.

11. *Labeo dyocheilus pakistanicus* Mirza and Awan

This species is less common as compared to *Labeo dero*. Its specimens were in the collection from Badain Zai, Brunj Kili, Naray Zai, Mughal Kot and Khajuri Kach. It is not found in Gomal upstream from Khajuri Kach.

It is a large-sized fish and is consumed as food. The longest specimen is 35.7 cm in length.

12. *Naziritor zhobensis* (Mirza)

This is the *Zhobi mahasheer* of Pakistan. It was originally described from Viala by Mirza (1967). Its range was sbsequently extended to NWFP (Mirza, 1990).

This is one of the commonest species of fishes found in the river Zhob. About one hundred specimens were collected from almost all the localities from where the collection was done from Muslimbagh to Khajuri Kach.

It is a large-sized fish and is consumed as food. The longest specimen collected is 33.7 cm in length and 338.8 gm in weight. Like the common mahasheer, it is also an omnivorous species mainly feeding upon diatoms, Algae and macrophytes along with aquatic insects *etc*. The fecundity of this fish is low. It is about ten eggs per gramme of body *weight* of the fish.

13. *Racoma labiata* McClelland

This species is one of the rarest species of this river. Only five (5) specimens were collected from the river Zhob at Safikot. The largest specimen is 23.8cm in length and 118gm in weight. Mirza and Naik (1969) reported this species from Kum Karez near Mulimbagh. It is usually found at higher elevations and might be quite common in upper tributaries of the river.

14. *Schizocypris brucei* Regan

This species is one of the commonest species of the rivers Gomal and Zhob. More than one hundred specimens were collected from almost all the localities visited. It is also common in the Wana Stream.

It grows upto about 17cm in length and is consumed as food.

15. *Schizothorax plagiostomus* Heckel

It is the rarest species in the river Zhob. Only one specimen, 23.7cm in length and 127gm in weight was collected from Safikot. It is less rare in Waziristan where it is quite common. The largest specimen in the collection from Waziristan is 28.5cm.

Mirza and Naik (1969) reported this fish from Kum Karez near Muslimbagh. This is a hill-stream fish living in cold and clear waters at higher elevations. So it may be common in the upper reaches of the tributaries of rivers Zhob and Gomal. Sexual dimorphism is conspicuous in this species. Males and females differ in breadth and depth of head, size and shape of snout, body form and length of barbels.

16. *Tor putitora* (Hamilton)

This is the famous *Himalayan mahasheer* or the common mahasheer of South Asia. It is quite common in the river Zhob and Gomal. More than 50 specimens were collected from various localities. The largest specimen is 51.5 cm in length and 1110 gm in weight. According to the local people, specimens more than a metre in length and upto 4kg in weight are common in the lower reaches of the river Zhob. It is the most popular game fish and is known as the "*pride of the anglers*." Its flesh is tasty and less bony.

The food and feeding of this fish was studied by Subhan and Hafeez (1994), who concluded that this mahasheer was omnivorous and adaptable to changing biotic conditions. The fecundity of this fish is quite low and was found to be about ten eggs per gramme of body weight (Subhan and Hafeez, 1993).

17. *Chela cachius* (Hamilton)

It is widely distributed in the subcontinent. From the river Gomal it was captured from Pir Kach and Basti Kowr. The largest specimen in our collection is 4.8 cm. It remains small and is of no economic importance.

18. *Puntius sophore* (Hamilton)

It is a small-sized fish, common in the lower reaches of the Gomal river, not recorded from Zhob and Wana streams. It was captured from Pir Kach and Tank from the river Gomal. The largest specimen in our collection is 68.00 mm. It does not grow to a large size and hence is of little food value.

19. *Puntius ticto* (Hamilton)

A small-sized fish, commonly found in the streams of lower mountanous regions and plains from Pakistan to Thailand. From Gomal river, it was captured near Pir Kach and Tank. It is not found in Zhob, Wana and upper reaches of Gomal. The largest soecimen is about 5cm in length. It is of no food value because it remains small in size.

FAMILY: COBITIDAE

20. *Botia birdi* Chaudhuri

This species is also one of the rarest species in the river Zhob. There is only one specimen 12.8cm long from Badain Zai. It is not found in the Gomal river.

This is a beautiful fish and is used as *an* ornamental fish all over the world. Its local name is Cheeta Machhli.

FAMILY: NOEMACHEILIDAE

21. *Noemacheilus corica* (Hamilton)

This species is also rarer in the river Zhob and Gomal. There are only a few small-sized specimens from Badain Zai (river Zhob) and only two specimens from Gomal river near Pir Kach. Its maximum size in the collection is about 4cm.

22. *Acanthocobitis botia* (Hamilton)

It was only captured from the Wana stream near Mughal Khel. No specimen is present in the collection from Gomal and Zhob rivers. It is a small sized fish hence of no food value.

23. *Schistura arifi* Mirza and Banarescu

This species was described from the river Zhob near Zhob city. There are 9 specimens from Zhob and Mina Bazar, a few specimens were collected from South Waziristan Agency near Wana camp and Mughal Khel village. Maximum length in present collection is 7.4cm.

It is a beautiful fish and can be used for ornamental purpose.

24. *Schistura punjabensis* (Hora)

This species is rare in the Gomal drainage system. A few specimens were collected

from the river Zhob near Zhob city and from Wana stream near Mughal Khel. In some specimens the vertical black bars are missing. It is also an ornamental fish.

25. *Schistura pakistanica* (Mirza and Banarescu)

This species was originally described from Kum Karez near Muslimbagh. It has subsequently been collected from the river Zhob near Zhob city and Wana stream near Ashraf Khel and Mughal Khel. It has vertical bars along both sides of the body. These bars fade away along with age. In a large specimen about 15cm in total length the bars were completely absent. This specimen was wrongly reported as *Noemacheilus rhadineus* Regan by Mirza and Angvi (1972) and as *Noemacheilus cristatus* by Mirza (1975).

ORDER: SILURIFORMES
FAMILY: SISORIDAE

26. *Glyptothorax naziri* Mirza and Naik

This species is quite common in the river Zhob and its tributaries. Eighteen specimens were collected from Zhob city, Viala and Khajuri Kach. It is a small-sized fish and hence is not used as food.

27. *Glyptothorax punjabensis* Mirza and Kashmiri

This species is rare in the river Zhob, only two specimens were collected from Viala and Badain Zai. It is not found in the river Gomal and Wana stream. The largest specimen is 9.4cm. This species remains small in size and hence is of no economic importance.

FAMILY: SILURIDAE

28. *Ompok pabda* (Hamilton)

This species was recorded by Regan (1914) from Wana Toi. It is not present in our collection.

SUPERORDER: ACANTHOPTERYGII
ORDER: MASTACEMBELIFORMES
FAMILY: MASTACEMBELIDAE

29. *Mastacembelus armatus* (Lacepede)

There is only one specimen of this species collected from Viala (river Zhob).
This species grows upto 60cm in other areas and is used as food. The specimen in the present collection is only 27.8cm in total length. It is also found in the Wana stream.

ORDER: CHANNIFORMES
FAMILY: CHANNIDAE

30. *Channa punctata* (Bloch)

It is a medium-sized fish common in the plains. It is not recorded from Zhob and Wana. A few specimens were collected from Gomal river (near Pir Kach).

ORDER: PERCIFORMES
FAMILY: BELONTIIDAE

31. *Colisa fasciata* (Bloch)

It is a beautiful fish and is common in streams of the plains. It was captured from Gomal river (near Pir Kach). The longest specimen in the collection is 3.8cm. There are coloured bands on the sides of the body. It is an ornamental fish.

FAMILY: GOBIIDAE

32. *Glossogobius giuris* (Hamilton)

One soecimen 6.9cm in total length and 5.4cm in standard length was collected from the Wana Toi near Muslim Kach between Wana and Khajuri Kach.

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# BIOCHEMICAL CHANGES DURING REGENERATION OF TRANSPLANTED EXTENSOR DIGITORUM LONGUS MUSCLE IN SPRAGUE DAWLEY RATS*

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**Abstract**: Effect of transplantation on the DNA, RNA and protein contents of the regenerated Extensor Digitorum Longus (EDL) muscle in rats have been studied. The transplantation was found to cause a decrease, initially in the level of DNA, RNA, total protein, insoluble protein and soluble protein contents. Within 24 hours of transplantation, DNA decreased 33% and RNA 39%. Later on, with the initiation of regeneration, nucleic acids content started increasing and peak level of DNA was achieved on day 10 and RNA on day 15. Both these contents decreased afterward, but at the end of the experiment, these were still 2.34 x (DNA) and 1.48 x (RNA) more than that of control. The total protein content first showed decrease and then increase with the progress of regeneration. At the end of the transplantation it was only 34% lower than the control value. The insoluble protein content also followed the same pattern, so at the end of the experiment insoluble protein content was 32% less than that of control. The soluble protein content was comparatively stable component of the EDL muscle. Maximum decrease in soluble protein was obtained on day 15 of transplantation. While during the remaining days, the soluble protein content increased due to progressive regenerative activity.

**Key words**: Muscle transplantation, muscle nucleic acids content, muscle protein contents.

## INTRODUCTION

It is well established that skeletal muscle can be grafted and the histological events of muscle transplantation conclude that muscle regeneration recapitulate the ontogenic events of normal muscle in that the regenerating myoblast arise from the original degenerating fibers. These myoblasts fuse with each other to form myotubes which then synthesizes myofibrils and become new muscle fibers (Carlson, 1978; Faulkner, *et al.*, 1989; Gill and Shakoori, 1995). A number of factors influence the success of muscle regeneration occurring within a graft. These include revascularization, hormones or growth factor, phagocytic activity and biochemical factors which support muscle regeneration.

The protein biosynthesis occurs more slowly in adult striated muscle as compared to secretory tissues like pancreas or liver. Early studies by Brachet (1941, 1950) and Davidson and Waymouth (1946) showed both the intensity of basophilia and the RNA content of muscle tissue are very low. The rate of protein biosynthesis as well as RNA content may vary considerably in different types of muscle cells, according to their specialized metabolic features and function. No systematic studies have so far been carried out to establish such a correlation. Indeed a few chemical analyses have been

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*Part of the Ph.D. thesis of first author, submitted to the University of the Punjab, Lahore, Pakistan.

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carried out on individual muscle, instead of groups of muscles. The values reported in the literature for the RNA content of muscle vary widely from author to author and according to the methods employed (Leslie, 1995; Muscatello, *et al.*, 1961; Perry and Zydowo, 1952; Schneider, 1945).

Winick and Noble (1965) for examining skeletal muscle growth frequently utilize DNA and protein/DNA as estimates of cell number and cell size, respectively. Because skeletal muscle is a multinucleated tissue, the DNA content (the number of nuclei) should not be equated with muscle cell number. Muscle fibers are multi nucleated, undergo structurally distinct growth in longitudinal and transverse dimensions appear capable of adding nuclei at any point along the fiber (Burleigh, 1974) and maintain the ability to add nuclei from satellite cells beyond the end of muscle growth (Young *et al.*, 1978). In addition to the rapid accumulation of nuclei during muscle regeneration, it will be demonstrated that changes in nuclear and sarcoplasmic basophilia precede the formation of structural proteins. These inter-related events offer favourable material for the study of cytological changes accompanying rapid protein synthesis. Results of a recent study, which examined the role of enzymes associated with glucose metabolism in regenerating free grafts of the extensor digitorum longus (EDL) muscle, were consistent with the interpretation that the grafts did not attain complete maturity, at least not in the biochemical sense (Wagner *et al.*, 1977; Schwartz *et al.*, 1985).

No quantitative data exist for the biochemical changes in free muscle grafts. So the objective of the present investigation was to determine the level of total muscle DNA, RNA and protein contents in regenerated EDL muscle following transplantation.

## MATERIALS AND METHODS

The experiments were performed on male Sprague Dawley rats of 210-255 gms. The animals were kept in semi-controlled temperature conditions and were provided with tap water and commercially prepared food *ad libitum*.

### *Transplantation*

The experiments were carried out under semi-sterile conditions. The EDL muscle was isolated from the anterior tibialis muscle and transplanted as described previously (Gill and Shakoori, 1995). The tendinous connections were securely stitched while the cut ends of the nerves and the blood vessels were left lying nearby. The operated legs were cleaned with 70% alcohol and acriflavin solution (5%) was applied over the wound to avoid infection.

After various time intervals, viz., 1, 3, 5, 7, 10, 15, 30 and 60 days, both right and left EDL muscles were dissected out and processed for biochemical analysis.

### *Biochemical analysis*

For estimation of nucleic acids, the method described by Shakoori and Ahmad (1973) was adopted. DNA was extracted in 10% perchloric acid (PCA) at 65°C for 30 minutes, while RNA was extracted in 20% PCA at 4°C for 24 hours. The hot PCA

extract was used for the estimation of DNA by Diphenylamine method according to Schmidt and Thannhauser procedure as described by Schneider (1957), while cold PCA extract was used for the estimation of RNA by orcinol method. The standard curve of DNA was prepared from calf thymus gland, while that of RNA was prepared from pure yeast.

For estimation of protein contents, the weighed amount of muscle was crushed in 0.89% saline, centrifuged and supernatant was used for the estimation of soluble proteins according to Lowry *et al.* (1951). The pellet obtained above was digested in 6N NaOH at 36-40°C for 24 hours, heated at 45°C for ten minutes till muscle was dissolved completely. This was used for the estimation of insoluble protein content. For the estimation of total protein content muscle was dissolved in 5N NaOH. The O.D. obtained was caliberated against standard curve prepared from BSA.

## RESULTS

*Nucleic acids and protein contents*

Figure 1 shows the changes in nucleic acids and protein contents. The control EDL muscle contained $0.64 \pm 0.03 \mu g$ DNA (n=5) and $1.15 \pm 0.05$ $\mu g$ RNA/mg muscle weight (n=5). One day after transplantation, both the DNA and RNA content decreased 33% and 39%, respectively. While with the initiation of regeneration, nucleic acids content started increasing and maximum level of DNA was achieved on day 10 which was 4.65 x more than that of control, and that of RNA was 4.36 x, which was achieved on day 15 of transplantation. After attainment of peaks on the above days of observation, the nucleic acids content started decreasing. The values of DNA and RNA contents had decreased to $1.50 \pm 0.03$ $\mu g$ (n=5) and $1.70 \pm 0.08$ $\mu g$/mg muscle weight (n=5), respectively, at the end of the experiment. These were still 2.34x (DNA) and 1.48x (RNA) higher when compared with the control levels. The RNA and DNA hold a ratio of 1.79 in the control muscle. After transplantation, the ratio between the RNA and DNA increased to 2.65 on day 7. While with the initiation of regeneration, ratio started decreasing and was 1.13 on day 60 of experiment (Fig. 2).

The total protein content of EDL muscle in control rat was $154.24 \pm 2.72$ $\mu g$/mg muscle weight (n=5). With the initiation of transplantation, total protein content started decreasing and continued to do so till day 10 when it was $60.92 \pm 1.43$ $\mu g$/mg muscle weight (n=5). During subsequent days of experiment *i.e.*, with the initiation of regenerative activity, total protein content started increasing and reached to $102.51 \pm 2.81$ $\mu g$/mg muscle weight (n=5) on day 60. The total protein content was 34% lower than the control value at the end of the transplantation period. The insoluble protein followed almost the same pattern. The insoluble protein component in control rat EDL muscle was $120.98 \pm 2.96$ $\mu g$/mg muscle weight (n=5) which decreased 61% on day 10 and 32% on day 60. The soluble protein was comparatively much stable component of the EDL muscle. The control EDL muscle had $33.34 \pm 0.90$ $\mu g$ soluble protein/mg mucle weight (n=5). The soluble protein content showed maximum decrease of 67% on day 15 of transplantation. During the later days of the experiment, the soluble protein content increased, but these contents were still 39% less than the control values.

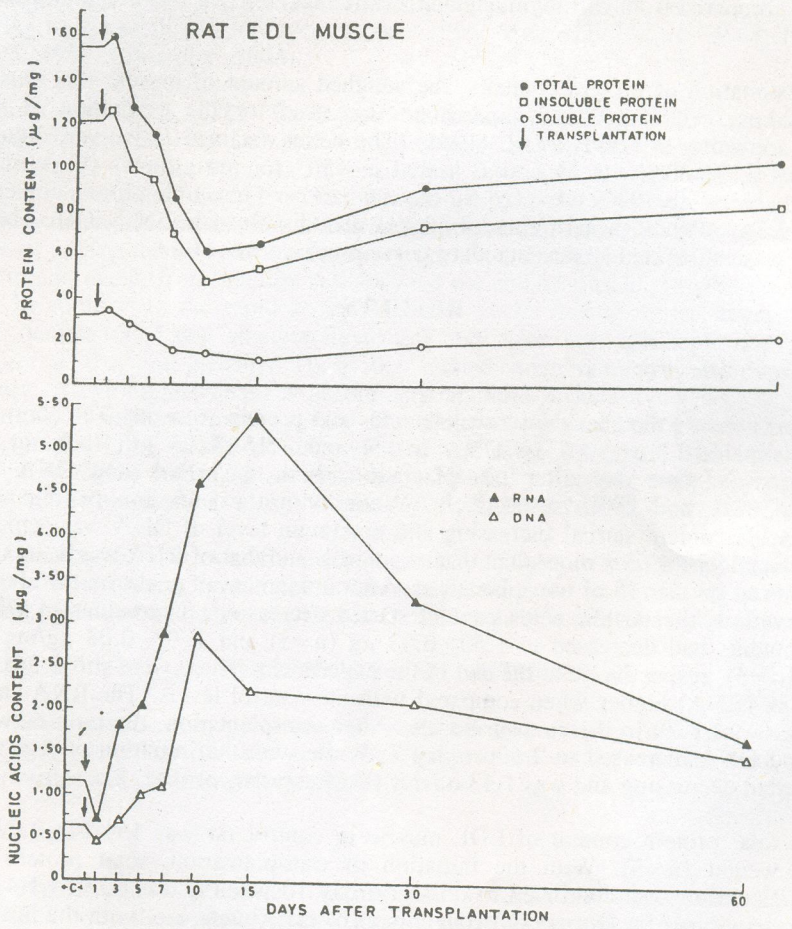


**Fig. 1**. Effect of transplantation on the nucleic acids and protein content of EDL muscle of rat.

The total protein hold a ratio of 4.62 with the soluble protein, the ratio reached to its minimum level on day 10, but then showed an increase afterward. The total protein and RNA content relationship showed some very distinct variations during the post operational period. This ratio in control was 134.12, which shot up to 226.57 within one day and then drastically reduced during the subsequent days (Fig. 2).

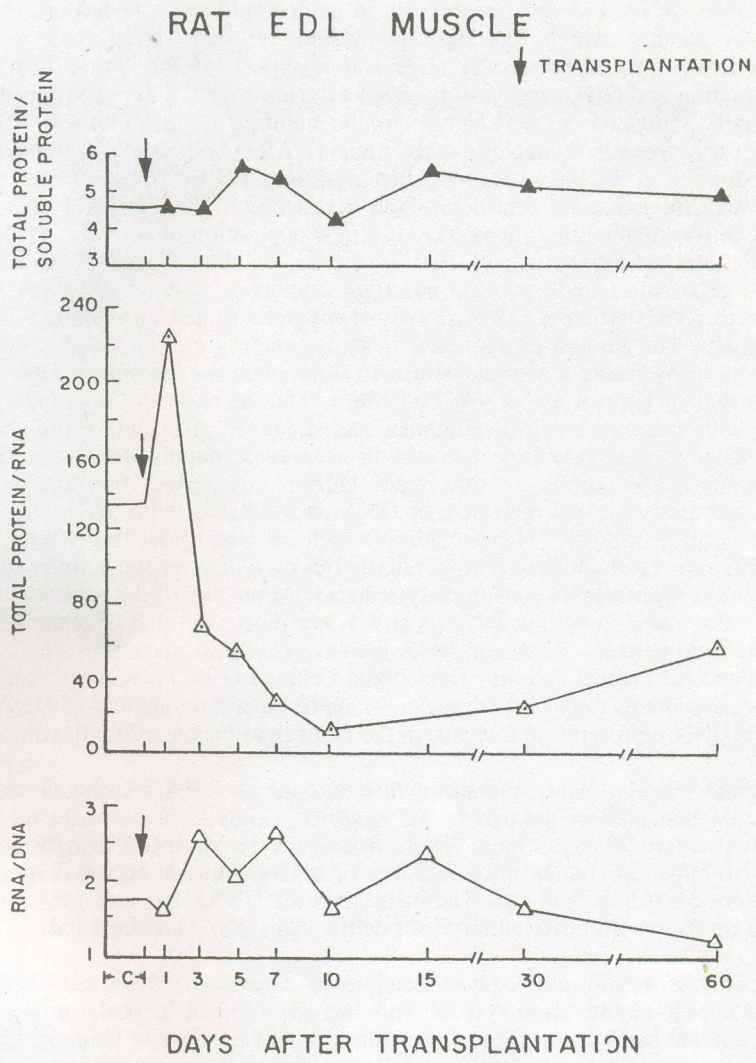


**Fig. 2**. Changes in ratios between RNA and DNA, total protein and RNA and total protein and soluble protein of rat EDL muscle after transplantation.

## DISCUSSION

Transplantation produces alterations in morphological, functional and biochemical characteristic of muscle. This experiment shows that DNA, RNA and protein contents are more important determinants of the success of a free muscle graft. The observation

made on DNA, RNA and protein contents in control and transplanted muscle show a quantitative pattern which corresponds accurately with what has been seen morphometrically and histologically (Gill and Shakoori, 1995). There is observed a rapid destruction and degeneration of the nuclei during first 1-5 days of transplantation, which is duly indicated by a reduction in the number of nuclei/muscle fiber and a decrease in the amount of nucleic acids content. After one week of transplantation, there is reduction in the quantity of soluble, insoluble and total protein contents which coincides with the extensive destruction and degeneration of the muscle fibers. By the 7-15 days of transplantation, emergence of a new population of actively dividing cells. These cells are predominantly of two types, as myoblastic and fibroblastic. The myoblastic cells, after undergoing a specified number of mitotic divisions fuse with each other to form myotubes. This process of myotube formation is quite conspicuous by the 7th day. The amount of total nucleic acids content *i.e.*, DNA and RNA increase sharply from a low value to a peak within 15 days when the amount of DNA is 2.46x more than that of control and RNA 3.65 times. This increase in the amount of DNA coincides with the increase in the number and diameter of nuclei. From the 15 day onwards, there is relatively little increase in myoblastic population, connective tissue and tendinous tissue within the transplant. During this period, the concentration of RNA is comparatively less than that of DNA, nevertheless both are higher than the levels of control muscle. These findings indicate that older regenerate are still considerably more cellular than normal muscle but protein synthetic activity of the cells comprising the regenerate is considerably reduced. At the end of the experiment *i.e.*, 60 days, the value came down but DNA is still 1.34x more than that of control and RNA 0.47x. The ratio between RNA and DNA increase after transplantation which is due to the disturbance of normal activity. Enesco and Leblond (1962) postulated that a normal muscle tissue contains only diploid nuclei which has a costant amount of DNA and any increase in DNA represents an increase in the number of nuclei within the muscle.

In normal muscle, while measuring the amount of DNA/muscle, Gordon *et al.* (1966) found that nuclear number in the quadriceps muscle of the male rat increased until approximately 90 days of age. These findings were confirmed using four different muscles. No apparent change in the number of nuclei / muscle occurred in any of the muscles from 81-165 days of age. The increase in the DNA content of growing fibers is dependent on the proliferative activity of satellite cells (Moss and Leblond, 1970).

Gallucci *et al.* (1966) noticed a tremendous increase in the DNA and RNA contents of minced muscle regeneration system. This increase reached its peak on day 15, when the DNA content had become almost four times that of the control value and RNA 2½-4 time that of normal. At day 60, the value remained high but came down to three time that of normal value. Moss (1968) and Burleigh (1974) believe that postnatal growth is achieved both by nuclear (DNA) proliferation and increase in protein / DNA ratio which are measures of cell size.

Gorin *et al.* (1989) mentioned that the magnitude and time course of DNA synthesis can be compared with changes in RNA and protein content during regeneration. The DNA synthesis reflects satellite cell proliferation and was maximal on postgraft day 5. As a result of which significant changes in total RNA content, mRNA content and muscle protein content during later regenerative times. Martin *et al.* (1990) suggested

that recovery of up to 1 year was insufficient for the normalization of several connective tissue matrix components and biochemical properties of the grafts.

In the present study, muscle regeneration after transplantation is associated with marked decrements in protein synthesis. The total protein content decreased to a considerable extent after transplantation which coincide with the destruction and degeneration of the muscle fibers. At the end of the experiment, the total protein content is 34% lower than the control value. The insoluble protein content also followed the same pattern and is 32% less than that of the control. The soluble protein content is stable component of the EDL muscle, so after transplantation maximum loss is obtained on day 15 whereas that of total and insoluble protein content is obtained on day 10. The ratio between the total protein and soluble protein and total protein and RNA is also disturbed after transplantation. The ratio between the total protein and soluble protein reached its minimum level on day 10 which is due to distinct behaviour of the soluble protein after transplantation. The total protein and RNA ratio shoots up within 24 hours of transplantation, this abrupt change is due to disturbance of normal mitotic activity. The ratio reached its minimum level afterward which is due to regeneration of degenerated fibers.

Kelly *et al.* (1984) while working on pre- and post- natal growth and protein turnover in skeletal muscle of rat postulated that the fractional rates of protein synthesis (measured *in vivo*) and break down in each muscle declined with age, the change in the former correlates with decrease in the ribosomal capacity of the muscles. Wagner *et al.* (1977) and Schwartz *et al.* (1985) mentioned that the EDL muscle grafts did not attain complete maturity at least in the biochemical sense. It is well known that any treatment or disturbance effect the normal activity of the muscle. Howard *et al.* (1989) and Steffen *et al.* (1990) observed alteration in the pool of mRNAs in immature muscles within hours of the initiation of hind limb suspension. Changes in the levels of protein synthesis and degradation (Goldspink *et al.*, 1986; Loughna *et al.*, 1986; Loughna *et al.*, 1987; Thomason and Booth, 1990) occur within the first 3 days of hind limb unweighting.

It is concluded from all the above mentioned information that it is more important to determine the level of total muscle DNA, RNA and protein contents in regenerated EDL muscle after transplantation. All these information support the idea of regenerative capability of muscles.

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# MORPHOMETRIC VALUES OF OVARY, OVIDUCT AND CERVIX OF PAKISTANI FEMALE CAMEL (*CAMELUS DROMEDARIUS*)

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**Abstract**: The biometrical values including size (length, width and thickness) and weight of the normal ovary, oviduct and cervix in three different age groups of Pakistani female camels (*C. dromedarius*) have shown that there were no differences in both left and right organs of same age group, however, have variations in different age showing the increase with the advancement of the age.

**Key words**: Biometrical values, camel, female reproductive organs, ovary, oviduct, cervix.

## INTRODUCTION

Camel (*C. dromedarius*) is an important mean of transport, meat and milk in the middle East and African countries. The reasonable population of this species is used for this purpose in Pakistan which is about 0.819 million (FAO, 1978). Compared to the importance of dromedary, there is a dearth of literature about it. The study of the reproductive organs in camels has remained incomplete, only very little information exists on the female camel (Icapal, 1987). Several studies have been done on the biometrical aspects of female genitalia in adult camels. Tayeb (1953), Shalash (1965), Joshi (1978) and Khan *et al.*, (1989) have observed cervices. Abdalla (1967) and Musa (1969) studied oviducts and Musa (1969, Chahrasbie and Goulbazhagh (1975), Musa (1979) and Arthur (1989) have examined ovaries in adult female camels. Al-Eknah *et al.*, (1992) have studied morphology of female genitalia of camel in three different age groups in adult stages.

The morphological developments of genital tract from pre-puleral stage to post-puleral stage are of great significance in several aspects. Such study is lacking in camel. The present work accords the biometrical changes that accompany from immature to fully developed stage in ovary, oviduct and uterus of female camel.

## MATERIALS AND METHODS

The animals used in this study were those which were brought for slaughtering in the Lahore Abattoir. A total of 130 samples were collected. Those were assorted into three different groups on the basis of their age. These were camel calf (below 2 years), heifer (2-4 years) and adult (above 4 years). One hundred and thirty internal genitalia, specimens were separated into normal and biometrical studies included size (length, width, thickness) and weight of normal organs. These were determined with a measuring tape, vernier caliper and analytical balance based on the techniques used by Khan (1985).

## RESULTS AND DISCUSSION

The biometrical values including length, width, thickness and weight were observed in 256 normal ovaries of 128 of these three groups were shown in Table I. These values were comparable with those reported by Musa (1979) and Iqbal *et al.* (1993). The length of the 260 normal oviducts were measured and shown in Table II which were comparable with Abdalla (1967) and Iqbal *et al.* (1993), while regarding 127 normal cervices only length and number of rings were noted and are shown in Table II. These values were comparable with those reported by Joshi *et al.* (1978) and Ali *et al.* (1992).

This data may serve to determine the normal status of female reproductive state in a camel for any biochemical, physiological, or hormonal studies based on slaughtering animals.

**Table 1. Biometrical values of normal ovary and oviduct of female camel**

| Age | Calf (n=15) | | Heifer (n=33) | | Adult (n=80) | |
|---|---|---|---|---|---|---|
| **Ovary** | | | | | | |
| Parameters | Left ovary | Right ovary | Left ovary | Right ovary | Left ovary | Right ovary |
| Length (cm) Mean ± S.E.M. | 2.5±0.10 | 2.42±0.07 | 3.06±0.08 | 2.90±0.07 | 3.63±0.05 | 3.45±0.06 |
| Width (cm) Mean ± S.E.M. | 1.89±0.15 | 1.90±0.08 | 2.24±0.06 | 2.11±0.06 | 2.62±0.05 | 2.57±0.05 |
| Thickness (cm) Mean±S.E.M. | 0.80±0.05 | 0.71±0.06 | 0.75±0.04 | 0.79±0.04 | 0.93±0.02 | 0.82±0.02 |
| Weight (g) Mean±S.E.M. | 3.65±0.46 | 3.50±0.37 | 4.52±0.12 | 4.24±0.11 | 4.76±0.08 | 4.33±0.08 |
| **Oviduct** | | | | | | |
| Parameters | Left oviduct | Right oviduct | Left oviduct | Right oviduct | Left oviduct | Right oviduct |
| Length (cm) Mean ± S.E.M. | 19.2±0.6 | 19.2±0.6 | 23.5±0.53 | 23.5±0.53 | 25.5±0.29 | 25.2±0.29 |

S.E.M. = Standard error of mean

**Table II. Biometrical values of normal cervix of female camel**

| Age | Calf n=15 | Heifer n=33 | Adult n=78 |
|---|---|---|---|
| Length (cm) Mean+S.E.M. | 3.22±0.09 | 4.06±0.08 | 4.68±0.06 |
| Range (cm) | 2.5-3.6 | 3-4.7 | 3.5-6.5 |
| No. of Rings Mean+S.E.M. | 3±0.13 | 3±0.05 | 3.38±0.07 |

S.E.M. = Standard error of mean

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# MAJOR PLASMA LIPIDS FOLLOWING GROWTH HORMONE TREATMENT IN FED AND STARVED MALE DWARF GOATS

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**Abstract:** Major plasma lipid fractions, separated thin layer chromatographically, were studied following growth hormone (GH) treatment (75 µg/ kg body weight) in six days starved and fed goats. GH significantly mobilized free fatty acids (FFAs) in fed state increasing their level to about 34%. Starvation, however, suppressed the consequent FFAs elevation following the hormone treatment. A non significant increase in plasma free cholesterol (FC) was noticed in fed animals following the hormone treatment. Conversely, in starvation, the circulatory level of the fraction was significantly enhanced by 25% compared to fed controls. GH failed to induce an appreciable effect on esterified cholesterol (EC) fraction in fed state, however, in starved state the treatment resulted in a non significant increase after two hours. A pronounced but non significant reduction of 19% in GH treated fed goats and a non significant elevation in starved state was exhibited in plasma triglycerides (TG) fraction, two hours following the treatment, as compared to fed controls.

**Key words**: Cholesterol, free fatty acids, growth hormone, lipids, starvation, triglycerides.

## INTRODUCTION

Several hormones particularly insulin, glucagon, growth hormone, etc. control and regulate lipid metabolism. Excess of growth hormone (GH) increased non esterified fatty acids and ketones by 50% without any effect on fasting glucose in human (Sherwin *et al.*, 1983). Its lipolytic activity in rabbit adipocytes with stimulated fatty acid production (Gorin and Goodman, 1984) and in rat adipocytes (Goodman, 1984) with other biological actions as well (Barenton *et al.*, 1984) has been observed. Lipolytic effects of bovine GH with increases in free fatty acids (FFAs) and plasma insulin have been reported (Hart *et al.*, 1985) with similar results in gilts (Kirkwood *et al.*, 1989). Circulatory levels of lipids also affect secretory capacity of the hormone as fatty acids increases markedly suppressed the immunoreactive GH levels (Hicks *et al.*, 1977).

Numerous factors such as fasting, cold exposure, excessive food intake also affect lipid metabolism (Sullivan *et al.*, 1971; Trenkle, 1970; Therriault and Mehlman, 1968). Triglycerides are catabolized to FFAs (Owen *et al.*, 1971) which are further oxidized to ketone bodies (Aoki, 1981). Prolongation of fasting turns free fatty acids an important metabolic fuel for liver and other tissues.

GH also changes with levels of nutrition. High secretions of GH are associated with restricted feeding and low concentrations with increased nutrition in female lambs (Foster *et al.*, 1989) and in sheep (Trenkle, 1989). Attention to the work on cholesterol,

another important circulatory lipid, has not been paid in such experimental state of ruminants as well as non ruminants.

Ruminants differ from non ruminants in energy metabolism as these use volatile fatty acids and are prompt in gluconeogenesis. Thus it is quite likely that responses of lipid metabolism are variedly indisposed in ruminants compared to non ruminants. Thus the present study is undertaken to investigate major metabolically responsive fractions of plasma lipids in fed and starved GH treated male dwarf goats.

## MATERIALS AND METHODS

Male dwarf goats weighing between 20-25 kg and 2-3 years of age of goat facility at Research Substation of Pakistan Atomic Energy Commission on Bedian Road, Lahore were used in the study. Ninteen goats were picked and initially categorized in two groups. Seven goats were deprived of food for seven days while being kept in a confined area. Other goats grazed during the day and were also confined, at night, to the area of fasting/starved goats. On seventh day, fed goats were further grouped into two batches of six heads each. One fed group was taken as control and injected with sterile saline; second fed group and the starved group were injected with bovine GH (Sa Novo Industry NV, Denmark), at a rate of 75 μg/kg body weight. Blood samples were drawn just before and two hours after the treatment. Plasma claimed following centrifugation of blood was stored at -20°C till used for biochemical analysis.

Free fatty acids, triglycerides and cholesterol fractions in plasma were determined after separation by one dimensional thin layer chromatography (Stahl, 1969). The concentrations of individual lipid fractions were quantified from the peak area obtained through densitometery (Sakura PDS. 15 with 8 mm wide slit). The total peak area was measured by polar planimeter (KE 62005) (Castellani *et al.*, 1975). Results were analysed and compared statistically by Wilcoxan Rank Sum Test at 5% probability level.

## RESULTS

*Free fatty acids*

GH distinctly affected plasma FFAs in fed goats. There was a 34% significant elevation of the fraction in GH treated fed goats compared to saline injected fed goats. Starvation suppressed the consequent FFAs elevation following GH treatment as the fraction in starved GH treated goats was not significantly different from fed controls. This effect was, however, pronounced between the comparison of GH treated fed and starved goats as starved goats showed 18% lower concentration of FFAs compared to fed goats following GH treatment (Figs. 1-2).

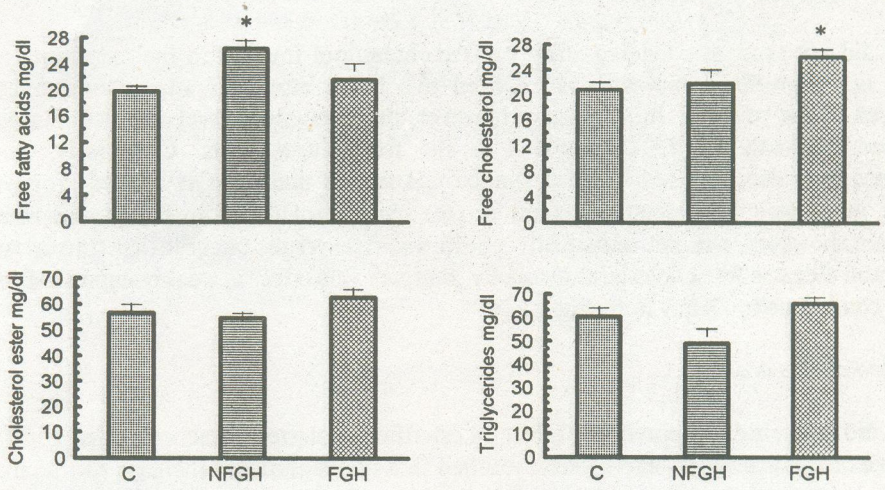


**Fig. 1.** Plasma lipids fractions (mg/dl) in control (C), non fasting growth hormone treated (NFGH) and fasting growth hormone treated (FGH) groups, two hours following the administration. Results shown are mean±S. E. *Least significance at P<0.05 when compared with zero hour control group.

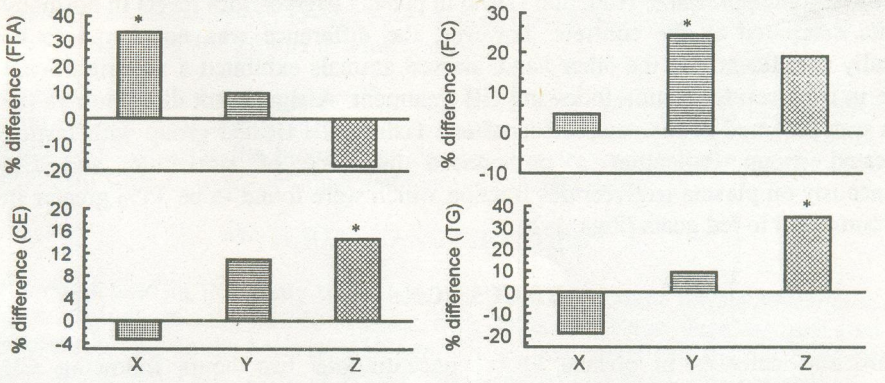


**Fig. 2.** Percentage difference comparison in the levels of free fatty acids (FFA), Free cholesterol (FC), cholesterol ester (CE) and triglycerides (TG), following growth hormone administration. X compares C and NFGH, Y compares C and FGH and Z compares NFGH and FGH. *Least significance at P<0.05 level.

*Free cholesterol*

GH did not show any marked effect on free cholesterol fraction in the fed animals, only a non significant increase was noticed two hours after GH administration as compared to the controls. In starvation, however, the circulatory level of fraction was increased markedly by 25% compared to the fed control goats. Conversely, the difference was also observed between starved GH treated and fed GH treated groups. Fasting group had 19% greater amount of free cholesterol than the fed groups; the difference, however, was not statistically significant. The results suggest that starvation affects and elevates free cholesterol distinctly, however this effect is weakly expressed in the fed conditions by GH as well (Figs. 1-2).

*Esterified cholesterol*

GH did not bring an appreciable effect on esterified cholesterol fraction in fed goats. The hormone, however, in starved state resulted in a non significant increase two hours after its administration compared to fed control. A pronounced effect of fraction elevation was observed in GH treated starved goats compared to the hormone treated fed goats. The results indicate that esterified fraction of cholesterol is enhanced noticeably by GH in starved state compared to the fed state (Figs. 1-2).

*Triglycerides*

GH caused an appreciable reduction (19%) in plasma triglycerides levels in normally fed goats compared to the controls, however, the difference was not found to be statistically significant. On the other hand, starved animals exhibited a non significant increase in triglyceride fraction following GH treatment. A significant difference in the fraction was observed in the comparison of non fasting GH treated group with fasting GH treated group indicating a pronounced influence of starvation and GH simultaneously on plasma triglycerides fraction which were found to be 35% greater in starved compared to fed goats (Figs. 1-2).

## DISCUSSION

Significant increases in plasma FFAs concentrations two hours following GH treatment in fed goats of the present study clearly exhibit the lipolytic activity of the hormone. Lipolytic activity of GH in rabbit adipocytes has been attributed to stimulated FFAs production (Barenton *et al.*, 1984). In starved state of dwarf goat, FFAs increases were not as pronounced which may be accounted due to prompt utilization of FFAs or comparatively suppression of lipolytic action of GH. The former assumption seems more likely as it has been explained that the major source of cellular fuel for fasting animals

are triglycerides of adipose tissue which are hydrolyzed to FFAs and transported across the adipose tissue membrane (Jackson and Winkler, 1969). During transition between fed and fasted state, body fat metabolism is alternately switched from anabolic state characterized by FFAs and triglyceride synthesis to a catabolic state in which triglycerides are converted to FFAs (Owen *et al.*, 1979); FFAs in turn are oxidized and converted to ketone bodies (Aoki, 1981). The possibility of the mobilization of FFAs due to lipolytic action of GH and their oxidation to ketone bodies cannot be ruled out. In ruminants, ketone bodies are the principal energy source in prolonged fasting and starvation. It is quite likely that GH during starvation promotes FFAs oxidation and the elevation of GH during starvation is the compensatory response to promote FFAs oxidation. Considerable studies have shown that high secretions of GH are associated with restricted feeding and low concentrations with increased feeding in female lambs (Foster *et al.*, 1989) and in sheep (Trenkle, 1989). Landefeld *et al.* (1989) also observed profound changes in synthesis, storage and secretion of the hormone during limited secretion in lambs.

In dwarf goat of the present study, in fed state, GH non significantly increased cholesterol both free as well as esterified fractions, however, significant increases of both the fractions were observed when GH was administered in the starved state. In general, in goat, GH and starvation together elevated circulatory cholesterol fractions. This response is quite in contrast to the reports in rat as far as starvation alone is concerned. In rats, starvation for five days caused lowering in total and free cholesterol and a decreased ratio of total cholesterol to cholesterol ester was observed whereas in refed state, a rise in the level of cholesterol ester and of total cholesterol but no change in free cholesterol in blood sera was observed (Gutkowski, 1970; Kerpel *et al.*, 1971). In guinae pigs, like in dwarf goats, an increase in cholesterol levels after five days of starvation has been reported. In literature, there is no comparable data in non ruminants on the effects of GH in fed and starved states on the cholesterol fractions. The parallel effects on both free and esterified fractions of cholesterol show that there is a general mobilization of cholesterol by GH and/or starvation. Naqvi *et al.* (1989) have reported increases in β-lipoprotein fractions during prolonged fasting with a rapid decrease of alpha/beta ratio which have been attributed to reduced anabolic activity of adipose tissue under starvation and lowering in the removal of β-lipoproteins from blood. As argued in FFAs, in cholesterol also, GH promotes the compensatory response like lowering in the removal of β-lipoproteins from blood resulting in enhancement of esterified cholesterol as well as free fraction of the cholesterol.

The appreciable decrease in plasma triglycerides levels of fed goats two hours after hormone injection probably reflect an indirect influence of GH on plasma triglycerides level. A close relationship is observed between the pancreatic hormones and the GH in pigs as increased circulatory GH elevated significantly the insulin in venous blood (Gustavson and Lundquist, 1982). Also a strong influence of insulin on plasma triglycerides

is observed in rats, where high levels caused more increase in rate of triglyceride removal from the circulation than the rate of triglyceride secretion into the blood (Kazumi *et al.*, 1988). In view of these studies it seems probable that GH injection in fed state increased insulin release and caused lowering in triglycerides from the blood plasma by removing into the liver or other tissues. Marked increase in plasma triglycerides was found in starved GH treated dwarf goat in the present study. In rats, prolonged fasting, however, reduced the serum triglycerides levels (Kerpel *et al.*, 1971 and Menahan and Sobocinski, 1983). After five days, fasting alone caused no significant effect on serum triglycerides levels in guinae pigs (Khalifa, 1986).

The results of triglycerides in starvation further support the argument of insulin involvement in lowering triglycerides during fed state as discussed earlier. In starvation, sensitivity of insulin is markedly reduced so as to sustain energy constituents of glucose and FFAs for their availability to the tissues. Thus it is quite likely that GH fails to elicit insulin axis in the clearance of triglycerides in starved state. The role of GH seems to be of significant importance in the starvation of a ruminant for the energy homeostasis. Further studies to elucidate this phenomenon would be of greater importance.

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# BABESIOSIS IN CROSS-BRED CATTLE (*BOS INDICUS* x *BOS TAURUS* AND BUFFALOES (*BUBALUS BUBALIS*)

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**Abstract**: Babesiosis (Redwater), a tick borne protozoal disease, poses a great threat to cows and buffaloes. It was recorded from different districts of Punjab *i.e.* Lahore, Kasur, Jhang, Faisalabad and Gujrat. The surveys revealed many animals to be infected with *Babesia begemina*. These infected animals showed pyrexia, loss of appetite, dysponoea, haemoglobinurea and decrease in the milk yield. Animals responded to treatment with Diminazine 20-30 ml, Berenil 1.05 gm/12 ml distilled water and Imizol (Imidocarb) 1ml/100kg body wieght.

**Key words**: Babesiosis, cross bred cattle, buffaloes.

## INTRODUCTION

Babesiosis is a debilitating, and often fatal disease of cattle. In Pakistan all breeds, *i.e.* the local breed (*Bos indicus*) as well as the exotic breed (*Bos taurus*) and crossbred (*Bos indicus* x *Bos taurus*) are vulnerable to it. Cross breds have anhanced susceptibility to infectious diseases, especially to babesiosis (Smith and Kilbourne, 1893; Ashfaque *et al.*, 1983; Mottelib *et al.*,1992). Buffaloes have also been reported to suffer from babesiosis (Lieu, 1986).

The organism that caused this disease is a protozoan parasite belonging to the genus *Babesia*. *B. bigemina* causes clinical disease in cattle and buffaloes. Most animals that are susceptible to the disease die from infection if not treated.

The present data represents part of a larger study comprising survey of out breaks of babesiosis, among other tick-borne cattle diseases in different parts of Punjab, assessment of damage and work along remedial lines has also been carried out.

## MATERIALS AND METHODS

A survey for the presence of babesiosis in cattle and buffaloes was carried out in Lahore, Kasur, Jhang, Faisalabad and Gujrat districts. Blood samples of the infected animals were taken at the spot and stained with Geimsas' stain. Other haematological studies were carried out using the standard techniques of Schalm *et al.* (1975). All sixty four animals were found to be suffering from babesiosis during the different surveys carried out.

Diminazine (Star) 20-30 ml, Berenil (Hoechest) 1.05 g/12ml distilled water, and Imizol (ICI) Imidocarbdipropionate 1ml/100kg (i/m) were used for treatment. B-complex 10 ml (i/m) was injected five times, once on every alternate day.

## RESULTS

Seven out of 15 cross-bred cows showed rise in temperature to 105 °F within one month *i.e.* middle of July to middle of August. Treatment with antibiotics like Oxytetracycline and Gentamycine brought no response. Cows also showed the symptoms of haemoglobinurea. Examination of blood smears during febrile stage revealed *B. bigemina*. Diminazine injection of the infected animals resulted in a positive response and to normalcy. *Boophilus microplus* were the vector ticks. Buffaloes showed rise in temperature, loss of appetite, haemoglobinurea. There was a significant decrease in milk yield. These cases were observed in different districts of Punjab and were treated with Diminazine, Berenil and Imizol successfully, (see, Table I). Buffaloes were also infected with another species *B. microplus*. After the treatment a complete cure occured and almost all the animals became normal in milk yield within 4 weeks.

**Table I. Cases of babesiosis recorded in cattle and buffaloes at private farms and clinics.**

| Source | Species | Affected cases | Animals cured | Deaths due to disease | Drugs used |
|---|---|---|---|---|---|
| Khalid Dairy Farm, Burki, Dist. Lahore | Cross cows | 7 | 6 | 1 | Diminazine |
| Dist. Lahore | Buffaloes | 15 | 13 | 2 | Imizol |
| Dist. Lahore | Cows | 5 | 5 | - | Berenil |
| Dist. Kasur | Buffaloes | 12 | 10 | 2 | Diminazine |
| Dist. Jhang | Buffaloes | 9 | 8 | 1 | Berenil |
| Dist. Faisalabad | Buffaloes | 12 | 10 | 2 | Diminazine, Imizol |
| Dist. Gujrat | Buffaloes | 4 | 4 | - | Berenil |

Haematological examination revealed decrease in packed cell volume (PCV), total erythrocytic count (TEC), haemoglobin (Hb), total leukocytic count (TLC; Table II).

**Table II** **Haematological profile of healthy and clinical babesiosis positive cases (2 cattle and 4 buffaloes) of each group.**

| Parameters | Healthy animals | Diseased animals |
|---|---|---|
| PCV (%) | 30.00±0.50 | 15.60±1.25 |
| Hb (g%) | 11.00±0.90 | 5.93±0.30 |
| TEC ($10^6$/cmm) | 6.30±0.77 | 3.50±0.11 |
| TLC ($10^3$/cmm) | 7.93±0.16 | 1.42±0.08 |
| Neutrophils (%) | 32.6±2.33 | 50.30±3.32 |
| Eosinophils(%) | 6.10±1.38 | 3.0±1.54 |
| Basophils (%) | 0 | 0 |
| Lymphocytes (%) | 54.30±2.33 | 42.00±3.03 |
| Monocytes (%) | 6.50±1.04 | 1.60±1.36 |

## DISCUSSION

Besides causing physical damage, the tick *B. microplus* is also responsible for the transmission of babesiosis in cattle and buffaloes in India and Pakistan. Dwivedi *et al.* (1979), Roychoudhry and Cautam (1980), Sanshez (1984), Rao *et al.* (1986) and others have aslo recorded the same results. *Babesia bigemina* are present in the red blood cells during the febrile stage of disease in the form of round or pear shaped bodies 2-4$\mu$m long by 1 to 2$\mu$m wide (Blood and Radostits, 1989).

Incidence of babesiosis was higher in the rainy season. It was mainly due to the increase of population of the ticks, although sporadic cases were aslo found throughout the year. These findings are in line with Mallick *et al.* (1983), James *et al.* (1985), Ouhelli (1985), Cooper (1989).

Haematological results revealed haemolytic anemia in diseased animals. The leukocytosis with neutrophilia was due to stress of acute babesiosis. Similar results were observed by Rogers (1971), Panday and Misra (1987), Bansal *et al.* (1990), Mottelib *et al.* (1992). Deaths occured due to the inadequate clinical management of haemolytic anaemia.

Subclinical cases in cows and buffaloes showed red water symptoms for one or two days and then self recovery was observed due to the acquired immunity which is also a character of *B. bigemina i.e.* it may occur in mild form as previously reported (Mahony, 1977). A number of drugs exist for the treatment of babesiosis. Imizol, Diminazine and Berenil were tried in cattle and buffaloes and gave good results as

obtained before by other workers (Karimov and Gafurov, 1984; Hashmi and Sharki, 1991; Irwin and Hutchison, 1991).

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# GRAM-NEGATIVE BACTERIA FROM INDUSTRIAL WASTES CONFERRING PLEOTROPIC METAL AND ANTIBIOTIC RESISTANCE

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**Abstract**: Ten isolates, MA-1 to MA-10, were isolated from the effluents of ICI Industry and Nalla Dek near Sheikhupura. They exhibited resistance to cadmium, chromium, mercury, lead, zinc, iron, nickle, copper, manganese, cobalt and molybdenium. They all tolerate salts of Cr, Ni and Co (50$\mu$g ml$^{-1}$), Cu (100$\mu$g ml$^{-1}$), Mn and Fe (150$\mu$g ml$^{-1}$), Pb (1000$\mu$g ml$^{-1}$) and Mo (7000$\mu$g ml$^{-1}$) in the medium. They tolerate kanamycin, chloramphenicol and ampicillin in the medium. These isolates differ morphologically, physiologically and biochemically from one another. But all of them had catalase and oxidase enzymes. They could reduce nitrate, hydrolyse gelatin and produce acid from arabinose. MA-5 could be affiliated with Pseudomonadaceae. MA-1, MA-2, MA-6, MA-7, and MA-9 belong to Gram-negative facultative anaerobic rods. MA-3, MA-4, MA-8, and MA-10 shared maximum characters with family Bacillaceae. Different strains favour different pH values (6-9) for maximal growth. Gel electrophoresis of total cell lysate revealed presence of plasmid only in MA-6 and MA-8. Conjugation experiments exhibited that plasmid residing in MA-6 was conjugative and confer resistace to molybdenum.

**Key words**: Metal resistant bacteria, antibiotics resistance bacteria, bacteria from industrial effluent.

## INTRODUCTION

The industrially polluted aquatic environment is frequently contaminated by jeopardous heavy metals. The toxic elements, after a part of life cycle of soil, enters the biotic strata and affects its biological activities (Capone *et al.*, 1983). The heavy metals show menacing effects by binding with essential functional groups, replacing the essential metal ion or modifying the active confirmation of biological molecules (Collins and Stotzky, 1989; Guzzo *et al.*, 1991). In addition, metal ions and their complexes have potential to cause genetic damage and has carcinogenic effects (Abbott, 1985). Their long half-lives and accumulation in tissues aggravate the danger.

Bacteria can tolerate heavy metal stresses due to prosence of cellular mechanisms of combating the toxic effects. These mechanisms include gene amplification (Beach and Palmiter, 1981), enhanced transcription of metallothionein gene (Hildebrand *et al.*, 1982), cadmium efflux (Burke and Pfister, 1986), alteration in cell wall and plasma membrane complex (Grindle, 1984), deposition of material in walls (Brown and Smith, 1976), release of bacterial exudates (Birch and Bachofen, 1990). Metal resistant bacteria are important as an index of pollution as well as clearing agents for heavy metals from the environment. In the present work isolation of bacteria, which confer pleotropic metal resistance, is being described.

## MATERIALS AND METHODS

For isolation of metal resistant bacteria, two samples from ICI Industry Sheikhupura, one from inlet effluents (odourless, colourless with black suspended solid particles, pH 2.45), other from outlet effluent (odourless, colourless with blackish suspended particles and pH 8.46) and one sample from Nallah Dek (with yellowish suspended particles, pungent smell and pH 3.11) were collected in sterilized glass bottles. Water sample (50$\mu$l) was plated onto nutrient-agar plates supplemented with 25$\mu$g ml$^{-1}$ of each $CdCl^2$, $CrO^3$, $NiCl^2$, $CoCl^2$, $CuSO^4$, $MnSO^4$, $FeCl^3$, $HgCl^2$, $Pb(NO^3)^2$, $ZnSO^4$, and $NaMoO^4$ for the selection of Cd, Cr, Ni, Co, Cu, Mn, Fe, Hg, Pb, Zn and Mo resistance, respectively. The isolates were purified and were taken to elevated levels. Ten strains, that could deter salts of Cr, Ni and Co (50$\mu$g ml$^{-1}$), Cu (100$\mu$g ml$^{-1}$), Mn and Fe (150$\mu$g ml$^{-1}$), Pb (1mg ml$^{-1}$) and Mo (7mg ml$^{-1}$) in the medium are the subject of this study. They were designated as MA-1, MA-2, MA-3, MA-4, MA-5, MA-6, MA-7, MA-8, MA-9 and MA-10. Ensuing Gerhardt *et al.* (1981) the isolates were characterized morphologically, physiologically and biochemically. Additional twenty biochemical and cytochrome oxidase tests were performed by using QTS-20 and Co-strips, obtained from DESTO Laboratories, Karachi. Spore forming ability was corroborated by the method of Moir (1981). They were also checked for thier sensitivity behaviour against antibiotics, ampicillin (Ap), kanamycin (Km), tetracycline (Tc), chloramphenicol (Cm) and streptomycin (Sm).

For genetic analysis, bacteria were screened for the presence of plasmid by total cell lysate method (Thomas, 1984). To characterize plasmid, conjugation experiments were performed (Willetts, 1984). MA-12 ($Tc^{r20}$, $Mo^{s1000}$) was used as a recipient.

## RESULTS AND DISCUSSION

Bacterial growth obtained, was purified and subjected to elevated levels, MA-1, MA-4, and MA-5 were isolated from sample 1, while isolates MA-6, MA-7, MA-9 and MA-10 were obtained from sample 2. MA-3 and MA-8 were from sample 3. All isolates could tolerate salts of Cr, Ni and Co (50$\mu$g ml$^{-1}$), Cu (100$\mu$g ml$^{-1}$), Mn and Fe (150$\mu$g ml$^{-1}$), Pb (1mg ml$^{-1}$) and Mo (7mg ml$^{-1}$). MA-1, MA-2, MA-3, MA-4, MA-9 and MA-10 could also resist 100$\mu$g ml$^{-1}$ of $ZnSO_4$, while remaining four isolates (MA-5, MA-6, MA-7 and MA-8) could tolerate only 25$\mu$g ml$^{-1}$ of this metallic salt. Only MA-9 could bear Cd and Hg salts (50$\mu$g ml$^{-1}$) in the medium, other strains were sensitive to these metallic salts. Both Gram-negative (Mergeays *et al.*, 1985; Nies *et al.*, 1989; Malik *et al.*, 1991; Hasnain and Sabri, 1991, 1992) and Gram-positive (Burke and Pfister, 1986; Mahler *et al.*, 1986) metal resistant bacteria have been reported previously. Multivariant resistance determining *Alcaligenes eutropus* (Ni, Hg, Co, Zn, Cd -- Mergeay *et al.*, 1985), *Staphylococcus aureus* (Cd, Zn -- Perry and Silver, 1982), *Bacillus* (Cd, Hg, -- Mahler *et al.*, 1986), (Cu, Ni, Sn, Mn, Ba -- Hasnain and Sabri, 1991), *Thiobacillus thiooxidans* (Cd, Zn -- Sakamoto *et al.*, 1989) members of Bacillaceae and Vibrionaceae (Ni, Cu, Co, Mn, Sn, Zn, Fe -- Malik *et al.*, 1991; Hasnain and Sabri, 1992) were described by different workers. None of them was qouted to confer resistance to Cr, Pb and Mo metallic salts. Chromium resistant *Pseudomonas* species (Ohtake *et al.*, 1987; Horitsu *et al.*, 1983, 1987) have not been described conferring resistance to other heavy metals. In *P. flourescence* genetic

determinant of resistance was plasmid coded (Ohtake *et al.*, 1987) Only *Alcaligenes eutrophus* which determine resistance to chromate also confer resistance to cobalt (Nies *et al.*, 1989), *Citrobacter* (Macaskie and Dean, 1987) and Pseudomonads (Hasnain *et al.*, 1993) have also been described. The isolates described here are unique in the sense that they also confer resistance to Cr, Pb and Mo. In the previous reports these resistances were not described in the same strain. The growth media, especially those that contain casein amino acids bound heavy metals and reduce the concentration of free ions, affects the bacterial growth (Ramamoorthy and Kushner, 1975) and the bacterial growth depends upon the kinds, forms and concentration of the salts in the medium (Onishi *et al.*, 1984; Hughus and Poole, 1991). These isolates could also tolerate antibiotics Ap (300 $\mu$g ml$^{-1}$), Cm (5 $\mu$g ml$^{-1}$), Sm (100 $\mu$g ml$^{-1}$) and Km (50 $\mu$g ml$^{-1}$) in the medium. They exhibited different behaviour for Tc resistance. MA-1, MA-2, MA-6 and MA-8 were sensitive to Tc (20 $\mu$g ml$^{-1}$) while rest of them could deter it in the medium. All strains were sensitive to Sm (500 $\mu$g ml$^{-1}$). These results are in accord with our previous results (Hasnain and Sabri, 1991, 1992) in that, in addition to conferring resistance to metals they exhibited multiple resistances to antibiotics. Ahmad and Yadava (1988) described Hg-resistant strains, majority of which showed either single or multiple antibiotic resistances whereas according to Mergeay *et al.* (1985) heavy metal resistant bacteria do not show antibiotic resistance.

**Table I. Colony and cell morphology of metal resistant bacteria**

| Isolates | Visual color | Elevation | Form | Margin | Size (mm) | Cell shape | Cell Size ($\mu$m) |
|---|---|---|---|---|---|---|---|
| MA-1 | Creamy | Convex papillate | Rhizodial | Entire | 1.0-3.00 | Pleomorphic rods | |
| MA-2 | Ochre | Umbonate | Circular | Entire | 1.7-2.5 | Pleomorphic rods | |
| MA-3 | Creamy | Raised | Rhizodial | Filamentous | 2.5-7 | Rods | 2x10 |
| MA-4 | Ochre | Raised | Myceloid | Erose | 1.4-3.3 | Rods | 0.5x3.0 |
| MA-5 | Bright yellow | Pulvinate | Circular | Entire | 1.7-2.0 | Cocci | 1 |
| MA-6 | Orange | Raised | Circular | Entire | 2.0-2.5 | Rods | 1.0x2.5 |
| MA-7 | Cream | Raised | Circular | Slightly undulate | 2.0-2.5 | Rods | 1.5-3.0 |
| MA-8 | Cream | Convex papillate | Circular | Entire | 4.5-4.7 | Rods | 0.5x2.0 |
| MA-9 | Cream | Flat | Circular | Entire | 1.5-1.8 | Rods | 1.0x2.0 |
| MA-10 | Cream | Low convex | Filamentous | Filamentous | 2.5-2.8 | Pleomorphic | |

**Table II. Biochemical characterization of metal resistant bacteria**

| Tests | Isolates | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MA-1 | MA-2 | MA-3 | MA-4 | MA-5 | MA-6 | MA-7 | MA-8 | MA-9 | MA-10 |
| Gram-staining | - | - | - | - | - | - | - | - | - | - |
| Sproe-staining | - | - | + | + | - | - | - | + | - | + |
| Urease | - | - | - | - | - | - | - | + | - | - |
| Catalase | + | + | + | + | + | + | + | + | + | + |
| Oxidase | + | + | + | + | + | + | + | + | + | + |
| Methyl red | - | - | - | - | - | - | - | - | - | + |
| Nitrification | + | + | + | + | + | + | + | + | + | + |
| Denitrification | - | - | - | + | + | + | - | - | + | - |
| OF test | + | + | + | + | + | + | + | + | + | + |
| Pigment test | - | - | - | - | + | - | - | - | - | - |
| ONPG | - | ++ | ++ | - | - | - | - | ++ | - | - |
| Sodium citrate | - | - | - | - | - | - | - | - | - | - |
| Sodium malonate | - | - | - | - | - | - | - | - | - | - |
| Lysine decarboxylase | - | - | - | - | - | - | - | - | - | - |
| Arginine dihydrolase | - | - | - | - | - | - | - | - | - | - |
| Ornithine decarboxylase | - | - | - | - | - | - | - | - | - | - |
| $H^2S$ production | - | - | - | - | - | - | - | - | - | - |
| Urea hydrolysis | - | - | - | - | - | - | - | - | - | - |
| Tryptophan deaminase | - | - | - | - | - | - | - | - | - | - |
| Indole | - | - | - | - | - | - | - | - | - | - |
| Acetoin | - | - | - | - | - | ++ | - | - | - | + |
| Gelatin hydrolysis | ++ | ++ | ++ | ++ | ++ | ++ | + | ++ | ++ | ++ |
| Acid from glucose | - | - | - | - | - | - | - | - | - | - |
| Nitrate reduction | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Acid from maltose | - | - | - | - | - | - | - | - | - | - |
| Acid from sucrose | - | - | - | - | - | - | - | - | - | - |
| Acid from mannitol | - | - | - | - | - | - | - | - | - | - |
| Acid from arabinose | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Acid from rhamnose | - | - | - | - | - | - | - | - | - | - |
| Acid from sorbitol | - | - | - | - | - | - | - | - | - | - |
| Acid from inositol | - | - | - | - | - | - | - | - | - | - |

- negative; + positive; ++ strong positive

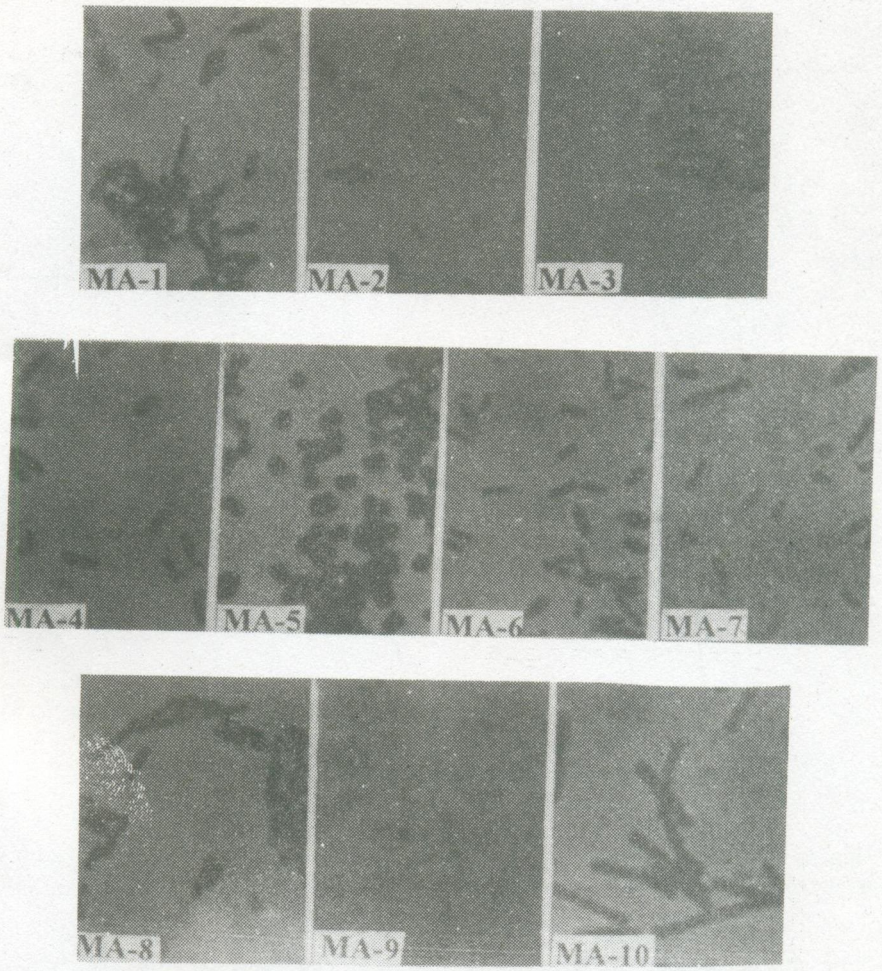


**Fig. 1**. Isolates from industrial effluents, MA-1, MA-2 and MA-10 pleomorphic Gram-negative rods; MA-3, MA-4, MA-6, MA-7, MA-8 and MA-9 Gram-negative rods; MA-5 Gram-negative Cocci.

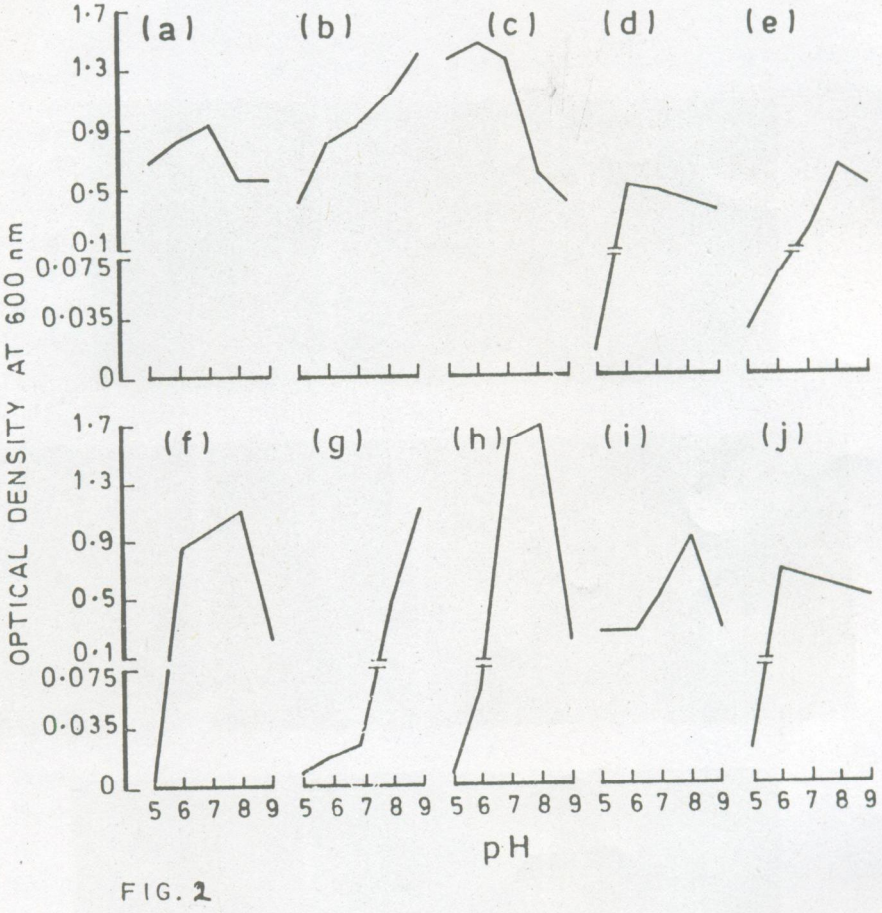


FIG. 2

**Fig. 2.** Effects of different pH (5, 6, 7, 8 and 9) on the growth of bacteria isolated from the industrial effluents.

For morphological characterization 24 hour old streaked colonies were used. Morphologically colonies differ in colour, shape, size and elevation (Table I). All cells were Gram-negative and motile but vary in shape and size (Table I, Fig. 1). As the concentration of metallic salts increased in the medium the bacterial growth as well as the cell size decreased. Which might be due to reduced cellular growth and metabolic activity under heavy metal stresses (Jonas *et al.*, 1984). All strains were able to grow on L-agar, nutrient agar but not on MacConkey's agar. Only MA-1 and MA-3 could grow on MacConkey's agar. All strains were facultative anaerobe and produce acid from both glucose and mannitol, only MA-10 showed gas production as well. MA-3, MA-4, MA-6 and MA-10 were spore former. Many Cd and Hg-resistant isolates are

spore former (Mahler *et al.*, 1986). All have catalase and oxidase enzyme. Only MA-5 could produce orange pigment on king's medium, while MA-2, MA-4, MA-7, MA-8 and MA-10 produce brownish pigments on media supplemented with salt of manganese. They share many biochemical characters, but differ from one another for some characters (Table II). On the basis of these biochemical characters MA-5 could be affiliated with Pseudomonadaceae, but this strain could grow anaerobically. The *P. nautica* (a denitrifying bacteria) isolated by Bonin *et al.*, (1989) could also grow anaerobically. This isolate MA-5 could resist very high concentration of Mo in the medium. Molybdenum co-transported quantitatively more in glucose, sucrose and mannitol growth cultures (Baxi and Modi, 1988) and its uptake is also dependent upon the metabolizable carbon source. Inhibition of glycolysis also inhibits molybdenum uptake (Baxi and Modi, 1988). Anaerobic breakdown of glucose and mannitol by this strain might be related to the Mo-resistance property of this isolate. Endospore forming, rod shaped MA-3, MA-4, MA-8 and MA-10 could be assigned to family Bacillaceae (Krieg and Holt, 1984). MA-1, MA-2, MA-6, MA-7 and MA-9 belong to Gram-negative, facultative anaerobic rods of Bergey's Manual (Kreig and Holt, 1984). Their further affiliation remained uncertain.

pH is an important factor affecting bacterial growth, bioavailibility, reactivity and complex formation of metals (Hughus and Poole, 1991). For physiological characterization these strains were grown in LB media (Sambrook *et al.*, 1989), adjusted to different pH (5, 6, 7, 8, and 9) levels, at 37°C (150 rpm) for 16 hours. After that the OD of the culture was monitored on spectrophotometer at 600 nm. Results of these strains demonstrate that different isolates prefer different pH levels for their growth. MA-3, MA-4 and MA-10 prefer pH6; MA-1 showed best growth at pH7; MA-5, MA-6, MA-8 and MA-9 showed maximum growth at pH8; while MA-2 and MA-7 favour pH9 for their growth. MA-1, MA-3, MA-6 and MA-8 could grow in pH range 5-8, while pH range for growth of MA-2, MA-4, MA-5, MA-7 and MA-10 was from 6-9. Only MA-9 showed comparatively narrow pH range (7-9) for its growth (Fig. 2). According to Rochelle *et al.* (1989) bacteria prefer environmentally related pH for their growth. It is not true for all the isolates obtained in this study. Only isolates from sample two (MA-2, MA-6, MA-7, MA-9) favours alkaline pH (8, 9) and pH of sample two was 8.46. Two other samples, one and three, were highly acidic (2.45 and 3.11 respectively) but the isolates obtained from these samples favoured pH6 (MA-3, MA-4), 7 (MA-1) or 8 (MA-5, MA-8) for their growth. It appears that these strains could survive at highly acidic pH, but their multiplication was restricted and on obtaining favourable conditions they showed extensive growth. MA-3, MA-4 and MA-8 were spore-formers, it seems that these strains were enduring extremely acidic pH by endospore formation. Further, metal resistances of bacterial strains are pH dependent (Wood and Wang, 1985). Gel electrophoresis of total cell lysate revealed the presence of single plasmid in each of MA-6 and MA-8. Hg, Cd, Cr, Co, Zn, Pb, and Fe has been reported plasmid encoded (Perry and Silver, 1982; Mergeay *et al.*, 1985; Mahler *et al.*, 1986; Nies and Silver, 1989; Nies *et al.*, 1989; Hasnain and Sabri, 1991, 92; Malik *et al.*, 1991). Whereas, few reports also dealt with non-plasmid borne cadmium-resistance (Mahler *et al.*, 1986). To determine whether resistance conferred by MA-6 and MA-8 were transcribed by plasmid/s or chromosome, conjugation experiments were performed. Since many broad host ranged plasmids form Gram-negative bacteria (Old and Primrose, 1985) can promote their transfer. MA-12 (Gram-negative, rod

shaped, $Cr^r$, $Pb^r$, $Cd^s$, $Mo^s$, $Ap^r$, $Cm^r$, $Km^s$, $Tc^r$) was used as recipient. Quite reasonable number (520) of transconjugant on Mo and Tc plates, when MA-6 was used as donor, demonstrate that Mo-resistance in this strain was encoded by plasmid. No transconjugants were scored when MA-8 was donor. These results do not necessarily reflect the lack of Mo-marker on this plasmid, it might be due to the operation of some host restriction mechanism. Failure in detecting the plasmid band by the electrophoresis of total cell lysate in the rest of strains do not exclude the possibility of plasmid determinants in the rest of strains. Mega-plasmids (cointegrate plasmids) may not be detected by this method (Thomas, 1984). Both plasmids (Mergeay, 1991) and transposons governed (Diels *et al.*, 1987) resistances to heavy metals have been reported. Multiple antibiotic resistances have been described both in plasmids and transposons (Watson, *et al.*, 1987; Bennett *et al.*, 1988). Whether pleotropic metal as well as antibiotics resistance in the strains described here are encoded by plasmids (cointegrate plasmids), transposons or chromosome separately or some integration between different genetic determinants exist for these resistance, remained to be determined.

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# A NEW SPECIES OF THE GENUS *HEXAPROTODON* FROM THE DHOKWAZIRA, DISTRICT JHELUM, PUNJAB, PAKISTAN

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**Abstract**: A well preserved skull is described from the Dhokwazira. In general the skull appears to be smaller in size as compared to those referred to *Hexaprotodon sivalensis*. The most distinguishing feature is the size of $P^1$, which is much smaller as compared to that of *H. sivalensis*. On the basis of these features the species under study is regarded as a new species and the name *H. dhokwazirensis* is being proposed which is after the name of the type locality.

**Key words**: Genus *Hexaprotodon*, Dhokwaziria.

## INTRODUCTION

The term *Hexaprotodon* was introduced by Falconer and Cautley (1836) as a subgenus of *Hippopotamus*. The main distinguising feature of this was condsidered to be the number of incisors. There are six incisors in *Hexaprotodon* as compared to four in typical *Hippopotamus*. Lydekker (1884) did not accept this and placed *Hexaprotodon* as a synonym of *Hippopotamus*. Later authors, Matthew (1929), Colbert (1935), Simpson (1945), Deraniyagala (1969) and Nanda (1978) contradicted this view of Lydekker (1884) and gave a generic rank to the term *Hexaprotodon*. Uptil now two species of this genus are known from the Siwaliks. These are *H. iravaticus* and *H. sivalensis*. Acoording to Colbert (1937) Siwalik Hippos range from Middle to Upper Siwaliks. But the recent studies of Swalik hippos (Sarwar and Akhtar, 1992) have shown that they were even present in the Lower Siwaliks. The specimen under study has been collected from Pinjor beds of the Upper Siwaliks. A comparison with the known species of the genus *Hexaprotodon* has shown that the specimen under study represents a new species of the genus. The name *H. dhokwazirensis* has been assigned to this new species. The specimen under study has been given the number with prefix P.M.N.H. (Pakistan Museum of Natural History, Islamabad). Measurements are given in millimeters.

## SYSTEMATIC ACCOUNT

| | |
|---|---|
| Order: | ARTIODACTYLA (Owen) |
| Suborder: | SUIFORMES (Jaeckel) |
| Infraorder: | ANCODONTA (Matthew) |
| Superfamily: | ANTHRACOTHERIOIDEA (Gill) |
| Family: | HIPPOPOTAMIDAE (Gray) |
| Genus: | *HEXAPROTODON* (Falconer and Caurley) |

*Hexaprotodon dhokwaziresis*, new species (Fig. 1).

*Type:* P.M.N.H. No. 87/114, a slightly damaged skull.

*Hypodigm*: The type only.

*Locality*: Dhokwazira, district Jhelum, Punjab, Pakistan.

*Horizon*: Upper Swilaiks.

*Diagnosis*: A Hexaprotodon smaller than Hexaprotodon sivalensis and with relatively small $P^1$.

## DISCUSSION

The specimen under study is a skull broken into two parts horizontally, so that the palatal part is separate from the top though both parts fit into each other. The canines of both sides and the incisors of the right side are represeted by their alveoli. Among the cheek teeth the left $P^4$ is well preserved and the rest are damaged to varying degrees. However, they clearly indicate their diagnostic features.

As is typical of the genus, the tooth rows are curved, they converge posteriorly and diverge anteriorly. The width of palate at $P^1$ is 106mm and at $M^1$ it is 66mm, which is typical of the genus and compares favourably with the figures given by Colbert (1935) and Hooijer (1950). As stated above, there are three alveoli of the incisors on the right side, the left side is damaged in this part of the skull. From the alveoli $I^2$ appears to be the largest one. The greater diameter of $I^1$ - $I^3$ are 17mm, 21mm and 19mm, respectively. Between $I^3$ and canine there is a diastema of 40mm. The same figure for *H. sivalensis*, described by Colbert (1935) is approximately 68mm as measured from the diagram. Similarly, for *H. sivalensis soloensis*, described by Hooijer (1950) it is 52mm. Thus, it appears that this diastema is relatively short in this species.

The canine is represented by its alveoli which shows the typical features of *Hexaprotodon*. It is broad anteriorly and narrow posteriorly. In its middle there is a longitudinal groove as in *H. sivalensis*. Like the latter it is longer than broad; these figures being 52mm x 40mm approximately. Between the canine and $P^1$ there is a diastema which is approximately 12mm. In *H. sivalensis* it varies between 22mm and 40mm (Hooijer, 1950). According to Colbert (1935) it varies between 28mm and 40mm. Thus, this diastema is, relatively, very short as compared to that of *H. sivalensis* even, about which Colbert (1935) says, "The canine premolar diastema is very short."

The most distinguishing feature is the size of $P^1$, which is much smaller as compared to that of *H. sivalensis*. The length x width of this tooth, which is represented by the base of the crown on right side is 25mm x 21mm. The same figure for *H. sivalensis*, (Am. Mus. No. 19776), described by Colbert (1935) are 44 x 28, as measured from the diagram. The length x width of $P^2$ and $P^3$ are approximately 38 x 28mm and 35 x 28mm respectively. These are represented by the bases of their crowns. $P^4$ of left side is relatively better preserved; it is damaged slightly on the labial side. Its length x width is 30 x 30mm approximately. It has two cusps, one on the lingual and

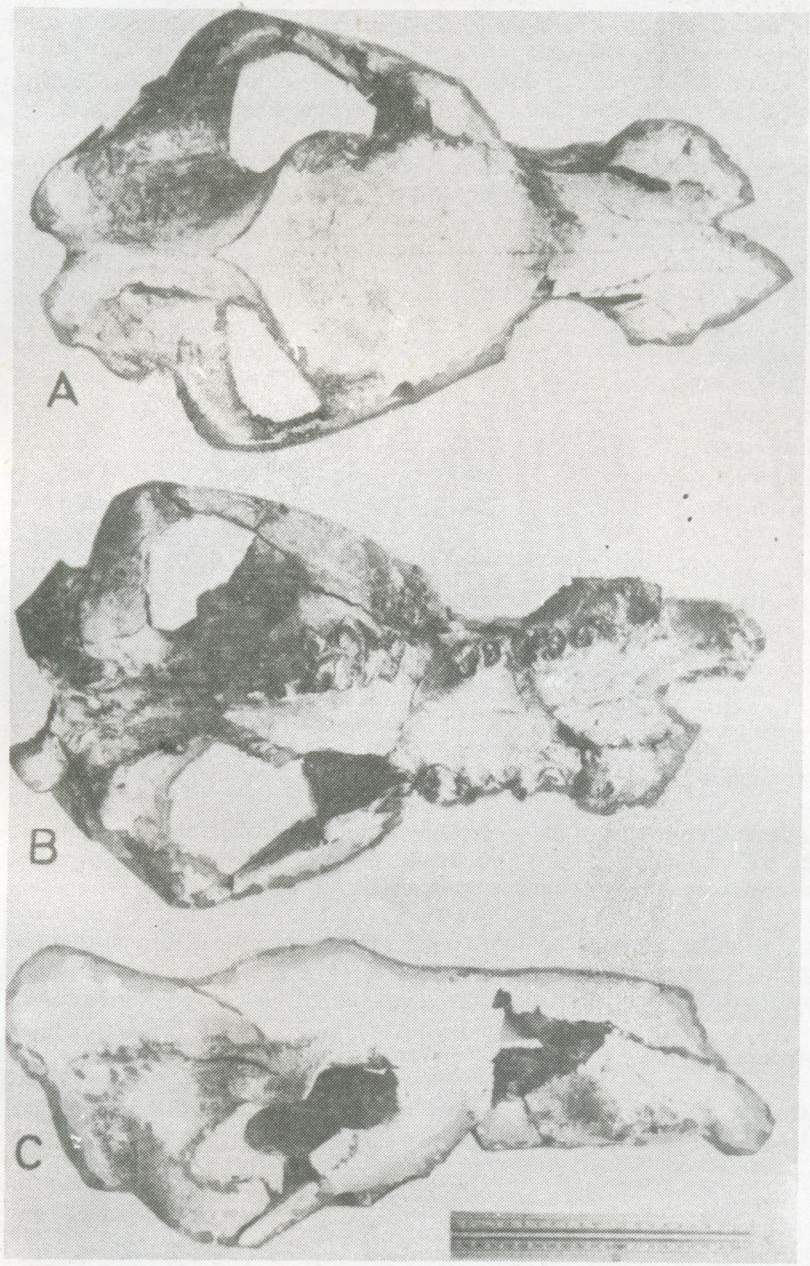


Fig. 1 A skull of *Hexaprotodon dhokwazirensis*. A, top view; B, palatal view; C, lateral view.

other on the labial side. The latter is damaged. The tooth is encircled by a cingulum. The molars, which are damaged, are present on the right side only. $M^1$ is the smallest and $M^2$ is the largest tooth. A distinct feature of $M^1$ is that it is narrow anteriorly and broad posteriorly. The same feature is visible, to some extent in $M^2$ as well.

**Table I: Measurements (in mm) of the specimen under study and those of *H. sivalensis* described by Colbert (1935) and Hooijer**

| | Specimen under | Colbert's specimens | Hooijer's specimens |
|---|---|---|---|
| Total length of skull | 500+ | 600-610 | - |
| Preorbital length | 280 | 330-335 | 314-340 |
| Postorbital length | 220 | 260-280 | 228-275 |
| Height x length of orbit | 60 x 62 | - | 57-65 x 55-73 |
| Width of condyles | 110 | 120-140 | 120-147 |
| Width of occiput | 210 | 220-263 | 224-263 |
| Width of zygomatic arches | 340 | 332-339 | 325-390 |
| Width of palate at $P^1$ | 106 | 104-125 | 104-125 |
| Width of palate at $M^1$ | 66 | 47-60 | 47-62 |
| Height of occiput | 190 | 165-185 | 164-188 |

+ approximate.

On the posterior part of the ventral side behind the palate two longitudinal ridges of palatine and pterygoid are very prominent. Behind these there are the auditory bullae which are rather short and compressed laterally.

In general the skull appears to be smaller in size as compared to those referred to *H. sivalensis*. However, it is relatively broader as seen in the Table I. The name *H. dhokwazirensis*, new species, has been assigned to this new form of the genus.

**Acknowledgement**

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# STUDIES ON THE PREVALENCE OF PATHOGENIC BACTERIA IN THE AIR OF LAHORE

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**Abstract**: The survival pattern of *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes* was studied in the air of Southern, Central and Northern part of Lahore. Colony forming units (CFU) were noted down on Endoagar, MacConkey agar, Mannitol salt agar and blood agar medium. The CFU of *S. aureus* were more in number in Central part (Railway Station, Regal Chowk) and Northern part (Shad Bagh) of Lahore. The number of this bacterium was more during hot months of May and June. The CFU of *E. coli* were lesser in number as ocmpared to *S. aureus*. However, the CFU of this bacterium was fairly prevalent in Central part of Lahore particularly during high temperature period of May and June, 1994. In general the CFU of *S. aureus* and *E. coli* were eight and six times more than *S. pyogenes* in summer months. Humidity was an important factor for higher CFU in the air of Lahore.

**Key words**: Pathogenic bacteria in air, air pollution, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*.

## INTRODUCTION

Powar and Daginawala (1988) discussed that air is not a natural ecvironment for the growth and reproduction of microorganisms. It does not contain the necessary amount of moisture and utilizable form of the nutrients. Yet microorganisms are found in air, though they have a transient survival. The sources of organisms are the soil, the organic wastes of man and animals, nasal and rectal passages of man and animals and from lung throgh cough and sneezes. The ultimate fate of air borne microorganisms is governed not only by a complex set of circumstances including the atmospheric conditions *e.g.*, humidity, sunlight and temperature, but also the size of the particle bearing the microorganism and the nature of the microorganism. The population of Lahore is increasing day by day. The waste disposal system is not up to the mark which can cause an increase in the number of CFU of bacteria in the air of Lahore. Farzana (1988) studied the presence of pathogenic bacteria in the air of various parts of Ganga Ram Hospital, Lahore. The results of this study showed that *Staphylococcus sp.*, *Streptococcus pyogenes* and *Enterobacter sp.* are present in the air of various wards. The present study was undertaken to know as to how far *Staphylococcus aureus*, *Escherichia coli* and *Streprococcus pyogenes* are present in the air of Lahore.

## MATERIALS AND METHODS

The areas slected for the study of bacterial air pollution were; University of the Punjab, (Quaid-e-Azam Campus), Model Town, Township, in Southern Lahore; Railway Station, Shah Alam Gate, Bhati Gate, Mozang, Ichhra, Regal Chowk, in Central Lahore and Shad Bagh in Nothern Lahore.

*Procedure*

Settle plate method as described by Cruickshank *et.. al.* (1973) was used for demonstrating the pathogenic bacteria in the air. Three petri plates (4 inches in diameter) containing the given medium were exposed to the air in the given locality for a period of three minutes. The bacterial colonies developed after 24 and 36 hours of incubation. The mean number of colonies growing on three petri plates was taken and tabulated to determine the number and types of organisms present in the air at a given time. Endoagar medium as described by Rhode (1973) was used for the detection of coliform and other enteric organisms. Mannitol salt agar as described by Cheesbrough (1984) is a differential and selective media. It was used for isolation of *Staphylococcus aureus*. MacConkey agar medium is a differential and low slectivity medium. It was also used to distinguish lactose fermenting from non-lactose fermenting bacteria. Blood agar medium, as described by Rhode (1973) was used for the detection of hemolytic activity of *Streptococcus spp*. Identification of the predominantial bacteria was carried out in accordance with the method as described by Bergey's Maual of determinative bacteriology (1975). The experiments were carried out in the given localities on 13th May, 7th June, 20th August, 20th November and 22nd December, 1994.

## RESULTS AND DISCUSSION

So far, a little published work is available on the presence of such pathogenic bacteria in the air which may be spreading upward and in all directions from the heaps of urban waste and open sewerage system. Keeping in view, these aspects, it was discovered that three types of bacteria were present in the air of Lahore: *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes*.

**Table I. Number of colonies of *Staphylococcus aureus* obtained on the isolation media which were exposed to the air of different localities of Lahore city in different months of the year.**

| Name of locality | Dates | | | | |
|---|---|---|---|---|---|
| | 13.5.94 | 7.6.94 | 20.8.94 | 20.11.94 | 22.12.94 |
| Railway Station | 31 | 36 | 32 | 27 | 24 |
| Regal Chowk | 37 | 40 | 35 | 29 | 28 |
| Shah Alam Gate | 29 | 25 | 20 | 21 | 17 |
| Bhati Gate | 30 | 28 | 18 | 15 | 11 |
| Ichhra | 30 | 26 | 21 | 9 | 9 |
| Mozang | 26 | 22 | 18 | 10 | 7 |
| Punjab Univ. | 19 | 20 | 18 | 14 | 14 |
| Model Town | 17 | 17 | 15 | 10 | 7 |
| Township | 21 | 22 | 19 | 18 | 15 |
| Shad Bagh | 34 | 39 | 30 | 29 | 16 |

*1. Staphylococcus aureus*

*S. aureus* is a pathogenic bacterium causing boils, pneumonia, scaled skin reaction, septicemia, otitis, sinusitis, and toxic shock syndrome. S. aureus is responsible for a variety of purulet infections as discussed by Ross (1983). The present study revealed that there was maximum pollution, as indicated by colony forming units (CFU) of *S. aureus* in the air of Central Lahore (Regal Chowk) and Southernn Lahore (Shad Bagh). In Regal Chowk, there were 37, 40, 35, 29 and 28 CFU on 13th May, 7th June, 20th August, 20th November and 22nd December, respectively. The air of another central part of Lahore (Railway Station) demonstrated 31, 36, 32, 27 and 24 CFU on the given dates, respectively. Whereas the air of Shad Bagh demonstrated 34, 39, 30, 29 and 16 CFU on the above mentioned dates. These results indicated that the air of all the three localities harboured maximum number of CFU.

The air on the clean roads and houses of central Lahore-Regal Chowk was expected to harbour lesser number of CFU of this bacterium. However, when this area was studied very closely, it was discovered that there were no lids on the man holes of underground drainage system and a foul smell was coming out in the air. It appeared that clogged underground drains supported the rapid growth of *S. aureus* which were being spread in the air by the wind and high traffic.

The raw skins of goat, sheep, and buffaloes are stored in various buildings situated behind Railway Station. It appears that because of such unhygienic conditions and due to presence of dense human and animal population the maximum number of CFU of *S. aureus* were obtained in the air of this locality. Furthermore, the dense human population, over crowded houses, and unhygienic conditions inside populated area of Central Lahore (Ichhra and Mozang) also found as an important factor responsible for the growth of CFU in the air of these areas.

A medium degree of pollution, as depicted by the medium number of CFU was observed in four localities of Central Lahore *i.e.* Bhati Gate, Shahalam Gate, Ichhra and Mozang. There were 30, 28, 18, 15 and 11 CFU in the air of Bahti Gate as studied on 13th May, 7th June, 28th August, 20th November and 12th December, 1994 respectively. The CFU in Shah Alam Gate, Ichhra and Mozang were more or less in the same range during the hot season of May, June and August. However, CFU in these areas remained less during November and December. Rest of the area showed minimum number of CFU in the air of each locality both during the summer and winter seasons. It appeared that the high temperature played an important role in increasing the number of CFU in the air of given localities.

*2. Escherichia coli*

*E. coli* is a pathogenic bacterium of urinary tract and wound infections (Cruickshank *et al.*, 1973). It can survive for several days when dried on clothing or in dust and pathogenic serotypes have been found to be viable and numerous in floor dust and in air. According to Ross (1983) a number of diseases such as enteritis, peritonitis and eystitis are caused by *E. coli*. In the present work, the density, in terms of CFU of *E.*

*coli* was found to be fairly less in each area as compared to *S. aureus*. On 13 May and 7th June, there were 21, 25 CFU of *E. coli* in the air of Central part of Lahore (Railway Station). Later on after a few months the CFU declined and there were 18, 11 and 9 CFU of *E. coli* on 20th August, 20th November and 22nd December, 1994, respectively.

**Table II. Number of colonies of *Escherichia coli* obtained on the Endoagar media which were exposed to the air of different localities of Lahore city in different months of the year.**

| Name of locality | Dates | | | | |
|---|---|---|---|---|---|
| | 13.5.94 | 7.6.94 | 20.8.94 | 20.11.94 | 22.12.94 |
| Railway Station | 21 | 25 | 18 | 11 | 9 |
| Regal Chowk | 17 | 19 | 13 | 8 | 8 |
| Shah Alam Gate | 18 | 25 | 20 | 10 | 10 |
| Bhati Gate | 19 | 23 | 15 | 12 | 11 |
| Ichhra | 15 | 20 | 13 | 11 | 12 |
| Mozang | 14 | 19 | 11 | 8 | 9 |
| Punjab Univ. | 12 | 17 | 9 | 6 | 6 |
| Model Town | 13 | 16 | 16 | 7 | 9 |
| Township | 18 | 19 | 18 | 15 | 13 |
| Shad Bagh | 21 | 22 | 16 | 12 | 9 |

In the air of central part of Lahore -- the Shahalam Gate, the CFU of *E. coli* were 18, 25, and 20 on 13th May, 7th June and 20th August, respectively. Later on, in this area there were just 10 CFU on 20th November and 22nd December, 1994, respectively.

In a densely populated area of Northern Lahore such as Shad Bagh, there were 21, 22, CFU of *E. coli* on 13th May and 7th June. Later on, there was a decline in this number and CFU were 16, 12 and 9, respectively on 20th August, 20th Nobember and 22nd December, 1994. These results indicated that during hot months of May and June, a high temperatue supported the prevalence of more bacteria in each locality.

*3. Streptococcus pyogenes*

*Streptococcus pyogenes* is responsible for a variey of inflammatory and supportive

conditions such as sore throat, scarlet fever, and wound infections (Cruickshank *et al.*, 1973). The most common route of entry of *S. pyogenes* is by the upper respiratory tract.

In the present study the maximum CFU were noted down in the air of central Lahore, such as Shah Alam Gate, Bhati Gate and Mozang particularly during the summer months of May, June and August (Table III).

**Table III. Number of colonies of *S. pyogenes* obtained on the isolation media which were exposed to the air of different localities of Lahore city in different months of the year.**

| Name of locality | Dates | | | | |
|---|---|---|---|---|---|
| | 13.5.94 | 7.6.94 | 20.8.94 | 20.11.94 | 22.12.94 |
| Railway Station | 1 | 3 | 3 | 2 | 1 |
| Regal Chowk | 3 | 3 | 4 | 3 | 3 |
| Shahalam Gate | 5 | 6 | 7 | 3 | 1 |
| Bhati Gate | 4 | 3 | 5 | 4 | 3 |
| Ichhra | 2 | 3 | 3 | - | 2 |
| Mozang | 4 | 5 | 5 | 4 | 2 |
| New Campus | 1 | 2 | 2 | - | 2 |
| Model Town | 1 | 3 | 4 | 3 | 2 |
| Township | 1 | 2 | 5 | 4 | 3 |
| Shad Bagh | 2 | 3 | 3 | 3 | - |

Since it is Beta-hemolytic, its presence was in less number as compared to other bacteria. Further studies are needed to know if there were bactericidal gases in the air which checked its survival in the local environment. Further research is also needed to know as to how far the diseases caused by this bacterium are prevalent in the local population.

*Impact of environmental factors on bacterial survival*

The environmental factors that play significant role in the survival of *S. aureus*, *E. coli* and *S. pyogenes* in the air are as follow;

*I. Temperature*

It has been discussed earlier that CFU of *S. aureus* and *E. coli* were found more in number during the hot months of May and June, whereas during the cold months of November and December, the CFU of these bacteria decreased. Studies of Ahmad (1982) showed that the number of pathogenic bacteria increased in the air of Lahore during the high temperature period of May as compared to the lower temperature period of March.

*II. Humidity*

It is known that high humidity is favourable for the growth of a number of specific bacteria in the air. In the present study it was found that CFUs of *S. pyogenes* were little more in number during humid period of August as compared to dry periods of May and June. Chantefort *et al.* (1983) measured air microbiological contamination. This study showed that gram negative bacteria could survive only in a special, very moist atmosphere. However, the present preliminary studies did not support this point. Ali (1991) studied bacterial air pollution of Lahore. His results supported the findings of Chantefort *et al.* (1983) that gram negative bacteria particularly *E. coli* were more in number during humid period of August as compared to dry period of May and June. Further studies are needed to test this point.

*III. Solar irradiation*

Solar irradiation is also known to affect the viability of bacteria. According to Teltsch and Katzenelson (1978) relative humidity and solar irradiation appeared to affect viable bacteria in the air. A positive correlation was found between relative humidity and the number of aerosolized bacteria. Similar studies are needed in the local environment.

*IV. Gases*

Various gases are known to be present in the air. According to Crookshank *et al.* (1978) helium at a pressure of 20 to 70 atm. in the presence of air was found to stimulate growth of *Streptococcus faecalis*, *E. coli* and *S. aureus* mainly by increasing the rate of exponential growth.

de-Mic and de-Groot (1977) by using the Microthread technique, studied the survival of *E. coli* in open air in different parts of the Netherlands. The presence of bactericidal compounds (open air factor = OAF) could be demonstrated on several days and quantitated in relative units of OAF concentrations. In the absence of ozone, the one concentration was always low. In the persence of ozone the OAF concentration was dependent on wind direction. At the selected microthread exposure sites air from areas with high traffic intensity contributed more to OAF production than air from industrial areas. OAF production is probably related to the nature of hydrocarbons in the air. Jafri and Shah (1992) discovered corrosive effects of toxic industrial gases on redstone of Lahore fort.

In the present study the prevalence of lesser number of CFU of *E. coli* as compared to *Staphylococcus aureus* and the prevalence of little number of *Streptococcus pyogenes* may be attributed to the presence of bacterial gases in the local air. Further studies are needed to know as to what kind of bactericidal gases are present in the peri-urban and urban areas of Lahore.

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# SOME METABOLIC ALTERATIONS INDUCED BY AN ORGANOPHOSPHATE INSECTICIDE, MALATHION, ON CHICK MUSCLE*

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**Abstract:** An organophosphate insecticide malathion (57 EC) was administered orally to seven week old broilers as three different doses *i.e.*, 600, 400 and 250 mg (a.i) /kg body weight/day for the total durations of 40 hours, 12 days and 4 weeks, and were designated as short term (ST), long term-I (LT-I) and LT-II experiments, respectively. After stipulated periods, a group of 3-5 birds were sacrificed along with a group of control animals, their muscle samples were quickly excised and processed differently for various biochemical analyses.

The muscle acid phosphatase (AcP) activity, although showed a gradual rise in ST experiment but it was significant, with 32% rise, only at 40 hours. In LT-I treatment AcP activity shot up by 21 and 79% at 3 and 6 days malathion feeding. The alkaline phosphatase (AP) activity in ST experiment showed a significant increase of 40, 45 and 38% at 10, 20 and 40 hours, respectively. The glutamate pyruvate transaminase activity remained uneffected in ST and LT-I experiment until day 6 after which 100% rise at 12 day and 33, 29 and 33% rise at 2nd, 3rd and 4th week insecticide feeding was observed, respectively. Significant increase in free amino acids (FAA: 338% and 145%) and glycogen (63% and 38%) contents was also noticed at 5 and 10 hour treatments, respectively in ST experiment. The rise in FAA contents was 54% (12 days) and 62% (2 weeks) in ST-I and ST-II experiments respectively. Glycogen content also increased by 55 and 54% at 6 and 12 days and 31 and 20% at 3rd and 4th week of insecticide feeding. Soluble proteins remained unchanged in ST experiment but showed variable results in LT-I and LT-II experiments. It is concluded that malathion is relatively less toxic to muscle tissue under the present experimental conditions as for as the above analysed parameters are concerned. This was probably due to the fact that muscle is not a primary target of foreign toxic compounds in the animal systems.

**Key words:** Malathion, chick muscle, biochemical effects, enzymes

## INTRODUCTION

Organophosphate pesticides are important agrochemicals which have wide range of activity against insect pests of crops, vegetables, fruits, and also active against the pests of medical and veterinary importance (Jayarathnam *et al.*, 1991; Kao and Tzeng, 1992; Stevens, 1992; Toma *et al.*, 1992; Aboul Ela *et al.*, 1993; Chakraborti *et al.*, 1993; Mourya *et al.*, 1993). These pesticides have replaced the earlier organochlorine compounds which are considered as highly persistent, due to their lipophillic nature, with consequent accumulation and induction of resistance in animals, plants and other systems of the environment (Mugambi *et al.*,

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1989; Sorokin and Zharov, 1992; Rao, 1992; Winter and Streit, 1992; Fischer and Pecher, 1993; Hellou *et al.*, 1993; Lin *et al.*, 1993; Urdaneta *et al.*,1995). Their degradation by animals and other systems of the environment was very slow, the factor which plays very significant role in the development of long term toxic effects in non-target systems, including man ( Shakoori and Haq, 1987; Ali *et al.*, 1988 a,b; Ali and Shakoori, 1990, 1993; Shahida and Solangi, 1990; Meera *et al.*, 1993; Rani *et al.*, 1993).

Organophosphate, being phosphate esters, have comparatively low cumulative potential. These are relatively easy to biodegrade by various systems of the organism and environment into non toxic or less toxic metabolites which indicated that these pesticides are less hazardous to the environment (Ali and Shakoori, 1981; Tripathi and Shukla, 1992). There are many reports regarding the development of toxic effects by these phosphate esters in non-target systems. These pesticides are well known for their ability to inhibit the acetylcholine esterase activity at the synapse with the result of loss of nervous co-ordination (Kumar *et al.*, 1992; Sawyer *et al.*, 1992; Satyadevan *et al.*, 1993). In addition, metabolic alterations induced by organophosphate pesticides have also been reported in different tissues of non-target organisms (Qadri *et al.*, 1987; Ali and Shakoori, 1988; Blasiak, 1993; Dutta *et al.*, 1994; Begum and Vijayavaghavan, 1995; Tsuda *et al.*, 1995).

The main objective of the present study was to investigate the effects of malathion, on some metabolically important biochemical parameters of chick muscle which is important as for as human nutrition is concerned.

## MATERIALS AND METHODS

*Experimental animals and their maintenance*

Seventy two one day old broiler chick *(Gallus domesticus)* were obtained from the local poultry breeders and kept in cages (25 cubic ft. area) in the animal house of the Department of Zoology, at controlled temperature (25.00±1.5 °C). Animals were provided with commercial poultry feed and water *ad libitum*.

*Administration of insecticide*

Sub-lethal doses of an organophosphate insecticide, malathion [*o,o*-dimethyl-S-(1,2-dicarboxyethyl) phosphoro- dithioate], were administered orally to chicks as a single strong dose (600 mg /kg body weight) and two weak doses (400 mg/kg and 250 mg/kg body weight/day) for the total durations of 40 hours (short term experiment), 12 days and 4 weeks (long term experiments), respectively, after consulting the $LD_{50}$ value of malathion for chicks.

*Experimental procedure*

For short term experiment, malathion was administered as a single strong dose to a group of 16 chicks and the sampling was performed at 5, 10, 20 and 40 hours intervals. In long term treatments, two groups, with 15 and 13 chicks in each, were administered

with weak doses (400 mg and 250 mg, respectively) of malathion. Sampling in former case was done at 3, 6 and 12 days while in later case it was performed at 2, 3 and 4 week after the beginning of insecticide feeding. A group of 4 chicks was used as a control alongwith each treated group in both short and long term experiments.

At the completion of stipulated periods in each case, a group of 3 to 5 malathion treated and a group of 4 control animals were anesthetizes. Their muscle samples were collected quickly from the legs and stored at -10 °C until processed further for various analyses

*Muscle processing for biochemical analysis*

Weighed amounts of muscle was processed in 0.89% saline, 0.5N sodium hydroxide and boiling ethyl alcohol, separately for the extraction of saline-soluble components, total proteins and nucleic acid (DNA and RNA) contents, respectively.

Saline homogenate was centrifuged at $4x10^3$ rpm for 15 minutes at 5 °C to obtain clear supernatent, which was used for the estimation of some enzyme activities *viz.*, acid phosphatase (AcP, *O*-phosphoric monoester phospho-hydrolase, E.C. 3.1.3.2.) according to Andersch and Szcyphinski (1947); alkaline phosphatase (AP, *O*-phosphoric monoester phosphohydrolase, E.C. 3.1.3.1.) according to the Bessey *et al.* (1946); glutamate oxalo-acetate transaminase (GOT, L-aspartate 2-oxoglutarate aminotransferase, E.C. 2.6.1.1.) and glutamate pyruvate transaminase (GPT, L-alanine 2-oxoglutarate aminotrasferase, E.C. 2.6.1.2.) both according to the method of Reitman and Frankel (1957)

In addition to enzymes some other muscle metabolites like total free amino acids (FAA) according to Moore and Stein (1954) and soluble proteins, according to Lowry *et al.* (1951) were also estimated from the saline muscle extract. Total muscle proteins were extracted in 3 ml of 0.5 N sodium hydroxide in water bath at 55 °C and estimated as soluble proteins. Glycogen was extracted and estimated according to the method mentioned in Hassid and Abraham (1957). Nucleic acids (DNA and RNA) were extracted as mentioned in Shakoori and Ahmad (1973) and estimated according to Schneider (1957).

## RESULTS

Some biochemical alterations induced by malathion at various dose (600mg, 400mg and 250mg) and duration (40 hours, 12 days and 4 weeks) levels are being mentioned in Tables I-III as percent variation from control values. Figures 1-3, on the other hand indicate the actual mean values of various parameters in case of control as well as three malathion feeding experiments.

In 40 hours malathion feeding experiment, most of the tested parameters of chick muscle remained unchanged with respect to control group (n=4). However, some prominent changes were observed in phosphatase (AP and AcP) activities, especially AP which was increased by 40% and 45% at 10 (n=3) and 20 (n=4)hours insecticide feeding studies, respectively. On extending the malathion treatment for next 20 hours

the AP showed 35% decrease. The muscle AcP activity exhibited 32% rise at 40 hours while no significant change was observed during the initial 20 hours

**Table I: Percent increase (+) or decrease (-) from control values (n=4) in some enzyme activities and metabolite concentrations of chick (*Gallus domesticus*) muscle after malathion administration (600 mg/kg body weight/day) for a period of 40 hours.**

| Parameters[a] | Malathion Treatment (Hours) | | | |
|---|---|---|---|---|
| | 5 (n=4) | 10 (n=3) | 20 (n=4) | 40 (n=3) |
| AcP activity | -1.89 | +9.43 | +22.64 | +32.08* |
| AP activity | +25.0 | +40.0* | +45.00** | -35.00* |
| GOT activity | +8.11 | +5.41 | +13.51 | -5.41 |
| GPT activity | -2.22 | -13.33 | -11.11 | +6.67 |
| Free amino acid | +337.68* | +144.93* | -33.33 | -10.14 |
| Glycogen | +62.5* | +37.50* | -31.25 | -37.50 |
| Soluble proteins | -22.38 | -3.38 | +23.74 | +27.60 |
| Total proteins | +0.89 | -10.18 | +3.59 | +22.98 |
| DNA content | +15.15 | +44.95 | +36.87 | +25.76 |
| RNA content | -23.97 | +23.86 | -22.77 | -20.70 |

Student's `t' test, *P<0.05; **P<0.01.

[a]**Abbreviations used**: AcP, acid phosphatase; AP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase.

of the experiment. Malathion administration, in this short-term (strong dose) experiment induced significant increase in muscle FAA (338% and 145%) and glycogen contents (63% and 38%) at 5 and 10 hour durations, respectively. The both components showed recovery during next 30 hour malathion treatment. The muscle GOT, GPT activities, soluble and total protein and nucleic acid contents remained unaltered during 40 hours administration of malathion as strong dose (Table I; Fig. 1).

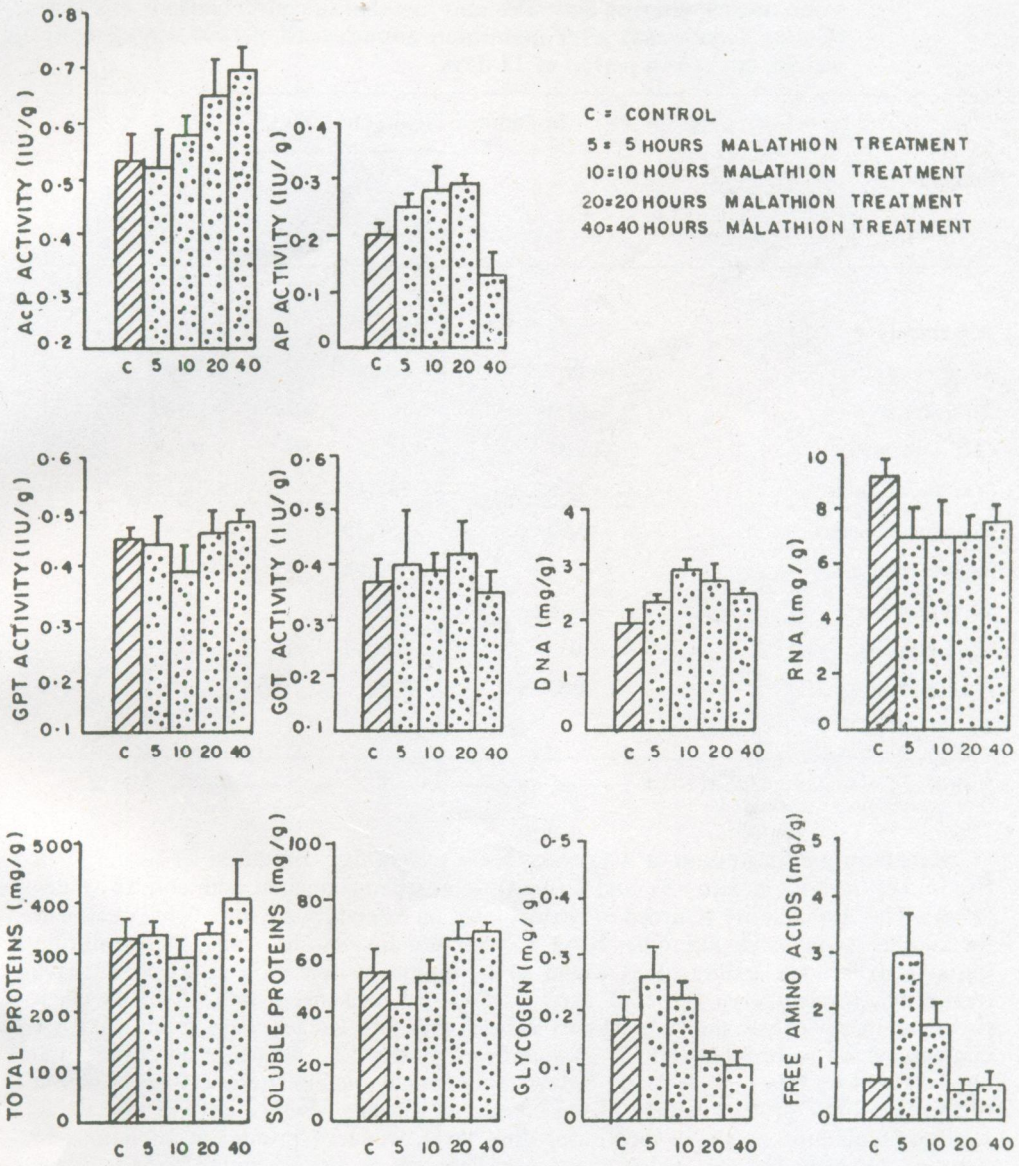


**Fig. 1.** Effect of malathion (600 mg/kg body weight), administered for a period of 40 hours on some muscle enzyme activities and metabolite concentration in chicks.

**Table II: Percent increase (+) or decrease (-) from control values (n=4) in some muscle enzyme activities and metabolite concentrations of chick (*Gallus domesticus*) after malathion administration (400 mg/kg body weight/day) for a period of 12 days.**

| Parameters[a] | Malathion Treatment (Days) | | |
|---|---|---|---|
| | 3 (n=4) | 6 (n=3) | 12 (n=5) |
| AcP activity | +20.83* | 78.95** | +17.39 |
| AP activity | -4.00 | -31.03* | -40.74* |
| GOT activity | +14.29 | -40.0 | +24.00 |
| GPT activity | -16.67 | -31.25 | +100.00 |
| Free amino acids | +25.81 | -24.24* | +54.17* |
| Glycogen contents | -40.00 | +54.55* | +53.85* |
| Soluble proteins | +11.63 | +30.92 | -41.52* |
| Total proteins | +14.92 | -5.59 | -16.59 |
| DNA content | -1.02 | +28.14 | -4.97 |
| RNA content | -24.63 | -5.40 | -5.76 |

Student's 't' test, *P<0.05; **P<0.01.
[a]For abbreviations, see Table I.

Malathion administration at 400mg/kg body weight/day, produced 21% and 79% rise in AcP activity at 3 (n=3) and 6 (n=4) days when compared with control values (n=4). The AcP activity returned to normal level on extending the insecticide treatment for another 6 days. On the other hand AP activity did not show any change during initial 3 days while exhibited 31% and 41% significant inhibition at 6 and 12 days treatments, respectively. The GPT activity remained unchanged until 12 hours when two fold increase was noticed. Almost similar type of changes were found in FAA contents which were increased (54%) significantly after 12 days while no change was observed before this *i.e.*, at 3 and 6 days of insecticide administration. The reduction (42%) in soluble protein content of muscle was noted only after 12 days of insecticide feeding. Malathion at above mentioned dose level produced prominent alterations in muscle glycogen content which, were significant at 6 (55%) and 12 (54%) days insecticide feeding. No change was found in GPT activity, total proteins and nucleic acid content (Table II; Fig. 2).

Some prominent changes were also observed after feeding this insecticide @ 250mg/kg body weight/day during four week study period.

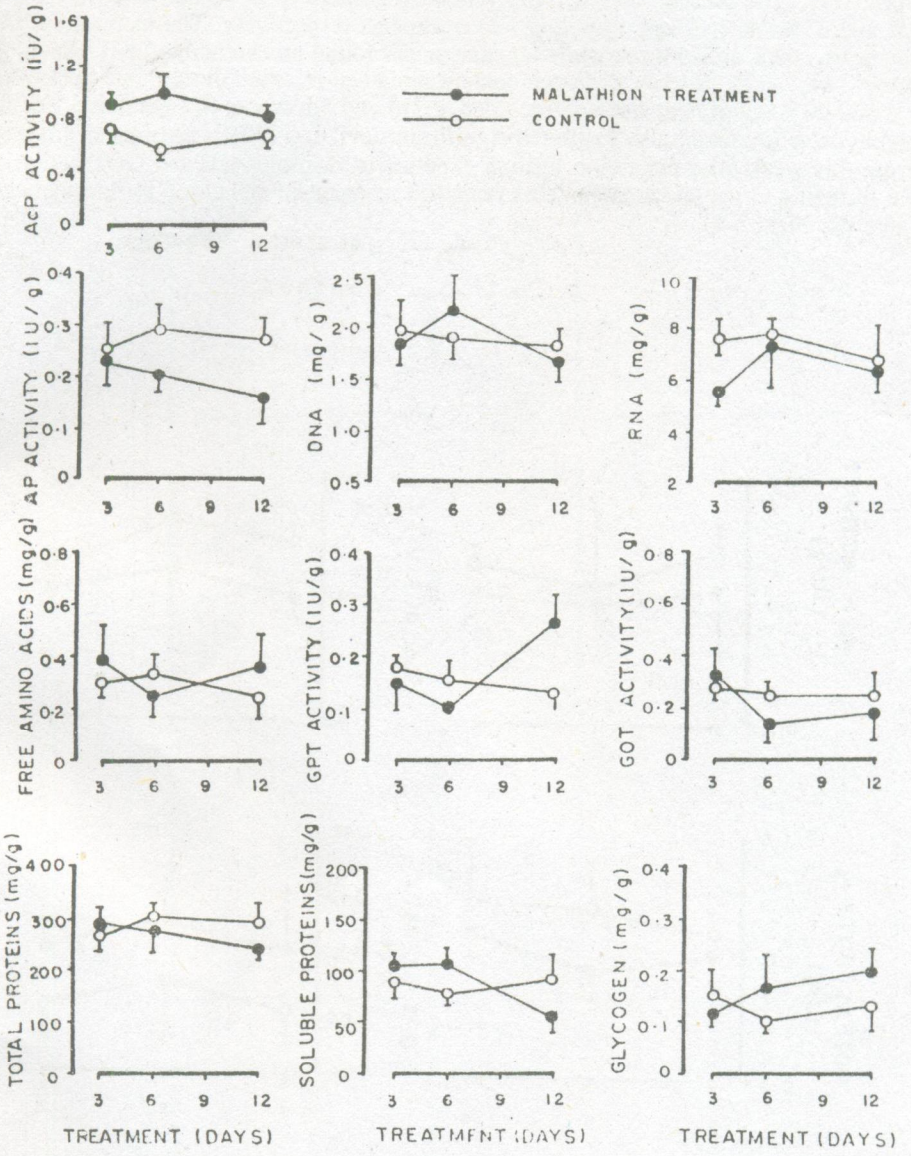


**Fig. 2.** Effect of malathion (400 mg/kg body weight/day), adminis- tered for a total period of 12 days on some muscle enzyme activities and metabolite concentration in chicks.

AP activity exhibited 38% and 28% fall at 2nd (n=4) and 3rd (n=5) weeks, respectively. The muscle GPT activity remained constantly at higher level *i.e.* (33%, 29% and 33%, at 2, 3 and 4 week (n=4) treatments respectively. The increase in FAA content was 62% at 3rd week while no change was found on extending the treatment for another two weeks. Muscle glycogen content remained resistant during 2nd week while 31% and 20% significant rise was recorded at 3rd and 4th weeks of insecticide feeding. Soluble protein content also exhibited significant deviation (70% increase) at 3rd week during this weak dose malathion feeding experiment. Muscle AcP and GOT activities, total protein and nucleic acid contents remained resistant to malathion during the study (Table III; Figs. 3-4).

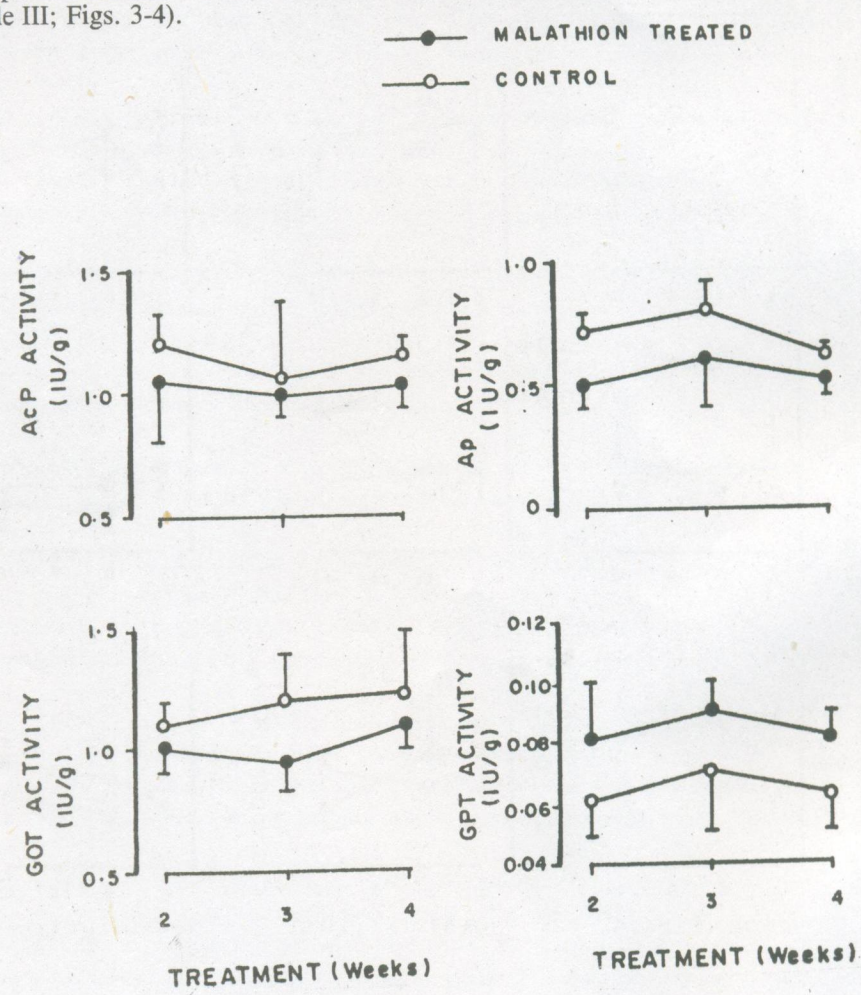


**Fig. 3.** Effect of malathion (250 mg/kg body weight/day) adminis- tered for a total period of 4 weeks, on some muscle enzyme activities in chicks.

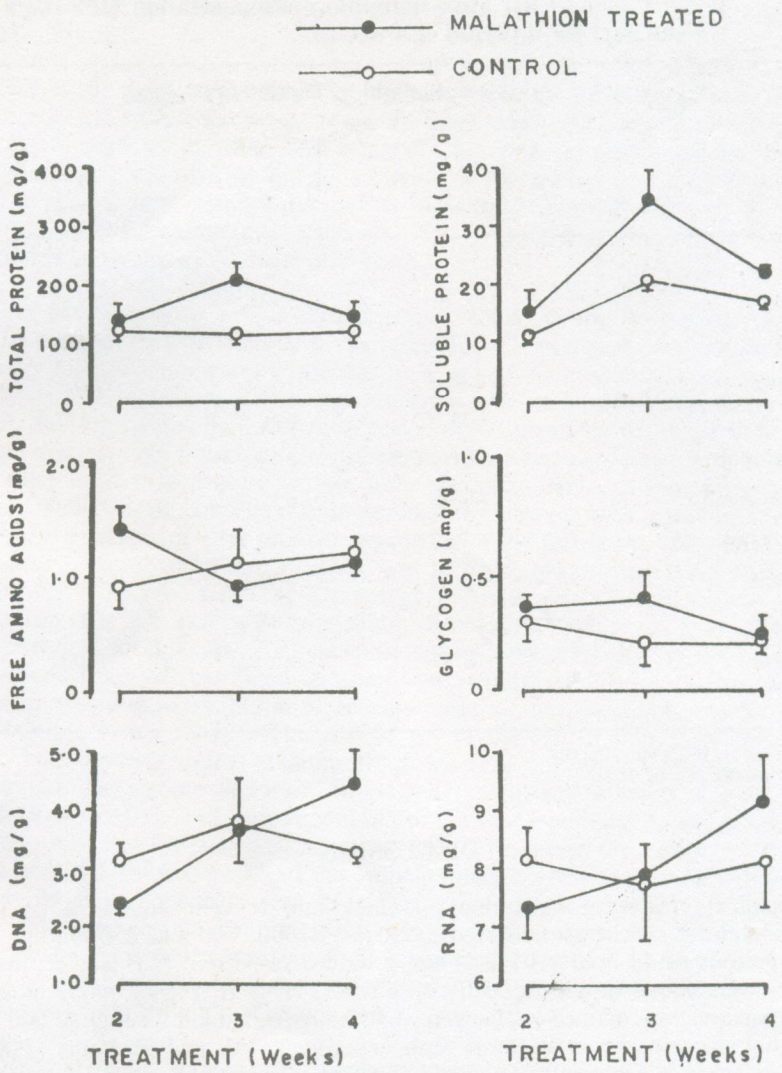


**Fig. 4.** Effect of malathion (250 mg/kg body weight/day) adminis- tered for a period of 4 weeks, on some muscle metabolite cocentration in chicks.

**Table III: Percent increase (+) or decrease (-) from control values in some muscle enzyme activities and metabolite concentrations of chick (*Gallus domesticus*) after malathion administration (250 mg/kg body weight/day) for a period of 4 weeks.**

| Parameters[a] | Malathion Treatment (Weeks) | | |
|---|---|---|---|
| | 2 (n=4) | 3 (n=5) | 4 (n=4) |
| AcP activity | -11.76 | -7.69 | -10.53 |
| AP activity | -37.84* | -28.21* | -5.88 |
| GOT activity | -8.85 | -18.26 | -9.32 |
| GPT activity | +33.33* | +28.57* | +33.33* |
| Free amino acids | +61.90* | -16.81 | -5.83 |
| Glycogen content | +9.68 | +31.03* | +20.00* |
| Soluble proteins | +30.32 | +69.94* | +32.36 |
| Total proteins | +8.46 | +29.50 | +18.13 |
| DNA content | -24.52 | -1.07 | +34.66 |
| RNA content | -10.59 | +0.52 | -7.00 |

Student's 't' test, *$P<0.05$.
[a]For abbreviations, see Table I.

## DISCUSSION

Biochemical effects of malathion on chick muscle were found to be mild to moderate intensity, when administered to chicks @ 600, 400 and 250 mg/kg /day for the total periods of 40 hours, 12 days and 4 weeks, respectively. However, maximum alterations were found in 400 mg daily dose level (Table II, Fig.2), while most of the analysed parameters remained unchanged in 40 hours malathion feeding @ 600 mg/kg, once during the experiment in short term experiment. Ali and Shakoori (1981) also reported that rabbits, generally remained resistant to malathion when administered @ 95 mg/kg body weight/day unintrruptedly for 15 months alongwith feed, except some significant decrease in cholinesterase (28%) and GPT (36%) activities, and raised AcP activity (55%). However hepatic GPT activity showed considerable (47%) inhibition during this study.

On comparing the effects of three doses of malathion for three different durations, it was observed that in most cases, the biochemical changes in chick muscle were not

induced immediately after administration of insecticide. However, the FAA and glycogen content seemed quite sensitive which showed significant increase within 5 hours of malathion feeding in short term experiment but normalized after 10 hours (Table I, Fig. 1).

Same is true in case of 400 mg/kg malathion feeding daily for 12 days. Only AcP is the exception which was increased within three days of insecticide feeding (Table II, Fig. 2). Moreover, the dose and treatment duration, at which maximum alterations were observed , was 400 mg/kg/day administered for a total period of 12 days. Various biochemical parameters showed a sort of recovery or normalization at the end of long term (4 week) malathion treatment (250 mg/kg/day) indicating induction of detoxification processes in the treated chick.

Acid phosphatase is a hydrolytic enzyme, found in the lysosomal fraction of the cell. Increase in its activity may be an indication of increased break down activities of waste cellular components produced as a result of insecticidal toxicity. Another possibility for increased AcP activity may be the increased biosynthesis of enzyme protein due to elevated demands in the cells. AP is generally regarded as liver function enzyme but it is also found in plentiful amounts in intestine, kidney and bone tissue. Its main function is to hydrolyse various monophosphate esters to split phosphoric acid, a reaction which is of considerable importance in various body processes. It is mainly rich in the tissues which are actively engaged in active transport. The rise in AP activity at 10 and 20 hour malathion feeding, was probably due to increased active transport process for supply of nutrients for energy generation to counter the toxic effects of insecticide. The AP activity showed significant decrease when the insecticide treatment was extended for another 20 hours, in strong dose experiment. In remaining two experiments, the AP exhibited a significant inhibition. It is not clear whether this inhibition was due to inhibition of enzyme protein biosynthesis or some other factor is responsible for this change. The normal pH in the cell is slightly alkaline (approx., 7.3). This enzyme requires alkaline pH in the range of 10.5. It can be suspected that malathion induces some alterations in the cell's environment which may inhibit this pH shift from 7 to 10.5, with consequent inhibition of AP activity. Muscle GPT activity is generally required for transamination purposes. The rise in the activity of this enzyme, at the end of LT-I and in LT-II experiments may be due to increased transamination process which is a primary step in utilization of amino acids for energy generation, required to detoxify the foreign toxic compounds. The elevated activities of AcP, AP, GOT and GPT have also been reported in another study (Awal and Malik, 1992) after administering a single oral dose of phosphamidon (an organophosphate pesticide) @ 20 and 40 g/kg body weight in *Bubalus bubalis*.

Amongst other biochemical components of muscle, FAA contents generally increased at the durations where rise in glycogen was also observed *i.e.*, at 40 hours and 12 days malathion feeding study. This indicates that during these periods, glucose was not available for energy generation in muscle, rather amino acids, which were available in higher amounts, were being utilized for various activities of the muscle tissue. Most probably the glucose was being routed towards glycogen biosynthesis. Normally, amino acids are utilized as fuel in the muscle cells for energy production when glucose and glycogen sources are not available. In this study, it was noticed that

FAAs are being utilized in the presence of glycogen reserves (Tables I-III), the condition which indicated the possible inhibition of glycogen utilization system. Lal *et al.* (1986) showed fall in liver and muscle glycogen content with simultaneous rise in plasma glucose in 16 day malathion treated fish @ 8 mg/l of water.

In weak dose (long term) insecticide feeding study, case was somewhat different. The FAA contents exhibited 62% rise after 2 weeks of insecticide treatment while glycogen was raised after 3 and 4 weeks. However, the percent increase was reduced to 31 and 20, respectively, which indicated the trends towards normalization. The normal FAA pattern during 3rd and 4th weeks may be due to re-availability of glucose for energy generation processes in the muscle which was supported by the decreased accumulation of glycogen. The 42% decline in soluble proteins may be due to some inhibition in protein biosynthetic pathway or it may be due to increased breakdown of proteins to amino acids which showed 54% rise during the experiment.

It is important to note that at the end of weak and strong dose experiments, almost all parameters, except few, showed recovery (Tables I,III). Moreover, the results in this study indicated, that the effects of malathion feeding to on chicks muscle biochemical components were in the range of mild to moderate as for as their intensity is concerned. This was, probably, due to the induction of drug metabolizing and other xenobiotic degrading enzymes in the liver because liver is considered as the major site for all types of biotransformations of the foreign toxic compounds. As a result, these pesticides may be transformed into various less toxic or non-toxic metabolites and excreted before reaching the other organs or tissues.

However, many workers have reported some metabolic changes in the muscle of different animals after insecticide exposure. Acetylcholine esterase (AChE) activity of the fish muscle showed significant depression at 5.88 ppm malathion concentration in 96 hours. According to this report, the activity was not fully recovered even six days after transferring the fish to pesticide free environment (Sulaiman *et al.*, 1989). Bashamohideen and Sailbala (1989) however, reported that inhibition of AChE activity was greater in white muscle as compared to red muscle. Significant fall in RNA and protein was observed by Jyoti *et al.* (1989) after malathion treatment in *Channa punctatus* but glycogen showed variations in different tissues. Similarly Husain *et al.* (1987) showed a significant increase in AP, GOT and GPT activities of liver, kidney and brain tissues of malathion treated (55 and 137.5 mg/kg orally for 32 days) rats.

It was concluded that malathion at the dose levels used in this experiment probably showed a moderate toxicity as for as biochemical parameters of chick muscle are concerned when compared with liver which is a primary target of all foreign toxic compounds ( Gupta and Paul, 1977; Ali and Shakoori, 1981; Thompson *et al.*, 1991; Ali and Ali, 1992).

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# EFFECT OF POLYTRIN-C AND NUVACRON ON THE ENTERIC EPITHELIUM OF *DYSDERCUS CINGULATUS* (FAB.) (HEMIPTERA: PYRRHOCORIDAE)

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**Abstract**: The adults of *Dysdercus cingulatus* were treated with 1.7, 0.8, and 0.4 ppm concentrations of Polytrin C in acetone whereas, 2.2, 1.1, and 0.6 ppm doses of Nuvacron were used. All the doses were given for 12 & 24 hours.

$LD_{50}$ for Polytrin C and Nuvacron was calculated to be 3.31 ppm and 4.37 ppm, respectively afrer 24 hours of the treatment. The effect of both Polytrin C and Nuvacron was found almost the same. Midgut seemed to be more effected as compared to the the fore- and hind-guts. Both insecticides disrupt the epithelial lining of the gut. Cells became elongated and narrow after treatment with smaller concentrations. Vacuolization also took place. Higher doses of insecticides had more acute effect. The cells lost all their contents and became flattened. They had almost no cytoplasm and the nuclei seemed to congregate.

Peritrophic membrane which was present in the midgut of control insects also broke-up and became absent over most of the surface. Cuticular intima of the foregut and hindgut became thinner and damaged. It broke-up in the foregut but in the hindgut it only became thinner.

**Key words**: *Dysdercus cingulatus*, Polytrin C, Nuvacron, insect entric epithelium, insecticides.

## INTRODUCTION

The cotton crop is prone to attack by a large number of major insect pests resulting in a considerable reduction in yield per acre. Various workers have given different estimates for the losses caused by insect pests to cotton crop. Hague (1972) estimated that different pests, on an average, cause 5-10% damage to cotton crops every year. Ghouri (1973) was of the view that cotton suffered the loss of about 16-20% due to this cause, while Chaudhry (1976) assessed this loss up to 32%. Ahmad (1983) and Yasmeen *et al.* (1985) have also discussed the increased damage to cotton crop by pests.

*Dysderous cingulatus* is widely distributed in Pakistan. Apart from cotton it also feeds on ladyfinger, maize, pearl-millet, wheat, clovers *etc*. The insects suck cell sap from leaves and green bolls of cotton. Heavily attacked boll open badly and the lint is of poor quality. The cotton also gets stained by their excreta. The seeds thus produced have low rate of germination. Moreover, the lint is stained further with their excreta or body juices as they get crushed in the ginning factories. The staining to the lint by the growth of certain bacteria inside the bolls is also believed to be initiated by the bugs. The chemical control is the most popular method, and different agents ranging from chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids have been introduced one after the other. Partial control is the minimum which can be achieved.

The development of resistance in insect population against various insecticides is the main reason for the failure and has forced the workers to use different biologically active chemicals against them.

The effect of the insecticides more or less depends upon the route of administration, it may be oral, tropical or intravenous (Awad and Kandil, 1980; Bariola *et al.*, 1984; Fahmy *et al.*, 1985; Powell *et al.*, 1980).

As the intestinal cell lining palys an important role in digestion, absorption and detoxification (Richards and Davies, 1977; Rastogi *et al.*, 1987) when any insecticide is given orally or topically, it is one of the tissues which is affected most. For this reason during the present study the entire epithelium of this adult bug was studied before and after the use of Polytrin C and Nuvacron.

## MATERIALS AND METHODS

The red cotton bugs, *Dysderous cingulatus* were collected from cotton fields at Bhai Phero, and from the vicinity of New Campus, Punjab University, Lahore. They were brought to the laborataory where they were sorted out according to their age. The different nymphal instars and adults were kept in separate jam jars and covered with muslin cloth. Small green leaves of different plants, especially those of cotton and shoe flower plants were given them as food. In this way a constant supply of the adult bugs was ensured. These insects were maintained in the laboratory at a temperature ranging 25 - 30$^{0}$C and 70$\pm$5% relative humidity.

*Insecticide treatment*

The adult bugs were treated with selected concentrations of Polytrin C (1.7, 0.8, and 0.4ppm) and Nuvacron (2.2, 1.1, and 0.6ppm) for 12 and 24 hours. Three grams of green leaves of cotton were soaked with 3.7 ml of each concentration of insecticides in petridishes. They were transferred to jars alongwith 10 adult bugs. Thus the insects fed on the treated food also came in body contact with the contaminated leaves. Besides the treated, the untreated control experiment was also carried on. For control, 10 adult bugs were fed on the untreated food.

*Histological Studies*

The adult bugs were dissected in 0.75% saline solution. Their alimentary canals were removed and put in watch glasses in the saline solution. The connective tissue present on the surface of the gut was scrapped off by means of a soft camel-hair brush. Different parts of the gut (fore-gut, mid-gut and hind-gut) were separated and fixed in Bouin's alcoholic fixative, embedded in paraffin wax and sections were cut serially with rotary microtome at 5$\mu$. They were stained with Mallory's Triple Stain and hematoxilin which was counter stained with eosin.

## RESULTS

*Alimentary canal of control insects*

The alimentary canal of *D. cingulatus* is a very long convolvulated tube extending

from the mouth to the anus. It is more than double the body length and typically divided into a fore-gut, a mid-gut and hind-gut. The Malpigian tubules are present between the junction of the mid- and hind gut (Figs. 1,a-c).

Fore-gut is the short portion of the alimentary canal. Its internal walls are thrown into many folds which are variable in depth. Internally the foregut is lined by a thin cuticular layer outside which is the epithelial layer. Its cells are normally tightly packed and either cuboidal or columnar with large nuclei. The folded parts are sometimes multilayered with tightly packed cells of varying dimensions. Their length varies from 16-20$\mu$m, while their width range is from 5-7$\mu$m. The nuclei which are generally centrally placed or slightly towards the base are rounded and 4-5$\mu$m in diameter. The cells have granular cytoplasm which sometimes may be slightly vacoulated. The chromatin is scattered randomly. They rest on a very thin basement membrane externally. Its thickness is never more than 0.5$\mu$m. The epithelial layer is surrounded by an inner layer of circular and an outer layer of longitudinal muscle fibres. They are well developed over the pharyngeal region. but over the oesophagus and the crop the longitudinal muscle fibres are poorly developed or even absent. The outermost peritoneal covering is very thin and almost non-existent. It has some tracheal supply. Haemocytes could be seen adhering to it at places.

Midgut is divided into two parts. The anterior part is in the form of a long tube while the posterior part is short.

The anterior midgut is a narrow and clearly demarcated from the foregut. It is lined internally by a very thin peritrophic membrane which is from 0.5 - 1.0 $\mu$m thick; outside this lies the epithelial layer which is of columnar cells. They are narrow and tall with a length range of 12-14$\mu$m and a breadth range of 4-5$\mu$m. They have granular cytoplasm. Their nuclei are small, only 3-4$\mu$m in diameter. They lie toward the basal part of the cells. The cytoplasm was seen to be vacoulated occasionally. Secretary granules could also be seen flowing into the lumen. The epithelial layer rests on a thin basement membrane which is around 0.5$\mu$m thin. The muscular layer is very thin, composed of an inner layer of longitudinal and an outer layer of circular muscle fibres. The wall of this part is also thrown into folds.

The posterior midgut region of the midgut has the same layers as the anteior portion, except that the cells show greater evidence of secretory activity. They are less tall, around, around 9-10$\mu$m in length, with well defined generally rounded or ovoidal nuclei. They are from 3 - 4$\mu$m in diameter, with conspicous uniformly scattered chromatin material. Big vacuoles are sometimes present in the cell.

A second type of cells, the regenerative cells are also found lying beneath these cells singly or in groups of two's or three's. At places a well defined space filled with granules is present between the epithelial and muscular layers. The muscular layers form many small diverticula with the continuation of this space and material inside them. This region of the midgut is also thrown into folds which are larger here as this region is aslo larger in diameter as compared to the anterior part. Enteric caeca were aslo present in this region.

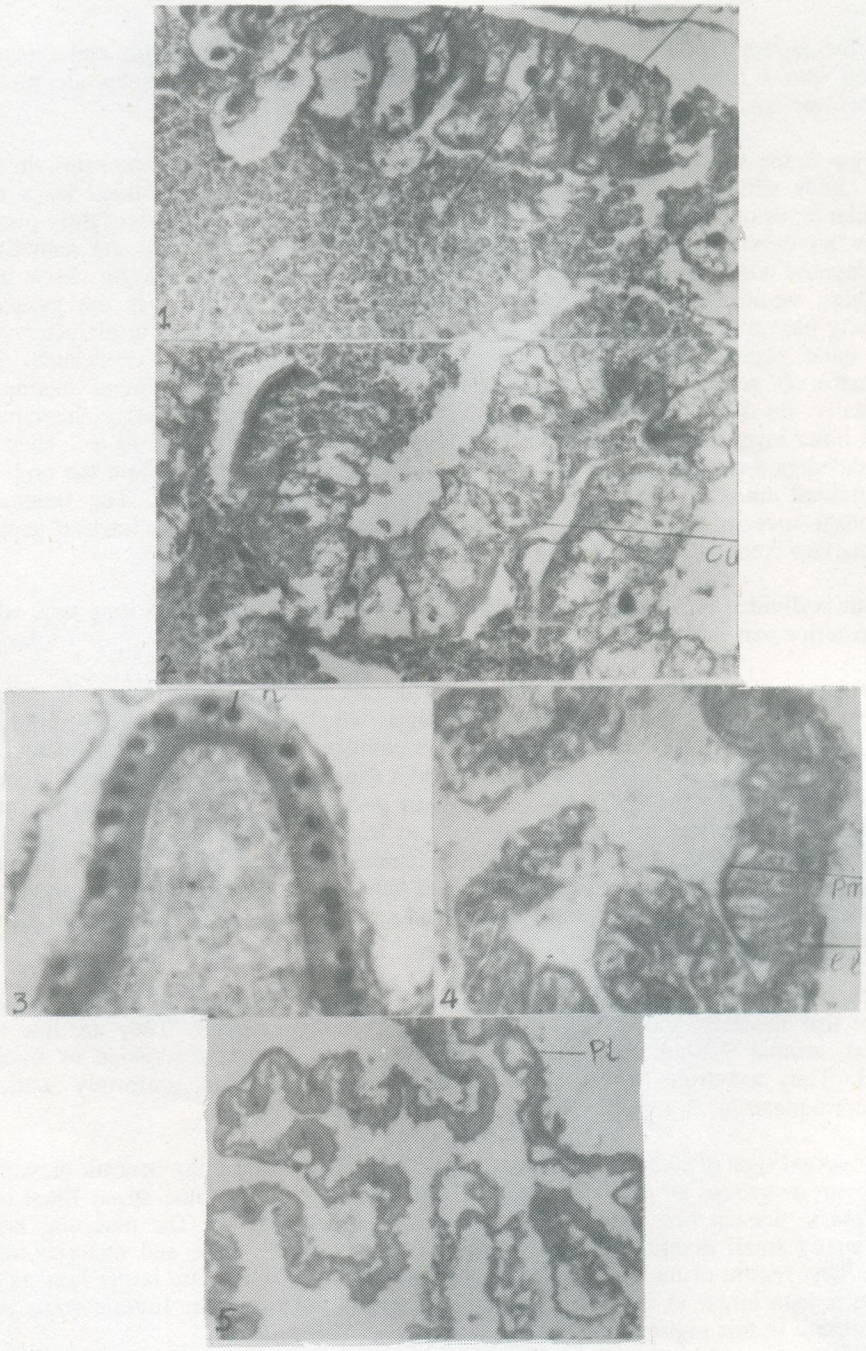


**Fig. 1.** T.S. of the various parts of the alimentary canal of red cotton bug. a, foregut showing large epithelial cells, 400x; b, foregut showing folding of epithelial lining, 400x; c, anterior midgut, 200x; d, posterior midgut showing darkly stained nuclei, 200x; e, hindgut, 200x

Hindgut is a short tube with a thin muscular layer. The epithelial layer has very narrow tall cells with small nuclei lying generally towards the base. Multinucleate cells are also common. These cells have many tightly packed vacuoles towards the lumen. The cuticular intima is very thick at places. Muscular layer is very poorly developed but peritoneal layer is well defined.

*Alimentary canal of treatede insects*

After treating the bugs with different concentrations of Polytrin C and Nuvacron for 12 and 24 hours, their effects on gut were studied (Figs. 2, a-e).

The effect of both the insecticides wa almost similar on the gut cells. Prolongation of the treatment intensified the effect. Generally the cells of the epithelial lining of the foregut became elongated and vacuolization took place. Twelve hours after treatment the epithelial cells which were squamous or columnar became tall and narrow, with an average height of 20$\mu$ and 3.5$\mu$ width. The nuclei either became elongated and narrow (6$\mu$ x 2$\mu$) or decreased in size up to a diameter range from 1.5$\mu$ to 2.0$\mu$. Vacuolization took place and clumping caused the presence of bigger granules in the cytoplasm. Partitioning of the cytoplasm also took place, so that the epithelial lining seemed multilayered at some points. Muscular layers were found to be the least affected.

After 24 hours of treatment, breakage of the too much elongated cells had taken place, and each cell of the inner layer had vacuoles towards the inner ends. Much cytoplasm had oozed out of the cells forming a regular layer, thus completely lining the lumen. The cuticular intima was consequetly all disrupted. The folds had become elongated and fewer.

The effect of these insecticidal doses on the midgut was the same. Here the epithelial lining was the most affected and the cell secretions had flown freely into the lumen. The muscular layer had become non-existent and no peritrophic membrane could be detected. In the hindgut also disruption of the cellular layer was quite conspicous but the cuticular layer was hardly disrupted.

*Effect of higher doses*

Higher concentration of the insecticide after 12 hours of the treatment had a similar effect as described for the lower dosages but of greater intensity. Many cells had become binucleate and general disruption and histolysis of the epithelium had taken place. At places the cellular layer had almost disappeared and only vacuoles could be seen. After 24 hours of the treatment the epithelial cells generally had become flattened all over the alimentary canal and cell boundaries had mostly diappeared. Cuticular intima of the foregut was found very much disrupted or absent and in the hindgut also it had become tinner. Pritrophic membrane could not be detected at all.

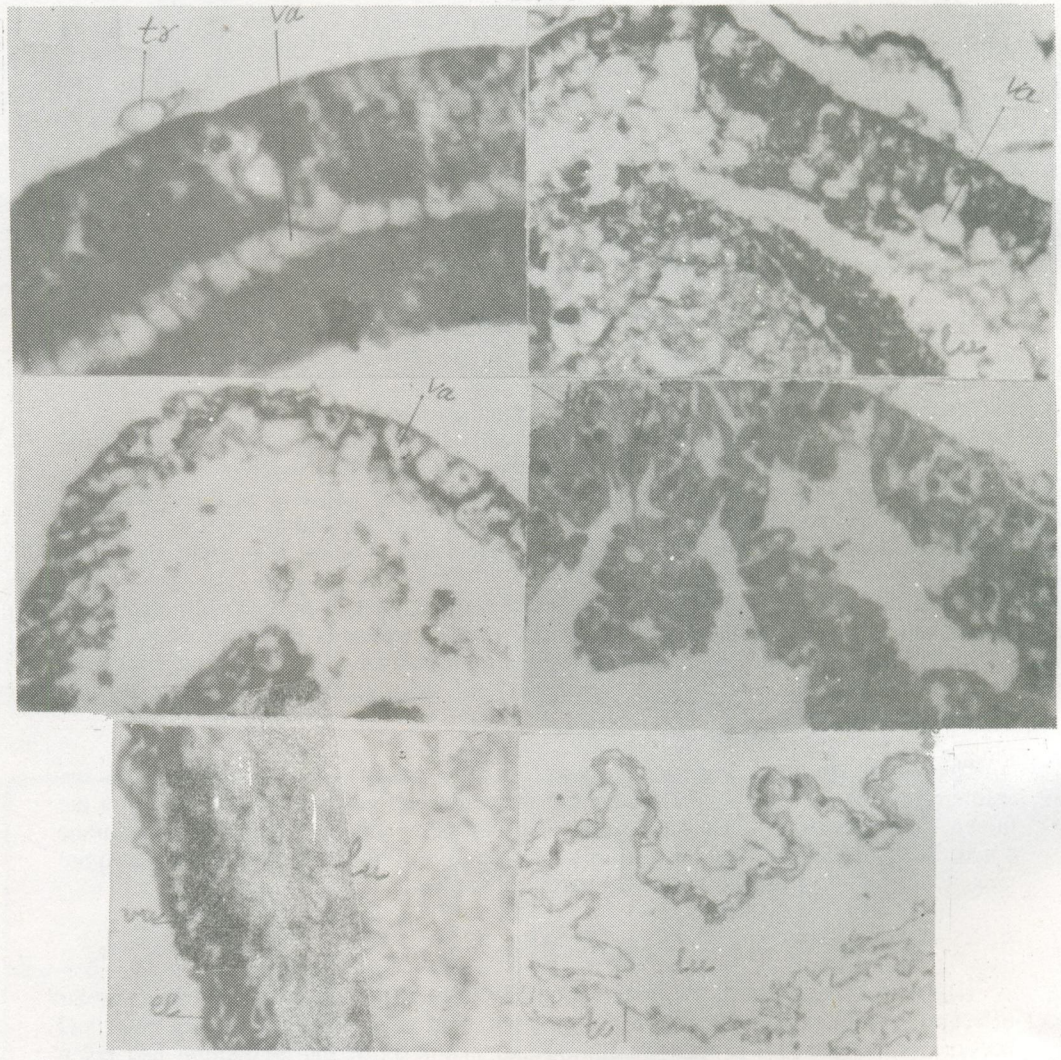


**Fig. 2.** T.S. of various parts of the alimentary canal of insecticide treated red cotton bug: a, foregut, treated with Polytrin C (0.8ppm) for 24 hours, 400x; b, the anterior midgut treated with Polytrin C (0.8ppm) for 24 hours, 400x; c, the posterior midgut treated with Polytrin C (1.7ppm) for 12 hours, 200x; d, the foregut treated with Nuvacron (1.1 ppm) for 12 hours showing epithelial layer, 400x; e, the posterior midgut treated with Nuvacron (0.6 ppm) for 24 hours, 400x; f, the hindgut treated with Nuvacron (2.2 ppm) for 24 hours showing cellular layer, 200x.

**Abbreviations used**: tc, thin cellular layer; lu, lumen; cu, cuticular layer; n, nucleus; el, epithelial layer; pm, peritrophic membrane; pl, peritoeal layer; va, vacuoles; tr, trachea; bn, binucleate epithelial cells.

## DISCUSSION

The alimentary canal of the adult red cotton bug is very long and tubular as is generally the case with fluid feeders (Chapman *et al.*, 1985; Richard and Davies, 1977). In this bug, apart from the pharynx, all the other parts of the gut have a very weak or no muscular layer at all. The midgut which is the main site of digestive activity is divided into an anterior and a posterior part. The division of the midgut into different portions is also common in fluid feeders especially those feeding on sap *i.e.*, petatomorphia has 4 recognizable divisions, or ventriculi while some others have three (Chapman, 1971; 1985). These divisions help to get rid of the excessive water content of the food as it flows along their length. Development of other devices like filter chamber in some insects like Cercopoidea is for the same purpose, otherwise the blood can becomes too diluted and thin. The ingested water enters the blood via the gut epithelium and other histological layers.

A fairly well defined peritrophic membrane was also found lining the midgut epithelium. It is said to be absent from most insects feeding on a liquid diet. However, it is present in *Cicadella*, mosquitoes and *Glossina* (Gouraton and Maillet, 1985; Moloo et al., 1970; Freeman, 1973) and accordig to Waterhouse (1953) may have been overlooked in other fluid feeders. It may have some other function apart from protection from food and act as a selective membrane and be secreted in response to a stimulus. This was first proposed by Stohler (1957) but later on many workers investigated it in various insects like *Simulium*, (Fallis, 1964; Lewis, 1950), Culicoides (Megahad, 1956), *Phlebotomus* (Gemetohu, 1974). In mosquito even a small blood meal can induce its secretion and a second meal can also stimulate the production of a second membrane which surrounds the first membrane (Waterhouse, 1953; Freyvogel and Jaquet (1965). Richard and Richard (1977) have given many other examples of this type of behaviour by insects. It seems that in the red cotton bug also the peritrophic membrane is secreted in response to feeding.

Alimentary canal plays an important role in the detoxification of various insecticides used in the field. The insecticides do not enter the insect body by cuticular penetration only, they are also ingested with the food and reach the haemolymph and target and non- target organs after passage through the gut wall. Transport through the gut wall is a passive deffusion process, because neither the presence of inhibitors of carbohydrate metabolism or couplers of oxidative phosphorylation, nor even the absence of oxygen has any effect. Its rate may be different in the different insects and also depending upon the type of insecticide (Shah and Gutherie, 1970; 1971).

In the red cotton bug Polytrin C and Nuvacron had almost the same effect on the gut. All parts of the alimentary canal were found affected. The maximum disruption took place in the epithelium lining of all gut divisions, especially those of the midgut. Higher doses and prologed treatment intensified the effect. Ultimaltely the enteric epithelial cells became flattened and vacuolated, with almost no cytoplasm *i.e.* histolysed to an extensive degree. The midgut epithelium is the site of secretion and absorptive activity. So this was observed to be the most vulnerable part. These insecticides proved fairly successful against this bug.

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# THE EFFECT OF AGE AND FEEDING AN ANABOLIC-ANDROGENIC STEROID (DIMETHAZINE) ON THE BODY AND ORGAN WEIGHTS OF FEMALE CHICKS

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**Abstract:** A study was undertaken to obtain reference values under local conditions on the effect of age on the organ weights of various internal organs during early ontogenetic development of female chicks (pullets). In addition, the effect of feeding, an anabolic-androgenic steroid, dimethazine, on the growth of chicks was also studied. During the first 100 days of life, the growth of liver, kidney, heart, adrenal, thyroid, ovary, and muscles pectoralis, gastrocnemius, and peroneus longus was different qualitatively and quantitatively. Feeding of anabolic-androgenic steroid increased the appetite of the birds but did not induce any changes in the FCE. The steroid fed groups were heavier ($P < 0.05$) than the control groups. Some of the organs studied had heavier weights (statistically significant in certain instances) in the steroid-fed groups when compared to the control groups. The study confirms some earlier reports that androgens can induce positive growth response in birds as they do in mammalian species. .

**Key words:** Chicks, age, body weight, liver, kidney, heart, muscles, thyroid, adrenal, specific growth rate, and food consuming efficiency.

## INTRODUCTION

The subject of growth and development has generated considerable interest among biologists and livestock scientists. Because of these studies, reference values have been reported for different body organs of various animals of economical interest. This type of research has been completely neglected in Pakistan. Although, new breeds have been imported from abroad where these reference values are available for these species. Nevertheless, it has been seen that these values cannot be regarded as representative here in Pakistan, because of many reasons like different physical environmental conditions, or nutritional regimes, etc., which at best are not optimal and normally can be termed poor, in which these animal are kept. In reporting organ weight changes during normal ontogenetic development, one cannot predict whether these differences in weights of internal organs are "normal" under the prevalent conditions or are due to the deficiency of nutritional factors or disease. Furthermore, in the absence of the reference values under local conditions, the researchers cannot make a clear decision as to whether the changes seen in their studies are true effects of growth and development or a combination or interaction of age with other experimental factors. Strain differences in weights of body organs have been reported in broilers and chickens (Hoffman *et al.*, 1953; Al-Dabagh and Abdulla, 1963; Daghir and Pellett, 1967). Similarly, differences in body organ weights of birds have also been reported by various workers under different nutritional regimes (Marion and Edwards, 1963; McCartney and Brown, 1977; Hargis and Creger, 1980; Marks, 1978a,b, 1979; Cornejo *et al.*, 1980).

Androgens, and their derivatives known as anabolic-androgenic steroids, have been shown to stimulate protein anabolism in a variety of laboratory animal species and livestock animals like cattle (Kochakian, 1976; Lone, 1996). Their use as anabolic agents in chicken has not been particularly productive. Evidence to date suggest that androgens neither affect feed efficiency nor growth however, some positive reports also have appeared in this direction (for more details see, Lone, 1996). Dimethazine, a potent anabolic-androgenic steroid used in human medicine, has a very favorable anabolic to androgenic ratio. The experiments done with rats and other mammalian and fish species (Lone and Matty, 1984) always gave positive results as far as positive nitrogen balance and myotropic effects are concerned. Keeping in view its higher anabolic efficiency with comparatively lower androgenic side effects it was decided to study its effects on growth of pullets when fed with diet from age day-one to day 97.

## MATERIALS AND METHODS

Newly hatched, three hundred (300) single comb, white Leghorn (layers variety) female chicks (Pullets) were obtained from a local commercial hatchery. The birds were housed in a well aerated and air-conditioned room where the temperature was kept between 25-30 °C. The room floor was thickly covered with sawdust which was changed periodically. Food and water were supplied *ad libitum*. The chicks were fed commercial broiler starter diet (proximate analysis: protein = 16.03%; fat = 4.93%; carbohydrates = 55.53%; moisture = 9.86%; ash = 7.83%). At two weeks of age all chicks were vaccinated against New Castle disease. Before the start of the experiment the chicks were divided into two (2) groups. One group was designated as control and the other experimental. The control group was given a normal diet without any hormone in it while the experimental group was provided with a diet containing dimethazine (2α-17α-dimethyl-17ß-hydroxy-5α-androsten-3,3'-azine; Roxilone,Richter, Italy) at a concentration of 5 mg/kg diet (5 ppm). The steroid was incorporated in to the diet by the method of Lone and Matty (1980). This method essentially means dissolving the steroid in absolute alcohol and then spraying the alcoholic solution on to the diet with the help of a chromatographic sprayer. The volume of alcohol used for the experimental diet is also sprayed on to the control diet but without any steroid. The alcohol is then evaporated in air and the diets are kept in a freezer for future use. For feeding, the chicks were provided diets in an appropriate feeder and the daily intake by the birds was noted. Chicks were weighed individually on the day of hatching and on every alternate day up to 14 days, every four days up to 38 days and weekly thereafter, up to 97 days. Weighing was done to the nearest gram.

At each sampling, in addition to taking the total body weight, different body organs like heart, liver, kidney, muscle gastrocnemius, pectoralis major, peroneus longus, thyroid gland, adrenal gland and ovaries were dissected out from eight birds and immediately weighed to the nearest mg. Moreover, comb weight, tibiotarsus weight and length and shank length were also taken. The sampling for these organs was done on the day of hatching and on alternate days up to ten days. Every fourth day up to the age of 38 days, every week up to the age of 52 days and fortnightly thereafter, till the end of the experiment. The weighing of organs was done to the nearest mg.

On the basis of the data obtained on the feed consumption of the control and

experimental chicks, feed consumed per chick per week and feed conversion efficiency (weight gain/food eaten X 100) were computed for each group. Specific growth rate (SGR) and tissue-somatic index (relative weight of organ) were also computed according to the following formulae.

$$SGR = \frac{Ln\ W - Ln\ w\ X\ 100}{Time\ (Days)}$$

where Ln is log natural and W and w are final and initial weights of the bird.

$$\text{Tissue-Somatic Index} = \frac{\text{Weight of the organ}}{\text{Body Weight of the Chick}} \times 100$$

Linear regression equations were also computed for all organs against age while the organs weights of the control and experimental birds were compared by two-tailed Student 't' test according to Sokal and Rohlf, 1973.

## RESULTS AND DISCUSSION

The total body weight of each chick was taken according to the weighing schedule detailed in the Materials and Methods above. At hatching mean body weight was $38.37 \pm 1.12$ g. This weight at the end of the experiment after 97 days was $828.29 \pm 34.18$ g and $900.85 \pm 37.17$ g for the control and experimental chicks respectively. The experimental birds were heavier (Fig. 1a) than the control birds and this difference was significant statistically ($P < 0.05$). Fig. 2a presents the weight gain per chick per two weeks. It is clear from the figure that weight gain of the experimental birds was better than the control birds. Theoretically, the specific growth rate (SGR) decreases with age. We saw the same trend in the SGR both in experimental and control groups (Fig.2b). Figure 2c presents data regarding the food consumed by the two groups of the pullets. It appears from it that the experimental birds were eating more (increased appetite) than the control birds. This divergence in food consumed was clearly seen around 52-day of the experiment. For example, the average food consumed by the control birds in the last fortnight of the experiment was 557.44 g while the same amount for the experimental birds was 937.50 g. This increase is around 68 % over the control values. Although, the experimental birds were eating more, their food conversion efficiency (FCE) however, was lower than the control birds. These data are more clearly shown in Fig. 2c and 2d respectively.

It has been shown many times that whereas estrogens may be potent in increasing the total body weight of the birds, anabolic-androgenic steroids did not give similar results (Belloff and Hsu, 1963; Trenkle, 1969; Lone, 1996). We have seen here results with dimethazine which are different as compared with other anabolic-androgenic compounds commonly used as growth promotants. These results however, are not unique in the sense that dimethazine is considered quite potent anabolic steroid in mammalian species. Dimethazine also increased the appetite of the birds which was apparent from the increased food intake however, this increase was not accompanied by

an increase in the FCE of the treated birds. Anabolic steroids generally increase the appetite of the animals and this has been reported for birds also. This factor may be the reason for not having any clear cut positive effects on the overall body weight of the birds in the previous studies for it has been reported that the birds which have been selected for higher body weight for a specific age consumed more food and converted this food to flesh more efficiently. Moreover, it has been shown in birds that appetite differences account for most of the genetic differences in growth rate (Sutton and Siegel, 1975; Reddy and Siegel, 1977; Marks, 1979, 1980a, b). In the present study although the hormone was capable of increasing the appetite of the treated birds it could not translate this increase in the appetite into higher FCE.

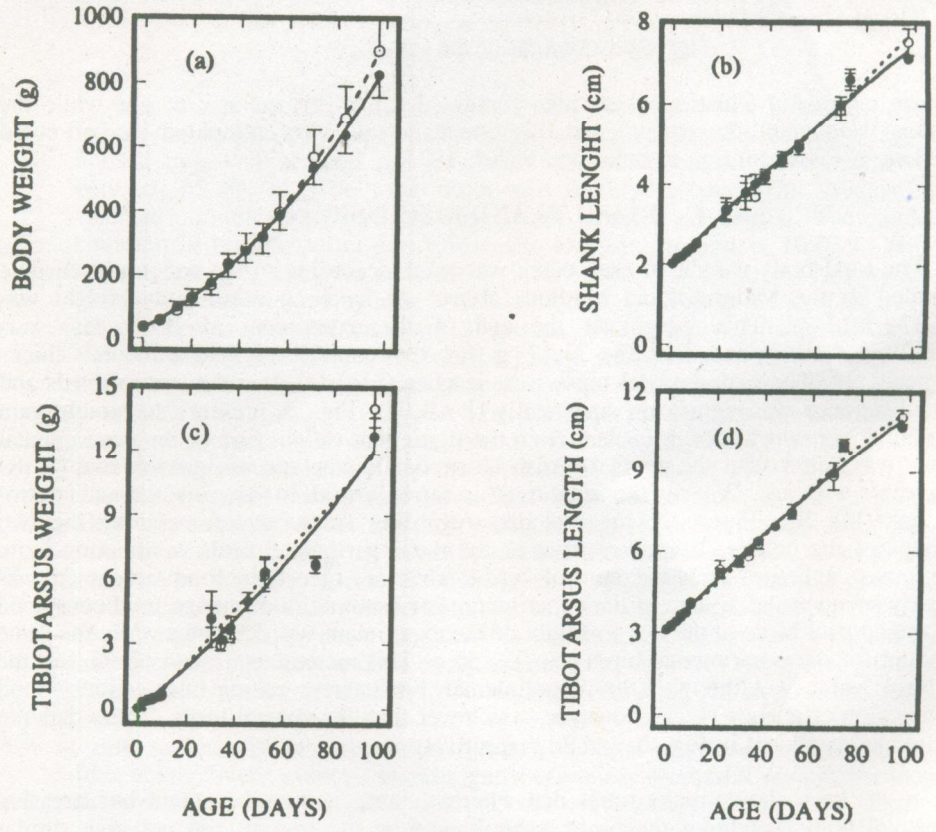


**Fig. 1.** Effect of feeding dimethazine on the body (a) and tibiotarsus (c) weight (g) and shank (b) and tibiotarsus (d) length (cm). Solid circles represent controls while open circles show the experimental data. Values given are mean ± standard deviation of at least 4 animals each.

There was no difference between the shank length (Fig. 1b) and tibiotarsus weight and length (Fig. 1c, 1d) of the control and treated birds. These parameters were studied in order to observe the effect of the steroid on the bone growth and height of the birds.

In addition to the body weight and other nutritional parameters, different body organs were removed from the slaughtered birds. Three muscles, i.e., pectoralis major, gastrocnemius and peroneus longus were removed and their total weights determined. The weight changes of these muscles are given in Fig. 3a, b, c, d and 4a, b. Whereas the body weight increased from zero-day to 97 days 21.39 times, the pectoralis, gastrocnemius and peroneus longus grew during the same time around 117, 34 and 58 times respectively. This means that these muscle were growing much faster than the body weight during the time of the experiment. Here, the steroid fed animals always

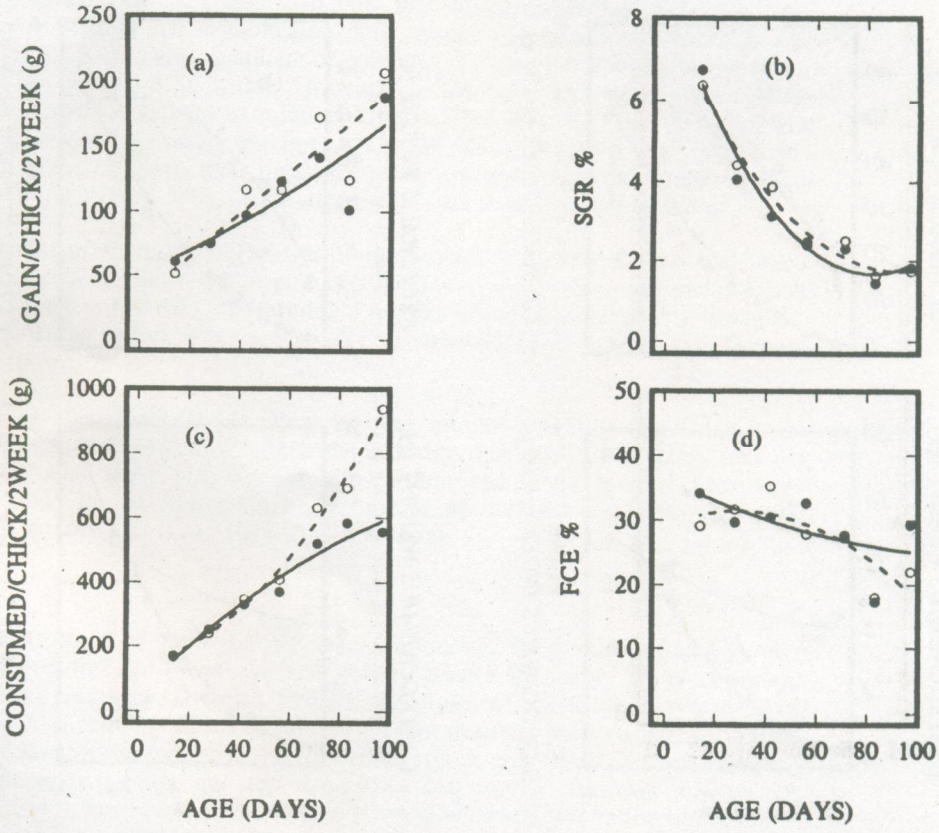


**Fig. 2**. Effect of feeding dimethazine on the weight gain (a), specific growth rate (g%) (b), feed consumed (c) and feed conversion efficiency (d) of female chicks. For more details please see Fig. 1.

had slightly heavier muscles than the respective control animals. For example, the pectoralis ($P<0.05$) and gastrocnemius ($P<0.001$) were 14 % and peroneus longus ($P<0.01$) was 22 % heavier than their respective controls at the end of the experiment. When seen in terms of the relative weight (tissue-somatic index) of the individual muscle, the growth pattern of the two leg muscles was different from the breast muscle (compare Fig. 3a and 4a). The growth pattern of the breast muscle was exponential while the growth of the leg muscles was linear in nature. Moreover, both leg muscles also differed in their growth characteristics. The growth of the peroneus longus was quite slow during the first month of life but became quite faster afterwards. These differences probably stem from the different function of the specific muscle and cellular events which are triggered because of the function. This can also be seen from the response of these muscles under experimental conditions of restricted nutrition or

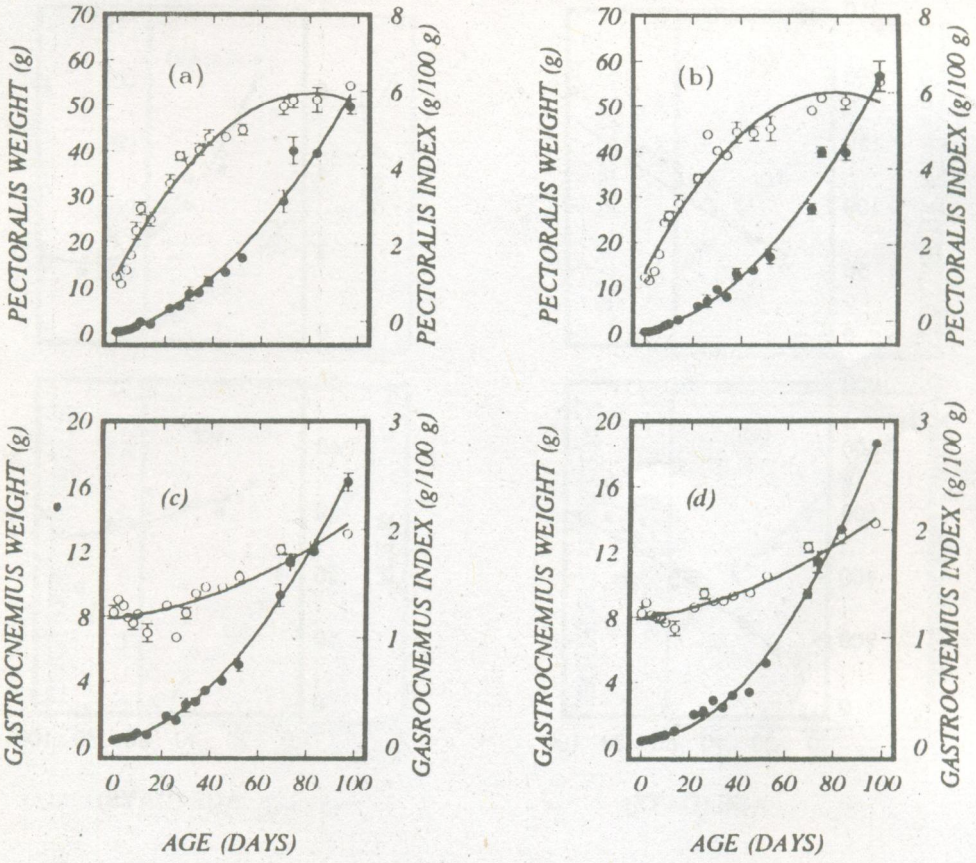


**Fig. 3**. Effect of feeding dimethazine on the absolute weight (g: solid circles) and relative weight (g/100g body weight: open circles) of muscle pectoralis and gastrocnemius. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

starvation. The muscle which perform more active function, for example, leg muscles, are affected most by the energy or feed restriction than those muscles which are less active functionally or metabolically (Burger *et al.*, 1962; Daghir and Pellett, 1967; Cornejo *et al.*, 1980).

Kidney, liver and heart weights were taken as representative of the most important internal organs. The growth pattern of these organs is presented in Fig. 4c and d and 5a, b, c and d. The weight increase of these vital organs seem to be more or less linear with age over the entire range of the experimental period of 97 days. As reported above the total body weight increase during the experiment was 21 times, the weight increase of the liver and kidney during this time was 20 and 19 respectively, showing that these two organs were still growing directly proportional to the body weight. The heart weight increase during this time was 15.5 times, exhibiting a slowing of growth with

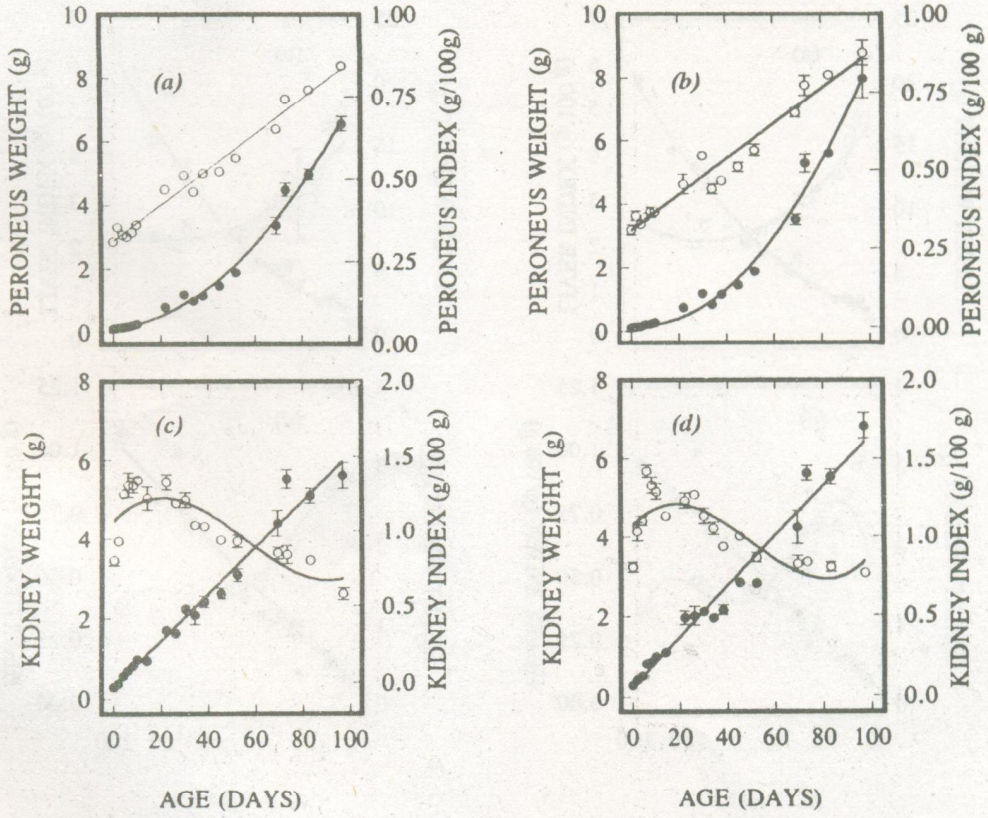


**Fig. 4**. Effect of feeding dimethazine on the absolute weight (g: solid circles) and relative weight (g/100g body weight: open circles) of muscle peroneus longus and kidney. Graphs labelled (a) and (c) are for control groups while (b) and (d) for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

age. Feeding of dimethazine caused the weight of these organs to increase (13.65, 21.89, and 15.49% increase in the final weight of the liver, kidney and heart respectively after 97 days of steroid feeding). This increase in weight over controls was significant ($P<0.01$) statistically. When the weight of these organs were calculated as the percentage of the body weight then it appears that the growth pattern of these three organs was different. The relative maximum weight of these organs in comparison to body weight (tissue-somatic index) was seen on 4th, 10th and 4th day for liver, kidney and heart. The experimental groups showed this maxima slightly earlier than the control values given above. After this maxima, the relative weight of these organs decreased steadily with increasing age. The qualitative pattern in this connection was similar between kidney and heart (compare Fig. 4c and 5c). The liver, on the other hand, showed a pattern similar to first order decay reaction. In this respect, our results are

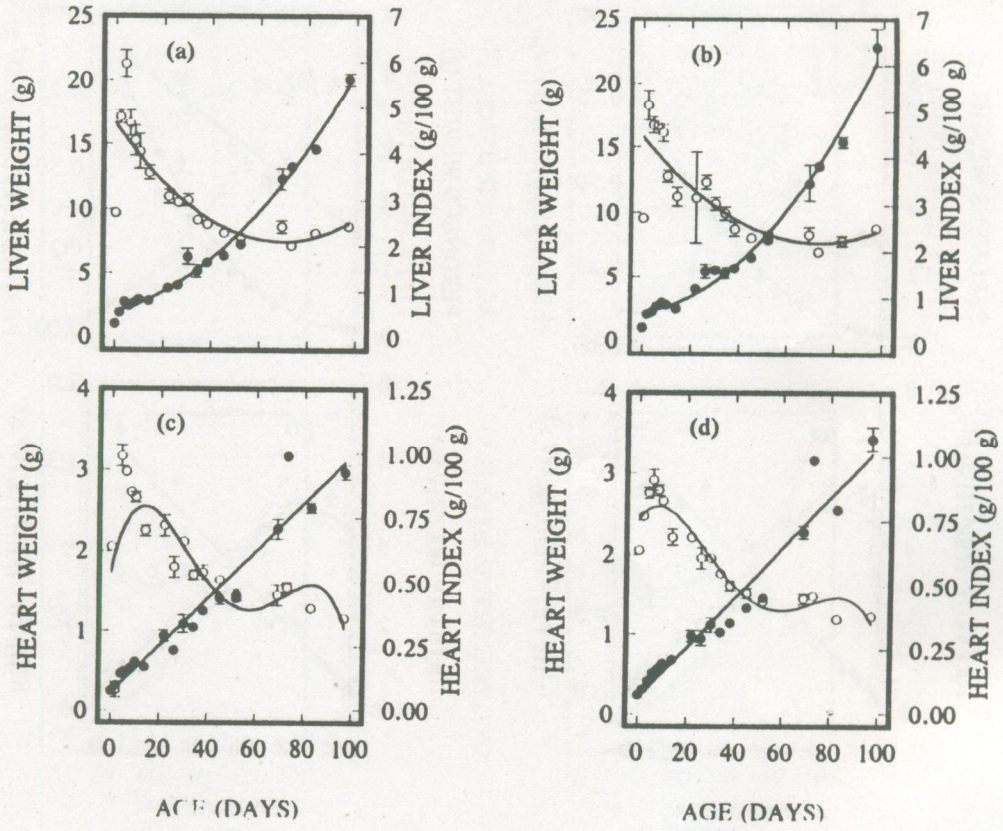


**Fig. 5**. Effect of feeding dimethazine on the absolute weight (g: solid circles) and relative weight (g/100g body weight: open circles) of liver and heart. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

similar to the earlier published reports in terms of qualitative (Burger *et al.*, 1962; Al-Dabagh and Abdulla, 1963; Daghir and Pellett, 1967; Cornejo *et al.*, 1980) changes but strain, nutritional and environmental differences caused changes in the quantitative pattern of the growth of these organs when compared with earlier published values.

In addition to the muscle and other vital body organs, some endocrine organs like adrenal, thyroid and ovary and a non-endocrine tissue, comb were also dissected out from the slaughtered birds and their growth in terms of organ weights was studied. The growth pattern of adrenal gland is presented in Fig. 6a, and b. The adrenal gland weight at hatching was $4.53 \pm 0.3$ mg. At the end of the experiment, at age 97 days, this weight was $67.38 \pm 3.13$ mg, i.e., an increase of some 15 times the weight at hatching. The weight increase was uniform over time and the hormone fed groups also

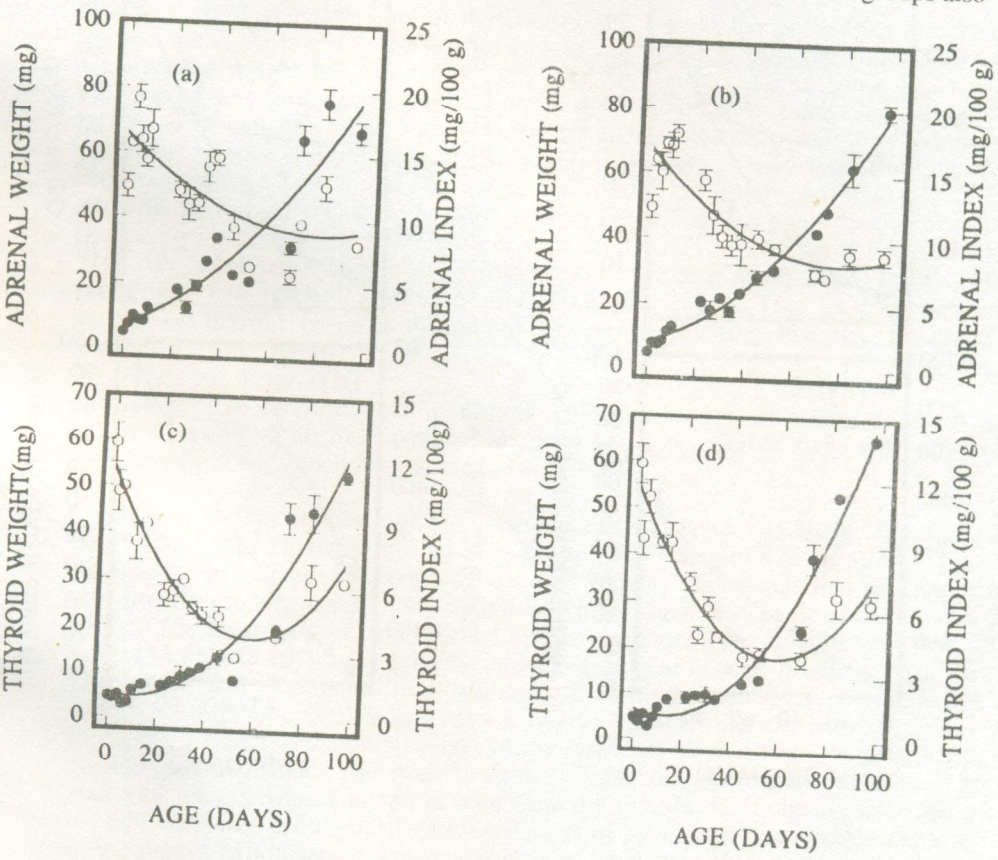


**Fig. 6**. Effect of feeding dimethazine on the absolute weight (mg: solid circles) and relative weight (mg/100g body weight: open circles) of adrenal and thyroid. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

showed similar growth pattern, however, the experimental birds had their adrenals 18% heavier ($P<0.01$) than the control birds at 97 days of life. The relative mass of adrenal in control birds was maximum($19.13\pm0.93$) at day-4 of the post-natal development while this maximum weight ($17.97\pm0.65$ mg/100 g) in dimethazine fed birds was seen after 10 days. This means that the dimethazine inhibited the growth of adrenal during early days of life, however, later on this inhibition is eliminated and adrenal weight catches up and becomes higher than the control values. After these maximum values the adrenal weights of both groups declined over time. At 97 days of life the relative mass of the adrenal in control birds was around 34% less than the hatching values of this parameter.

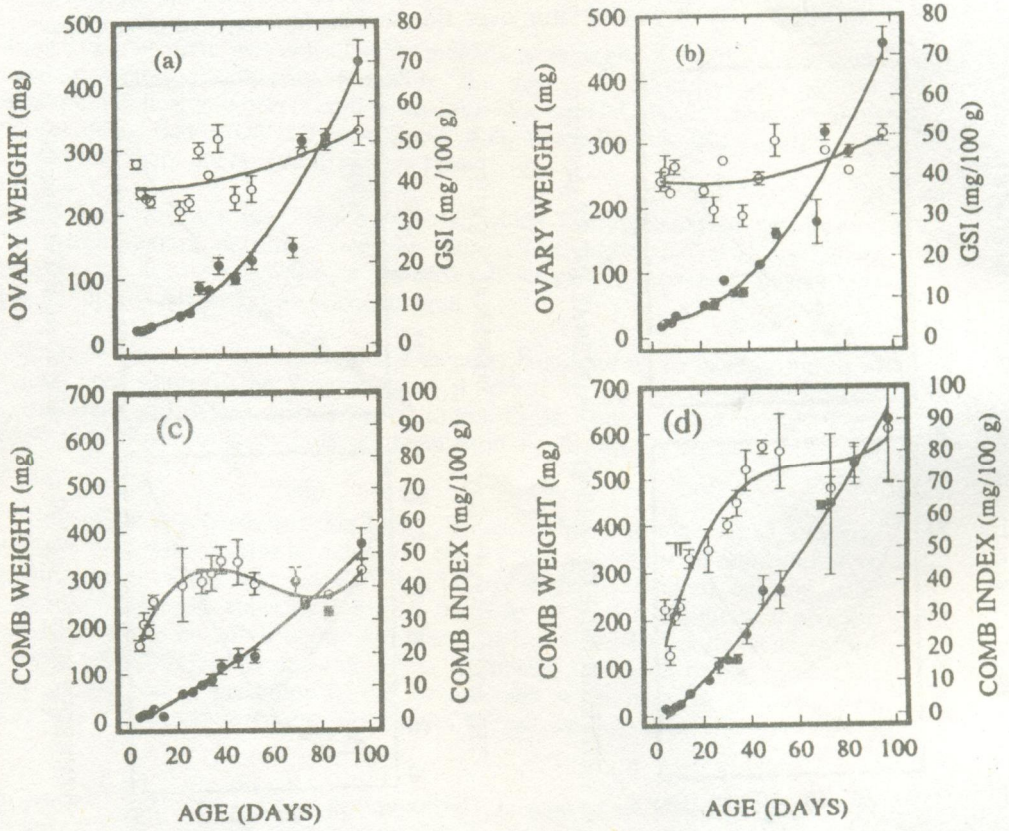


**Fig. 7.** Effect of feeding dimethazine on the absolute weight (mg: solid circles) and relative weight (mg/100g body weight: open circles) of ovary and comb. Graphs labelled (a) and (c) are for control groups while (b) and (d) are for respective experimental groups. Values given are mean ± S.D. of at least 4 animals each.

It has been shown in many birds species that during the first few weeks after hatching the absolute weight of the adrenal gland increases but the body weight increases are proportionally more. As a result, a sharp decline in the relative mass of the gland occurs during post-natal development. An observation also seen in the present study. It may be mentioned here that once birds mature, a constancy in the relative mass of the adrenal weight is also achieved. However, in mature birds, increase or decease in adrenal weight frequently occur in response to seasonal and environmental changes and these changes reflect the functional attributes of the gland. Chronic stress and social standing of the organism also cause a change in the adrenal weight of the birds (Holmes and Cronshaw, 1980). In the present study, the birds didn't reach maturity, which is generally achieved at the age of 18-20 weeks (Gilbert, 1971) therefore, the changes reported above in the relative weights of adrenal were not observed.

The thyroid gland weights of the control and steroid fed pullets are presented in Fig. 6c and d. At hatching the thyroid weight was 12.32 $\pm$ 0.89 mg and 97 days after hatching the weight has reached 53.08$\pm$1.42 mg, an increase of some 11.34 times. This means that during this time the thyroid gland growth was nearly half of the body growth. This can also be seen in the relative weight of the control birds which showed maximum relative thyroid mass at hatching. At 97 days of age the relative mass has decreased some 49.49 %. At this stage, the steroid fed group had higher (23.40 %) absolute thyroid weights than the control birds ($P < 0.001$) but there relative mass were similar. After reaching a minimum value of 2.83$\pm$0.23 mg/100 g of body weight at 52nd day, the relative weight of thyroid started increasing again and at the end of the experiment had reached a value of 6.42$\pm$0.22 mg/100 g. This increase in relative weight of thyroid is probably related to the approaching maturity of the birds (Falconer, 1971, 1984).

Like adrenal, thyroid gland also show variations because of season, iodine content of the diet and strain of the birds. Maturity and reproductive stage also affects the thyroid weight and function (Falconer, 1971, 1984).

Ovarian weight at 4 days of age (when ovaries were removed for the first time) was 20.65$\pm$0.55 mg. This weight reached at the end of 97 days to 436.68$\pm$33.81 mg. This amounts to an increase of 21 times the initial weight. This means that ovarian growth was directly proportional to body weight which also increased 21 times during this time period. The ovaries of the experimental birds (receiving 5 ppm dimethazine) at this time were 454.65$\pm$25.19 mg, a 4.12 % increase from the control group's organ weight. This means that during the first 100 days the anabolic-androgenic hormone did not affect ($P > 0.05$) the total weight of the ovary. The fact that the ovarian weight was increasing parallel to the body weight of the birds can be seen from the progress of the gonado-somatic index. The gonado-somatic index did not change much during the course of the experiment as can be seen from the Fig. 7a, b. In the experimental birds, the GSI decreased a little during the early days of the experiment (androgenic effect (?) of the steroid fed) however, in the later days it was comparable to the control birds.

The comb of chicken is considered a very sensitive target organ for the androgens. This organ was included in the present study in order to know whether the birds were

getting enough steroid from the food and also to know its androgenic potential. As can be seen from the weight increase and the relative mass of the comb (Fig. 7c and d) that the birds fed dimethazine with the food had absorbed it from the gut. The experimental birds always had higher comb weights from the corresponding control birds at any time. The comb growth was linear during the course of the experiment in both groups however, the slope of the curve for the two groups was different. The relative mass of the comb was maximum in the control birds on 38th day of the experiment while this maxima was not achieved in the experimental birds till the end of the experiment. At the end of the experiment the comb weight was around 70 % higher ($P<0.05$) than the control birds. Another observation made during the study was that the steroid-fed groups had bigger wattles than the control birds. This again show that the steroid was present in the body and that androgen sensitive tissue did respond to it.

**Table 1: Linear regression equations (Y=a+bX) of age versus organ weights of control and dimethazine (5mg/kg food) fed pullets during the first 97 days of life.**

| Parameter | Control Y= a+bX | $r^2$ | Dimethazine Y= a+bX | $r^2$ |
|---|---|---|---|---|
| Body weight | -26.39+8.04 | 0.975 | -43.71+8.77 | 0.965 |
| Shank length | 2.03+0.06 | 0.990 | 1.972+0.06 | 0.993 |
| Tibiotarsus weight | -0.299+0.12 | 0.959 | -0.462+0.13 | 0.932 |
| Tibiotarsus length | 3.164+0.08 | 0.990 | 3.13+0.09 | 0.990 |
| Weight gain | 43.39+1.24 | 0.745 | 33.41+1.61 | 0.820 |
| SGR (%) | 6.15-0.05 | 0.794 | 6.27-0.05 | 0.880 |
| Feed consumed/chick/2weeks | 104.17+5.24 | 0.949 | -13.21+8.99 | 0.959 |
| FCE (%) | 34.82-0.12 | 0.346 | 35.22-0.14 | 0.564 |
| Muscle Pectoralis | -4.13+0.51 | 0.942 | -4.34+0.53 | 0.930 |
| Muscle Gastrocnemius | -1.06+0.15 | 0.930 | -1.275+0.17 | 0.905 |
| Muscle Peroneus longus | 0.46+0.06 | 0.920 | -0.64+0.07 | 0.881 |
| Kidney | 0.280+0.06 | 0.974 | 0.217+0.06 | 0.967 |
| Liver | 0.632+0.17 | 0.943 | 0.412+0.19 | 0.926 |
| Heart | 0.220+0.03 | 0.936 | 0.209+0.03 | 0.939 |
| Adrenal | 2.42+0.67 | 0.838 | 2.649+0.67 | 0.942 |
| Thyroid | -1.51+0.47 | 0.809 | -1.83+0.55 | 0.832 |
| Ovary | -34.12+4.07 | 0.880 | -34.91+4.11 | 0.886 |
| Comb | -23.06+3.06 | 0.943 | -57.12+6.86 | 0.980 |

In the end we can conclude that the birds did absorbed the steroid from the food and dimetazine was able to induce significant somatic growth in the pullets. This study generated data on the ontogenetic development of the pullets and provides data during the first 100 days of life on the weight of the internal organs. These weights can be used as reference values for the determination of either the organ weight or the total body weight or age if either of the two is available by using the regression equations provided in the paper (Table I).

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# PRELIMINARY LABORATORY OBSERVATIONS ON THE PREDATION OF EGGS AND JUVENILES OF MEDICALLY IMPORTANT PULMONATE SNAILS BY A PROSOBRANCH SNAIL

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**Abstract**: The present study is an attempt to investigate the fate of newly hatched eggs and one week old juveniles of *Lymnaea acuminata*, *L. rufescens*, *Indoplanorbis exustus* and *Physa acuta* in the presence of well fed and starved adult (72 week old) and juvenile (one week old) *Bellamaya bengalensis* Lamarck. Results revealed that both juvenile and adult *B. bengalensis* cause damage to the eggs and newly hatched juveniles of the above mentioned pulmonate snails. It was further observed that extent of damage was more with the increasing number of unfed *B. bengalensis* as compared to the well fed *B. bengalesnsis*.

**Key words**: Biological control, pulmonate snails, viviparid snail.

## INTRODUCTION

*Bellamaya bengalensis* (Lamarck) has been proposed as a biological control agent of those molluscs which contribute to the transmission of diseases to man and his live stock (Tanveer and Khan, 1991). Tanveer (1991) further reported that *B. bengalensis* suppress the population of lymnaeid and planorbid snails in competitive interactions and damage to the pulmonate snails increased with the increasing number of *B. bengalensis*. Keeping in view such results it was planned to investigate the fate of newly hatched eggs and juveniles of *Lymnaea acuminata*, *L. rufescens*, *Indoplanorbis exustus* and *Physa acuta* in the presence of well fed and starved adults (72 week old) and one week old juvenile *B. bengalensis*.

## MATERIALS AND METHODS

Gastropod snails used in the present study belonged to four different families *i.e.*, Lymnaeidae (*Lymnaea acuminata*, *L. rufescens*), Planorbidae (*Indoplanorbis exustus*), Physidae (*Physa acuta*) and Viviparidae (*Bellamaya bengalensis*). For collection sites and maintenance see Tanveer and Khan (1989) and Tanveer *et al.*, 1989.

### *Experimental design*

For obtaining eggs, 14 small earthen pots of surface area 624.52 $cm^2$, with one liter water capacity and 20 cm water depth were stocked with 4 *L. acuminata* (age 32 week, wight 0.5 908$\pm$0.00021 g; shell length 26.08$\pm$1.8 mm). Fresh lettuce leaves was the only food provided to them. Lettuce leaves also icrease the surface area for egg laying. Laboratory temperature was kept at 25.0$\pm$1.5°C. Lymnaeid, Planorbid, Physid snails used for obtaining eggs were 32-36 week old. Water was changed very carefully and new fresh tap water was filled up to the mark daily. No aeration was provided. After 4 days a desirable number of egg masses were deposited on the base, side walls of the pot

and on the lettuce leaves. Then adult *L. acuminata* were removed, water was replaced and number of egg masses and eggs were counted very carefully by a hand lens. Only desired number of eggs were kept in the pots while extra egg masses were removed. These pots were daily filled with fresh tap water, lettuce was supplied as food and submitted to the action of adult *B. bengalensis* (age, 72.0±6.00 weeks; weight 2.056±0.0031 g; shell length 20.33±1.19 mm). The number of *B. bengalensis* was gradually increased as 1, 2, 3, 4, 6, 8, 10 in each pot. Two replicates for each density were run and two pots, run without sdult *B. bengalensis*, were termed as control groups. The exact number of eggs and egg masses were recorded before the introduction of test snails (*i.e.*, at zero day) and the number of undamaged eggs and egg masses were counted on 8th day and after this time the juveniles start hatching. The number of juveniles hatched were counted on 16th day and average of percentage values +S.D. have been presented. Similar experimental set up was made for *L. rufescens*, *I. exustus* and *P. acuta*. The above experiments with similar protocol were also repeated without any food. Fate of egg masses of *L. acuminata*, *L. rufescens*, *I. exustus* and *P. acuta* was also observed in the presence of their respective adutl snails. Two experimental groups one with food and other without food were run with the protocol mentioned above.

In another series of experiments fate of newly hatched juveniles of *B. bengalesnsis* in the presence of adult *L. acuminata*, *L. rufescens*, *I. exustus* and *P. acuta* was determined. For this purpose newly hatched actively crawling juveniles (up to the age of 24 hours) were kept in earthen pots (as described above) alongwith 1, 2, 3, 4, 6, 8, 10 adult *L. acuminata*, a control was run without any adult snail and one with 5 adult *B. bengalensis* (a number which was half of the number of test snails as mentioned earlier). The experimental protocol for maintenance and time for observations was similar to that mentioned earlier.

## RESULTS

The results of the present investigation revealed that both juvenile and adult *B. bengalensis* caused damage to the eggs and newly hatched juveniles of *L. acuminata*, *L. rufescens*, *I. exustus* and *P. acuta* (Figs. 1-2, Table I).

The extent of damage was greater by adult *B. bengalensis* as compared to the juvenile snails. This damage was also increased with the corresponding increase in the number of adult *B. bengalensis* and in the absence of food. However, in a reciprocal experiment it was noted that juvenile *B. bengalensis* were not affected by the presence of adult *L. acuminata, L. rufescens*, *I. exustus* and *P. acuta* even in the absence of food.

It is also evident from the Table I that in the control groups of competing snail species less than 70 % eggs developed into juveniles, while other 30 % died or destroyed due to unknown reasons (may be due to some born defects or any other reason not known). The maximum damage in the experimental groups took place in the pots where 10 snsils per pot were kept (*i.e.* only 10-14 % eggs left in this group after 16 days) the similar value for the group without food were 5-9 %. The minimum reduction was found in the group where only one *B. bengalensis* was kept *i.e.* 23-33 %

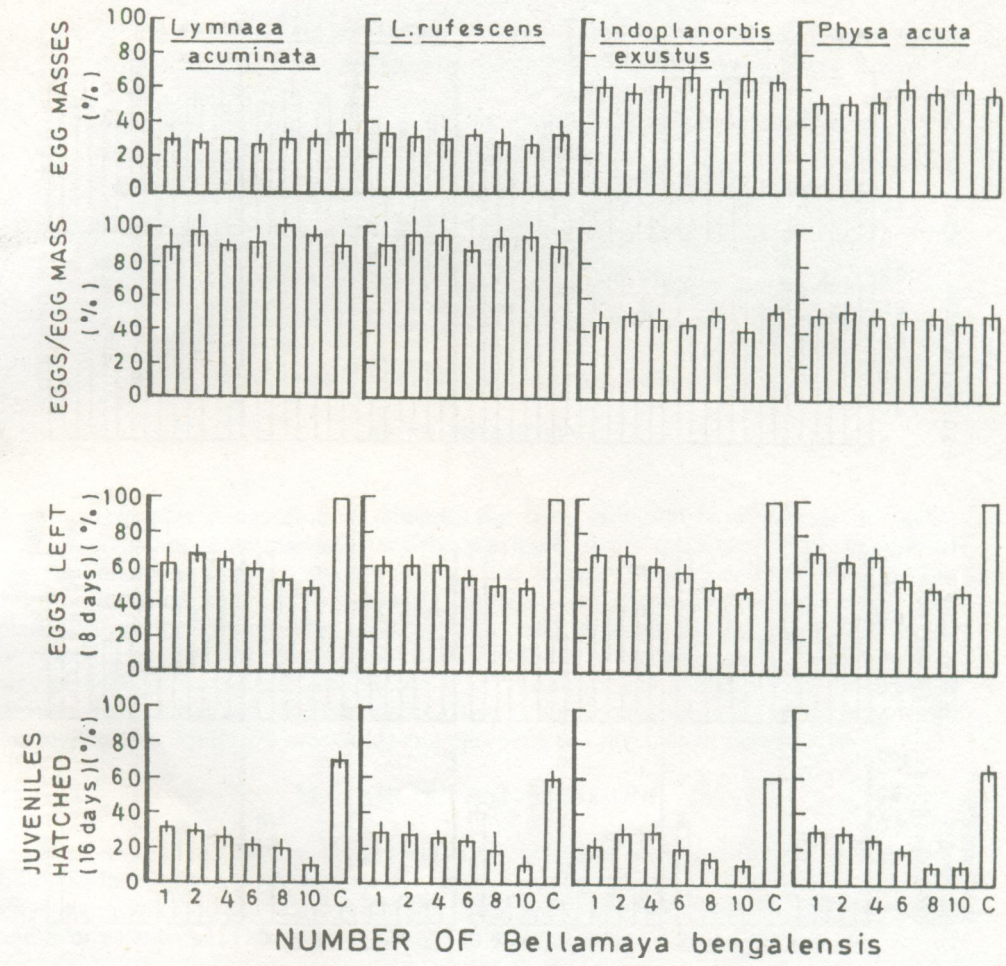


**Fig. 1.** Fate of eggs of *Lymnaea acuminata*. *L. rufescens*, *Indoplanorbis axustus* and *Physa acuta* in the presence of well fed adult *Bellamaya bengalensis*. Values given are mean±S.D. of two replicates of each group for 16 days.

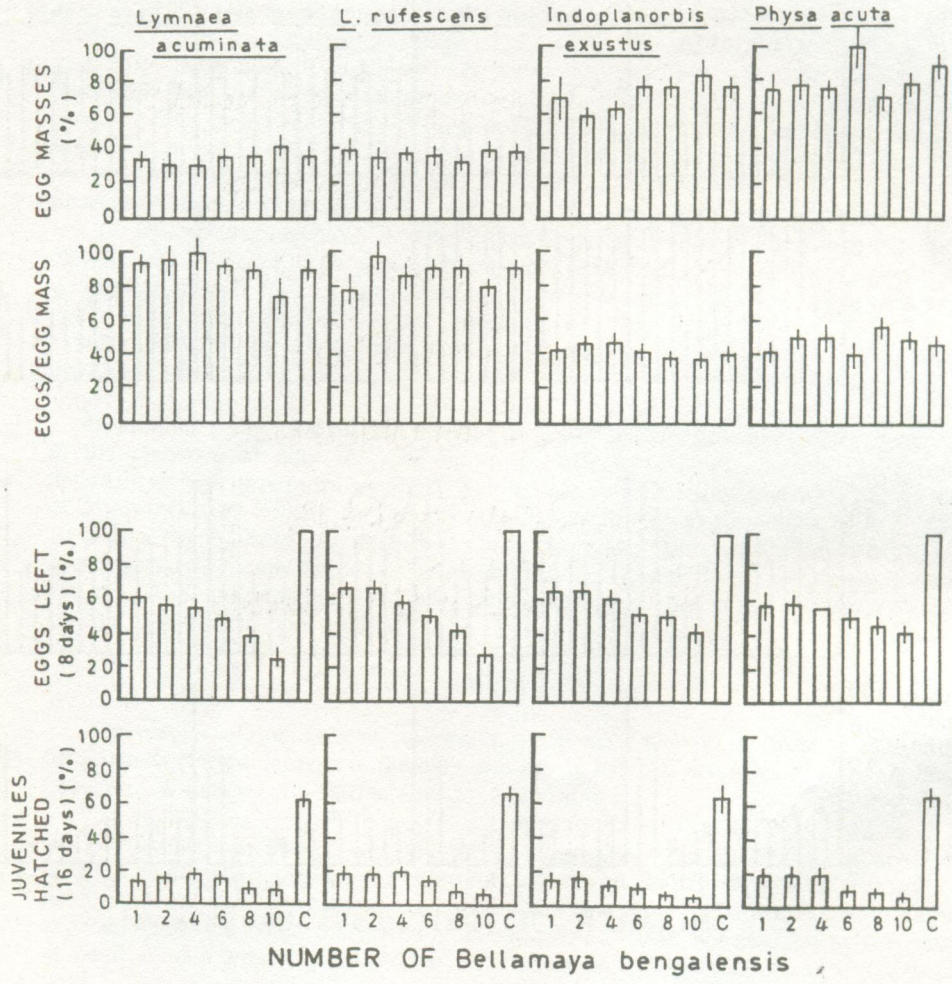


**Fig. 2.** Fate of eggs of *Lymnaea acuminata*. *L. rufescens*, *Indoplanorbis axustus* and *Physa acuta* in the presence of unfed adult *Bellamaya bengalensis*. Values given are mean±S.D. of two replicates of each group for 16 days. .

**Table 1.** **Fate of eggs of *Lymnaea acuminata*, *L. rufescens*, *Indoplanorbis exustus*, and *Physa acuta* in the presence of juvenile *Bellamaya bengalensis*. Values given are mean ± S.D. of two replicates. Food supplied is indicated by + and food not supplied by - .**

| Eggs | Juveniles | Food | Total No. of egg masses | Total No. of eggs | Eggs left after 8 days (%) | Total No. of juveniles | Juveniles left (%) |
|---|---|---|---|---|---|---|---|
| *L. acuminata* | *B. bengalensis* | + | 6.5±0.707 | 421.5±10.60 | 56.67±12.16 | 20.0 | 92.5±3.53 |
| *L. acuminata* | *B. bengalensis* | - | 5.5±0.707 | 371.5±4.95 | 7.79±2.18 | 20.0 | 85.0±7.07 |
| *L. acuminata* | - | + | 7.0±0.0 | 435.0±7.07 | 98.45±0.90 | - | - |
| *L. acuminata* | - | - | 6.5±0.707 | 451.5±12.02 | 88.55±5.33 | - | - |
| - | *B. bengalensis* | + | - | - | - | 20.0 | 100.0±0 |
| - | *B. bengalensis* | - | - | - | - | 20.0 | 92.5±3.53 |
| *L. rufescens* | *B. bengalensis* | + | 5.5±0.707 | 315.0±7.07 | 61.18±6.53 | 20.0 | 97.5±3.53 |
| *L. rufescens* 4 | *B. bengalensis* | - | 6.5±0.707 | 460.0±1.14 | 7.82±3.05 | 20.0 | 80.0±14.1 |
| *L. rufescens* | - | + | 6.5±0.707 | 394.5±6.36 | 97.72±0.35 | - | - |
| *L. rufescens* | - | - | 5.5±0.707 | 465.0±21.21 | 88.26±4.02 | 4.02 | - - |
| - | *B. bengalensis* | + | - | - | - | 20.0 | 97.5±3.53 |
| - | *B. bengalensis* | - | - | - | - | 20.0 | 92.5±3.53 |
| *L. exustus* | *B. bengalensis* | + | 9.5±0.707 | 331.0±14.14 | 53.82±9.35 | 20.0 | 97.5±3.53 |
| *L. exustus* | *B. bengalensis* | - | 8.5±0.707 | 374.5±7.78 | 8.58±3.58 | 20.0 | 92.5±3.53 |
| *L. exustus* | - | + | 10.5±0.707 | 360.0±0 | 97.13±1.74 | - | - |
| *L. exustus* | - | - | 9.5±0.707 | 399.5±13.43 | 95.29±3.03 | - | - |
| - | *B. bengalensis* | + | - | - | - | 20.0 | 97.5±3.53 |
| - | *B. bengalensis* | - | - | - | - | 20.0 | 92.5±3.53 |
| *P. acuta* | *B. bengalensis* | + | 11.0±1.41 | 364.5±21.92 | 53.41±2.99 | 20.0 | 100.0±0 |
| *P. acuta* 1 | *B. bengalensis* | - | 12.5±0.707 | 359.0±14.14 | 26.1±2.80 | 20.0 | 92.5±10.6 |
| *P. acuta* | - | + | 12.5±0.707 | 377.0±4.24 | 98.01±0.92 | - | - |
| *P. acuta* | - | - | 11.0±1.41 | 352±12.02 | 90.03±3.51 | - | - |
| - | *B. bengalensis* | + | - | - | - | 20.0 | 97.5±3.51 |
| - | *B. bengalensis* | - | - | - | - | 20.0 | 97.5±3.51 |

for those in which food was provided while the similar values were 15-21 % for the groups in which food was not provided, while the other values lie between these two extremes. Having a look on Table I it is clear that juvenile *B. bengalensis* caused damage to the egg masses of *L. acuminata*, *L. rufescens*, *I. exustus* and *P. acuta* (during the 8 days) the snails left in the pots were between 50-62 % when the food was supplied in maximum. However, these values reduced to 8-27 % when food was not supplied. Among the four starved, competing snails maximum reduction took place for *L. acuminata* and *L. rufescens*, then *I. exustus* and minimum reduction took place for *P. acuta* eggs (Table I). Juveile and adult *L. acuminata*, *L. rufescens*, *I. exustus* and *P. acuta* showed no tendency to destroy their own egg masses or juveiles, even in the absence of food. The negligible mortality faced by these groups was due to mechanical interference which they made in search of food. However, when the time for hatching of juvenile (from the eggs) of the competing snail species was noted, and compared with the control groups, there was found no difference.

## DISCUSSION

The main objective behind this study was to observe the fate of eggs and juveniles of medically important pulmonate snail species in the presence of gradually increasing number of well fed and starved *B. bengalensiş*.

As for as the fate of egg masses of *L. acuminata*, *L. rufescens*, *I. exustus* and *P. acuta* is concerned it was noted that not only the egg masses of these snail species were predated or destroyed by adult and juvenile *B. bengalensis* but the detrimental effect of *B. bebgalensis* was also evident on the newly hatched juveniles of the above snail species. While the effect of these snail specied (Lymnaeid, Planorbid and Physid) on the juvenile and adult *B. bengalensis* was not found. Almost similar findings have also been reported by other workers like Abdallah and Nasr (1973) showed the effect of *Helisoma duryi* on *B. glabrata*, Madsen (1979b) showed the effect of *Pomacea* spp. on *B. glabrata*, Malek and Malek (1978) explained the effect of *H. duryi* on *Biomphalaria cumerunensis*). It was further observed during the present investigation that this predation increased when food was in short supply and egg masses thus seem to be used as an alternate food source. The absence of food probably increases the searching activity of snails thereby making the incidental passage over egg masses more frequent. Storey (1971) was of the opinion that the rate of movement of *L. peregra* was three times faster on surface without food than on surface with food, when no alternative food source (*i.e.* algal growth on the sides of the plastic bowls) was available for the snails.

It was also noted that *B. bengalensis* did not effect its own juveniles by its presence in the absence of food. This may be due to the fact that juveniles of *B. bengalensis* can escape any predatory activity by actively moving away from the adult snails, while the egg masses of *L. acuminata*, *L. rufescens*, *I. exustus* and *P. acuta* are helpless creatures so they have to face the mechanical injuries caused by the movement of adult snails. It is also evident from Table I that in the control groups of competing snail species less than 70 % eggs develop into juveniles while others died or destroyed due to unknown reasons. The predation on egg masses was considered to be a solid reason for the suppression of reproductive potential of *B. camerunensis* in the experimental aquaria

(Madsen and Frandsen, 1979; Madsen, 1979)). They furter reported that water pollution (*i.e.* metabolic waste products from snails and bacterial activity and various chemical substances leaching from lettuce or other food sources was also held responsible as an important inhibitor for the development of egg masses in older balanced aquaria.

Mandahl-Barth (1970), Abdallah and Nasr (1973) and Malek and Malek (1978) suggested that excretions or secretions from Helisomes also affect the reproductive potential of *B. glabrata* but finding of Madsen (1979) did not provide evidence of inhibitory substances secreted by *H. duryi*. However, during the present investigation chances of fouling the aquaria were negligible because of the carefully routinized schedule of food and water change every day.

In view of above mentioned results, although prosobranch snail *B. bengalensis* suppresses the population of pulmonate snails by predating their egg masses and juveniles (under laboratory conditions) however, the significance of such predation for the competitive relationship between *B. bengalensis* and medically important pulmonate snails under field conditions in not known because in the field it is important whether food is a limiting facter for these snails or not and also upon specific trophic level of the species involved. It is also precautionary to note that the results of laboratory experiments should not always be indicative of what happens in natural habitat.

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# AIDS: STATUS OF OCCURRENCE AND MANAGEMENT IN PAKISTAN

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CONTENTS



**Abstract**: Acquired immune deficiency syndrome (AIDS) was first recognized as a clinical condition in 1981 and was thought to be confined to a specific population group - homosexuals. However, since then AIDS has received great media coverage which has revealed that it is both a homosexual and heterosexual disease with a fatal outcome. It is known that the disease spread from central and western Africa to the Caribbean eventually manifesting itself in the United States and Europe. In Asia, the disease still remins a taboo and is not well documented. Due to this reason that the spread of the AIDS virus remains un-checked in many Asian countries. According to WHO, the AIDS virus has infected over 2.5 million individuals throughout the world. The HIV (Human immunodeficiency virus) epidemic in Asia is growing at an alarming rate; in Thailand the estimated cases of infection have risen ten-fold and in India it has tripled since 1992. Thus it is estimated that by the end of the century more than 10 million Asians will be infected with HIV. In Pakistan, three social taboos are driving the spread of disease, effectively blocking prevention and care - denial, discrimination and disempowerment. Today a very conservative estimation of HIV infections in Pakistan, as reported by the National Institute of Health (NIH), Islamabad, stands at 850 HIV-positive cases, of those 46 developed AIDS and have since died. Compared to the figures released by the same organization a year ago there were 733 HIV cases - an increase of 123 cases in a matter of one year. These estimates do not reflect the true scenario. If the first case of HIV was detected in 1987, as is presently thought then the estimated cases of HIV could be around 10,000-20,000 persons, based on epidemiological parameters. However, if there were cases before 1987 but not diagnosed as HIV/AIDS cases - which is not unreasonable to presume - then based on the statistical parameters the estimates could be much higher - closer to 50,000 - 100,000. In 1990, India officially had 2,700 HIV positive casess; by 1993 the official figures rocked to 500,000 while the actual estimates hover around 2.7-3.5 million. According to an AIDS researcher, Pakistan is at the same stage at which India was three years ago, which necessitates immediate action on strategies for the prevention and control of HIV/AIDS. This includes universal precautions; compulsory testing of all donated blood; surveillance; comprehensive training of health workers; safer sexual behavior; sexually transmitted diseases STD management; condom procurement and distribution; counseling and rehabilitation of HIV infected individuals; management, monitoring and evaluation of the program. The largest hurdle of resistance that such a campaign can encounter is that from the nation's clergy. Such a reaction should be treated with patience and candidness and the clergy should be convinced that such a campaign is necessary to combat this disease and that any misleading information will have a detrimental effect on the whole nation.

**Key words**: AIDS, Pakistan, Status.

## INTRODUCTION

When AIDS was first recognized as clinical entity in 1981, it was thought to be a disease present only in the United States of America and among a population group, namely homosexuals; hence the first suggested name GRID (Gay-related immune deficiency). It was realized soon after that the disease was not restricted to USA alone,

but cases were reported in other countries in Europe, south and central America in 1982 and 1983.

The term AIDS describes only the most serious form of an infection which is caused by a specific virus called the human immunodeficiency virus (HIV). The viral origin of this disease was disccovered in 1983/84, simultaneously in the USA and France. In 1984, Gallo and co-workers described numerous isolates of a retrovirus from homosexual and hemophiliac patients with AIDS and AIDS related syndromes. Subsequent work by a number of investigators has named the AIDS related retroviruses as type-1 (HIV-1). More recent work on HIV has shown the existence of simian immunodeficiency viruses $SIV_{sm}$ and $SIV_{mac}$. Lymphadenopathy associated virus-2 now appears to be a member of a distinct family of human lentiviruses called HIV type 2 (HIV-2).

Between 1985-89 there was considerable media coverage about AIDS to create the much needed awareness, thereby becoming the focus for priority attention by politicians, public health workers and eventually the general public. Now there is conclusive evidence that AIDS is a new disease - the disease of the 90s - with a fatal outcome, and that there are no specific drugs for it.

In recent history, there has been no disease which has appeared so rapidly and caused so much anxiety and dread over the entire globe. Moreover, it is not known how and when the disease reached man, or what will happen to humanity, over a long perios of time due to AIDS, if it is not conquered.

No one knows how the infection spread to man, but it seems to have occurred in Africa in the area south of the Sahara desert, it spread to the rest of the world was probably via Haiti and thence carried by the tourist to the United States in the 1970s. As with any viral illness in an unprotected population the disease has spread rapidly and fatally.

HIV/AIDS virus is a retrovirus that infects T-cells (CMI) and macrophages having RNA genomes only with reverse transcriptase and a protein capsid with a bi-lipid layer with receptors sites. RNA injected into the T-cells with the help of envelop gene enzyme and RNA reverse transcriptase with the help of cell metabolites, forms the ground work gene strands of virus that enters the nucleus and initiates the synthesis of mRNA (protein synthesis), and genomic RNA. Eventually, the viral proteins assemble at the cell surface and organization of the virus occurs at this site; the virus is then pinched-off. Mode of action by the virus could be described as: early action latency pattern.

*Early action*: CD4 is the receptor protein of HIV located on the macrophages and T-cells. However, CD4 is not the sole requirement. Some CD4 free cells can be infected, hence receptor mediated infection in not the only method of viral infection. Atrocities of B-cell, skin cells, bowl cells etc. could be infected by HIV.

*Latency pattern*: HIV virus has developed a latency pattern as well to lengthen the age of the host which can be as long as 12 years, but the host is still a carrier. Later on,

the virus enters the lytic cycle/productive stage; eventually the virus kills the host. HIV attacks both activated and inactivated cells. Activated cell infection leads to rapid viral growth, host cells are killed and a full grown infection results. Inactivated cell infection leads to latent periods, "Post-integrated latency" and "Pre-integrated latency."

Macrophages are reservoirs of viral particles. Building-off of virus within the cell can occur. A complete virus can be maintained within the macrophage and the virus does not kill it.

*Spread of viral infection*

HIV-1 infection has been reported throughout the world in both developed and developing countries and is found predominantly in homosexual and bicexual men and intravenous drug users. Hemophiliacs, transfusion recipients, sexual partners among infected persons, and infants born to infected mothers are also at high risk. In many parts of Africa and the Caribbean, HIV-1 is found predominantly in heterosexual, transfusion recipients, and infants born to infected mothers. HIV-2 is found predominantly in west Africa, Portugal and Brazil.

However, both HIV-1 and HIV-2 are spread through sexual contact, exposure to contaminated blood or blood produsts, and from an infected mother to her offspring. In 1990 it was estimated that between 800,,000 and 1.3 million individuals in the United States were infected with HIV, it is not known what proportion of these individuals will develop AIDS. The average incubation period has been worked out to be approximately ten years.

*Clinical manifestations*

During the initial phase of infection with HIV there is an acute syndrome with symptoms which include fever, sweats, myalgia or arthralgia, sore throat, lymphadenopathy, nausea, vomiting, diarrhea, headaches and rashes. During the acute infection there may be a decrease in the number of circulating T4 lymphocytes. Later on, there is a long and variable asymptomatic period of infection. During this period there may be a slow, progressive decline in T-4 cell numbers and an inccrease in T8 cells. Some individuals may, however, maintain a constant, normal level of T4 cells during the asymptomatic course of the disease.

AIDS is a late outcome of infection with HIV which is a disease characterized by a marked depletion of T4 cells, resulting in a reversal of the T4/T8 cell ratio; normally 1.5:1 to 2.0:1. The ever increasing immune deficiency renders the patient vulnerable to a wide range of life-threatening infections and neoplasms, the most common being *Pneumocystis carinii* pneumonia and Kaposi sarcoma. Another manifestation of HIV is the AIDS-dementia complex (AIDS encephalopathy). This manifestation is characterized by neurologic abnormalities, progressive dementia and peripheral neuropathy. This may occur in the absence of opportunistic infection.

*Transmission of HIV*

It is transmitted by both homosexual and heterosexual contact. In the case of infected males the virus occurs in the semen. It also occurs in the blood of infected males and females. During sexual contact, the virus gains entrance to the blood stream of an uninfected individual, male or female, via the microscopic breaks in the mucous membrane lining of the genital tract or the rectum in case of male homosexuals. This virus can also be transmitted by residual contaminated blood on hypodermic needles and syringes that are shared among intravenous drug users. It is important to note that not only those who use so-called street drugs, but also those who use injectable substances such as steroids and insulin, can become infected if they share needles and syringes. HIV can aslo be transmitted by blood transfusions with comtaminated blood. Another important route through which HIV can be acquired is from a HIV positive mother to her child. Not all children born to infected mothers become infected. Viral transmission can take place either before birth, or during the process of birth through exposure to mother's blood or other infected fluids. A few cases indicating HIV trasmission through breast milk are also on record.

HIV is mostly present in semen, vaginal secretions and blood of infected individuals. It is also found in the saliva, tears, urine and the feces of the infected individuals. However, HIV virus cannot be acquired through casual contact involved in daily life, such as hand-shaking and coughing. Besides this, transmission of the virus from an AIDS patient to healthy individuals through the sharing of food, towels, cups, razors and even tooth brushes has not yet been documented.

Among doctors and nurses, who have been exposed to HIV infection, although very rare, they have had accidental infection through pricks of contaminated needles and the sudden splash of blood. Nevertheless, health care personnel are at a higher potential risk than the general population, therefore, `universal precautions' are now available for the health care workers, and should be practiced while taking care of this kind of patient. The universal precautions are being reproduced for the readers later on in this paper.

*Can HIV be transmitted via insect-bites?*

It is known that small amounts of residual infected blood on contaminated hypodermic syringes and needles can transmit HIV among drug users. Mosquitoes and other arthropods that also suck blood, hypothetically can play a similar role and can be safely claimed as "flying syringes". They could carry the HIV virus from an infected person and inoculate it into healthy individuals. Although, theoretically the possibility does exist that insects may transmit the AIDS virus, in practice it is now evident that HIV cannot be transmitted through mosquito or other insect bites.

In laboratory studies, researchers at the centers of disease control (USA) have shown that even in case of mmosquitoes injected with HIV-contaminated blood, the virus survived for only one hour in the insects and does not multiply either in the mosquito or in the tissue cultures of mosquito cells. Moreover, current efforts to transmit the virus from the insects to human cells in the laboratory have been

unsuccessful.

*Global AIDS situation*

According to WHO Global Program on AIDS, it is estimated that in south Asia and south-east Asia, HIV infections are now over 2.5 million, one million more than just a year ago. Furthermore, the HIV epidemic in Asia is growing at an alarming rate, for example estimated infections in Thailand have risen ten-fold since early 1990 and in India they have tripled since 1992. According to WHO estimations, by the year 2000, more than 10 million Asians will be infected with HIV, if this trend continues. Worldwide, over 6,000 people are becoming infected with HIV everyday, more and more of them in Asia.

WHO further estimates that another 3 million men, women and children have been infected with HIV. The total thus now stands at over 17 million.

Three societal taboos are driving the spread of the disease and are effectively blocking prevention and care - denial, discrimination and disempowerment. It is clear that until we overcome these social prohibitions we will never bring this deadly epidemic under control. Discriminatory laws and practices must be over ruled. Political leaders must take courage to provide leasership in disease control measures.

There is now global consensus that with sufficient resources and political will, millions of new infections can be avoided. Education is the foundation of change, hence it is essential to educate the people not only about HIV and AIDS but also on other health promoting basic and social information. Starting points for health education should be schools, colleges, universities and, last but not the least, remote areas where illiteracy rules supreme. This is further true of Pakistan where literacy rate is meager 26%.

WHO calculates that implementing the basic prevention programs in Asia would cost between US$ 0.75-1.5 billion. This cost appears to be too high for developing countries, but cost should not be used as an excuse, since it represents less than 0.03% of Asia's total economic output. An investment of this kind would prevent an estimated 5 million new infections by the year 2000. The only possible conclusion from these figures is to act now, before it's too late!

*AIDS in Pakistan*

The first case of the disease (AIDS) in Pakistan was reported in 1987. During 1986-87, 1,363 subjects were screened for HIV infection in Karachi, two were confirmed positive. These two were married females who had received multiple transfusions and denied other risk factors. Another three confirmed cases of HIV infection were recorded in a group of 413 individuals screened from Karachi in 1990; two were foreign nationals from Tanzania and Uganda and the third was a Pakistani residing in Saudi Arabia who had received multiple blood transfusions following a car accident in 1981. There is great paucity of AIDS data and studies in Pakistan, only a few publicatios are available so far.

There seems little doubt that the AIDS horror has finally hit home, with the steady rise in the number of people testing HIV positive in Pakistan, and the Gulf states cntinuously deporting AIDS infected Pakistani workers. Over the last few years, in fact, the magnitude of the deadly virus has suddenly spiraled into a frightening statistical countdown. HIV estimates, meanwhile, have crossed such dangerous levels that the usually tight lipped officials at the National AIDS Prevention and Control Program have been forced to concede that the number of infected cases is much higher than they had ever acknowledged before.

It was the most embarrassing situation for the officials of National AIDS Prevention and Control Program when members of the College of Physicians and Surgeons challenged their figures on the state of AIDS in Pakistan in a seminar held in December 1993, before they had even finished presenting their report. Representatives of Agha Khan University Hospital in Karachi claimed that they themselves had confirmed 56 cases of infection with HIV, while another 21 cases had tested positive but awaited confirmation. A Pakistani doctor working in UAE pointed out that the Emirate has deported a batch of 353 Pakistanis who tested positive for HIV. This figure alone is more than the 251 HIV positive and 36 full blown AIDS cases that the National AIDS Program (NAP) had acknowledged in more than six years of operation since its inception in August 1987. Such controversies over the recorded figures do prevail and require investigation.

In January 1994, the official figures of HIV and AIDS cases for December 1993 (251 HIV + 37 AIDS) were revised to 733 cases of HIV and 38 cases of full blown AIDS. Where did these new cases come from in a period of less than one month? There are no breakdowns given by the reporting agency. Nothing is reported about where these cases were detected. If they actually reflect cases confirmed over one month, then this is a monumental increase. If these cases existed before, they seemed to have escaped NAP's attention. One may again wonder if the new figures reflected reality.

The presently projected figures paint a frightening picture. They seem to indicate that the AIDS menace is finally taking-off in Pakistan. Compare these figures with March 1990, when only 33 people had been confirmed as HIV positive while 13 had developed AIDS. This clearly refutes the myth of non-existence of AIDS in Pakistan and if at all it existed, it was limited to foreigners and those Pakistanis who have been infected abroad. By showing the existence of indigenous cases (those who have contracted the virus within the country) the reality of AIDS has now been confirmed in Pakistan, even though the Government still continues to deny the existence of the disease in the country.

According to the official statistics, more than 250,000 individuals have been screened for HIV-1 infection in Pakistan and as of January 1995 a total of 850 cases of sero-positivity have been detected. Of those 46 who developed full blown AIDS, 45 have since died and the fate of the remaining one is unknown; presumably dead.

Even if the National Institute of Health (NIH) projections are correct, there is a lot of evidence from around the world that they represent the launching pad for a devastating epidemic. For example in 1990, India officially had about 2,700 cases of

HIV. By 1993, the official figure had gone up to 500,000 while the estimates of actual cases now range from 2.7-3.5 million. In Bombay alone, where over one third of the prostitutes are infected, there are an estimated 1000 new infections every 24 hours. Proportionate to population, according to an AIDS researcher, Pakistan is at the same stage at which India was three years ago. This necessitates immediate action on a war footing.

In Pakistan, the practice of screening of blood products for HIV infection is practically absent. Only a few large medical centers in the country are screening blood products. It is essential to screen all blood products, the risks in Western nations bave been minimized by screening samples of donated blood. We are still awaiting for by-laws and mechanisms to implement blood screening on a large scale. If immediate action is not taken perhaps it will be too late for controlling this dreaded disease.

*Scale of AIDS in Pakistan today*

No one can be sure of the scale of AIDS in Pakistan today. Studies that have been conducted can only help in making estimated projections. According to Toor (1995) there have been a total of 850 HIV-positive cases, of those 46 showed signs of full-blown AIDS; of these 45 have died and one has disappeared. Of the 46 full-blown AIDS cases, 13 were reported from the Federal territories, 11 from Punjab, 10 from NWFP and Tribal Area, 8 from Sindh and 4 from Baluchistan. The NIH has compiled this information from various sources, using the `unlinked anonymous survey'. The known sources are the Agha Khan University in Karachi, a few blood screening centers and the studies conducted by the researchers at NIH. Of course there will always be difference in the actual cases and the ones reported. From the available data it can be concluded, however, that there is a steady rise in the occurrence of HIV-positive cases as well as full-blown AIDS cases in Pakistan. According to EPI, an international model used to determine the estimates in Pakistan, the threat and the magnitude of the AIDS epidemic in January 1994 was 733 HIV cases and 40 full-blown AIDS cases. In a matter of one year there has been increase of 123 HIV-positive cases and that too according to the official reports. Using the EPI with these figures, the estimated cases could be around 10,000-20,000 persons, and in actuality the number could easily be ten times greater than what is gradually estimated.

If the first case was detected in 1987, as is presently thought, then the aforementioned estimate appears to be relatively accurate. However, if there were cases before 1987 but were not diagnosed as HIV/AIDS cases, then, based on the statistical parameters the estimates could be much higher.

The prevalent mode of transmission of the HIV virus in Pakistan is the same as in Western countries, *i. e.* sexual intercourse - heterosexual, followed by bisexual and homosexual intercourse, blood transfusion using contaminated blood and vertical transmission from mother to infant. Recent studies conducted during November-December 1993 have compiled data from 30 centers around the country and reported on the disease from urban areas and targeted the incidence of HIV/AIDS in high-risk groups. Many of those tested were, however, from rural areas and have had moved to the cities in connection with their jobs. The high-risk groups which were included in

these studies were, sexually transmitted disease (STD) patients, prostitutes, truck drivers, intravenous drug users, trans-sexuals, prisoners and TB patients.

Cumulative records concerning the occurence of HIV/AIDS, as reported by various surveys from 1987-95, covering major urban areas has revealed the existence of infection. In Lahore, 2-3% of the prostitutes and 2-6% of the homosexuals; in Peshawar 1.6-2.0% among STD patients and 5.0% among those who received blood transfusions; in Rawalpindi 2% among truck drivers have tested positive for HIV. Of the 38 total cumulative AIDS cases, 12 were reported in 1993, 11 of whom were clinically diagnosed by a trained doctor in Islamabad. In January 1995, this figure rose to 46 full-blown AIDS cases and over a wider area throughout the country.

A recent report from the UAE claimed that of the 660,000 Pakistani nationals working in the UAE when screened for HIV, 353 were found to be positove. This signifies the possible role of these Pakistanis in the spread of the disease in their homeland after repatriatiion from abroad. A similar situation is also possible with Pakistanis working in other Middle Eastern countries as well as in the East and West.

With the revelation of the above information the Government of Pakistan has given AIDS prevention the top priority it deserves and has considered immediate action to keep the general population informed of the nature of the infection and its mode of transmission.

Experts have also identified prostitutes and their clients, drug users, immigrant workers, STD patients, truck drivers, homosexuals, prisoners and the youth as the groups most vulnerable to HIV infection.

*Strategies for HIV/AIDS prevention and control in Pakistan*

The government, after national and international consultations and recommendations by different experts, has provided strategies ober a broad spectrum to control and prevent the spresd of this epidemic. Some of the precautions are now universally approved and need no verification. These are being presented in the following pages:

*Universal precautions*

These are recommended by the Centers of Disease Control (July 1991).

*a*. All health care workes should adhere to universal precautions and comply with current guidelines for disinfection and sterilization of devices reused in invasive procedures; *b*. Exposure-prone medical and dental invasive procedures be identified; *c*. Health care workers should know their HIV status; *d*. Infected health care workers should not perform exposure-prone, invasive procedures unless permitted to do so by the experts; e. Patients should be told of the health care workers' HIV status before performance of any exposure-prone procedure; *f*. Always use gloves when treating bleeding patients, when drawing blood, and for procedures involving contact with mucous membranes or potentially infectious material; wear masks, protective eyewear, and gowns; *g*. Wash hands and skin immediately if contaminated after removing

gloves, and always before taking care of another patient; *h*. Prevent injuries caused by needles and scalpels. Needle-stick exposure is the main route of transmission of HIV to workers. Needles should never be recapped, removed from didposable syringes, or bent or broken by hand. All sharp items should be placed in a puncture-resistant container; *i*. Minimize mouth-to-mouth resuscitation; use a protective device; *j*. Workers with weeping lesions should refrain from direct patient care and handling patient-care equipment; *k*. Pregnant health workers must be familiar with and must strictly adhere to precautions. The infant is also at risk of infection if the mother is infected.

*Prevention of transmission through contaminated blood*

This is a priority for Pakistan as it is one of the few countries that has has not instituted a safe blood transfusion system. Therefor, a comprehensive safe blood transfusion system based on recruitment of screened donors, rational use of the blood and the screening of all blood - blood products - before transfusion/use should be provided. Attention is essential to ensure aseptic conditions in all health care facilities as well as application of universal precautions of `sterilization' of all skin piercing instruments. The habits of drug users should be closely monitored to assess the damage and appropriate strategies aimed at reducing the harm be developed.

*Surveillance*

The system of Sentinel Surveillance, alresdy established to monitor the epidemic, should be strengthened. Reporting of AIDS cases with an accent on counseling and care of opportunistic infections ought to be instituted.

*Comprehensive training package*

The need for training of health workers has been recognized in all areas. A package consisting of programs to provide knowledge on the under mentioned areas should be developed.

*a*. Modes of transmission of HIV and prevention methods; *b*. Clinical diagnosis; *c*. Clinical management, nursing care, counseling and home care; *d*. STD diagnosis and management; *e*. Provision of safe blood; *f*. Universal precautions.

Training programs should be carefully planned with a facility to monitor the results in terms of `action' to be performed by the health worker.

*Safer sexual behavior*

It is essential to disseminate HIV/AIDS/STD related information on the media to reach the general population as quickly as possible, requesting responsible behavior. Focussed and targeted attention should be given to special population groups such as prostitutes, frequent travellers, drug users, migrant workers and the youth.

*STD management*

A comprehensive STD case management program based on a syndromic approach should be introduced throughout the country using such specialized services, as MCH/FP, gynecology and dermato/venereology. Private practitioners and basic health care workers should also take part in such a program. It should also include the promotion of health care seeking behavior of the public.

*Condom procurement and distribution*

Distribution channels adequate for current needs and additional distribution channels needed to be established, which are specially targeted to high risk population groups.

*Counseling and rehabilitation of HIV infected individuals*

Facilities should be provided which will be accessible to the people in all major cities, and provincial centers for the treatment of opportunistic infections (TB, *Pneumocystis carinii* pneumonia, oral thrush, diarrhea). These will provide clinical management, nursing care, counseling and home care of AIDS patients.

*Management*

Task forces at the federal level may continue to assess the status of the epidemic and program implementation. The Federal committee on AIDS and its technical sub-committee may continue to provide advice and direction. The staff and the facilities of the National AIDS program at the center and provinces need to be strengthened. Provincial task forces may be established with the same terms of references as those of the Federal task force.

*Monitoring and evaluation of program*

All activities and programs as a whole may be monitored and evaluated through the use of indicators and targets. An appropriate survey may be used for the collection and tabulation of the information.

*AIDS infections soar in Asia due to fear and denial*

While the world's leaders continue to deny the magnitude of the threat of HIV, public health experts have adamantly warned the Asian countries that there is little hope of controlling the AIDS epidemic. Arguments have to be put forth that even modest spending could avert millions of new infections in regions where the virus is now rapidly spreading. According to WHO's global program on AIDS despite all the efforts by the health educators and researchers, more people were infected with the HIV virus in the past 12 months than any previous years. WHO suggests that it is denial which keeps society's leaders from taking the pandemic seriously and investing the much needed resources. Secondly, discrimination by governments against HIV-positive people, for example in mandatory testing, is futile and often harmful to true prevention.

Thirdly, the most vulnerable people are deprived of the information and power to protect themselves from infection.

Governments need to invest in prevention and research for new drugs and a vaccine, and fill the colossal gaps in health services that leave many of the world's people with AIDS without care. Political leaders must find the courage to provide leadership and all discriminatory laws must be abolished.

In rare cases, where AIDS is taken seriously, the effects are measurable. In Thailand, for example, reported cases of sexually transmitted diseases has dropped more than 75% over the past 7 years. Epidemiologists believe the fall is the result to intensive HIV education in groups at risk and the mass promotion of condoms. Thailand is one of the few Asian nations putting impressive national resources into AIDS prevention and it is getting excellent results. Other nations should also follow this pattern.

The Office of AIDS Research at the UN is of the opinion that persuading people to use condoms is not the whole answer. Vaccine production is also critical in the prevention of the spread of AIDS and vaccine production remains a high priority. The AIDS research office firmly believes that the only way to eradicate AIDS is by vaccines. The research includes the possibility of using live, attenuated forms of HIV to protect people from the virus. Critics fear the dangers of such a vaccine, but the benefits seem to outweigh any of the risks involved, especially in areas where HIV is spreading rapidly.

One key question that researchers are facing is why some people with HIV remain healthy for more than a decade, while others develop AIDS. A team of scientists at the National Institute of Allergy and Infectious Diseases USA, have offered some explanations. The team has already shown that the lymph nodes of HIV-positive people are packed with HIV particles even when none can be detected in their blood. The virus appewrs to destroy the lymph tissue then overwhelms the immune system. The team then studied a small number of people who were still healthy more than ten years after becoming infected. They showed no sign of the disease, nor did they show the characteristic decline in the number of T-cells that signals disease. Studies of their lymph nodes brought an even greater surprise; the tissue was `perfectly normal.' In some cases, this was explained by a defective virus causing the infection, but often the virus was capable of replicating quite normally.

The work of this team has overturned earlier ideas about the kind of immune response that protects against disease. For example, their studies showed that no particular group of T-helper cells is linked with staying healthy or progression to disease. Those who stayed healthy for a long time had high levels of antibodes to the virus, once thought to indicate a failing immune system.

*Conclusions*

The real magnitude of the AIDS threat in Pakistan is still a dilemma. The official figure is 850 HIV-positive cases. Actually this figure could easily exceed 1000 and this

too may be multiplied 100 times over because each of the infected individuals can come in contact with any number of people within various parts of the country. Because there is no absolute treatment and cure for AIDS, a HIV carrier can transmit the virus to another person and that victim onto another for the rest of his life.

The government launched an AIDS awareness program in March 1994 but despite such efforts, the plans to curb the spread of the disease have fallen short of what they should have achieved in the last five or six years.

Another reason for the rapid spread of AIDS is the ever increasing population of Pakistan. A lot of people come to Pakistan from abroad and there is an increase in illegal immigration from countries where AIDS is a full-blown menace. These immigrants belong to communities which are not connected with any established cultural society and where the social norms about interaction between men and women are not as strict as in more settled societies.

To prevent such an uncontrolled and devastating spread of the disease, the government should create greater awareness using the electronic as well as the print media. The present government in January 1994, began to introduce a much stronger and more intense campaign to promote awareness among the masses, not only through the television and radio, but also via the print media by distributing brochures and handouts. This information should be made availabe to hospitals, colleges, dispensaries, medical shops and research institutes. In addition seminars, talks, symposia and review papers should be held and published at regular intervals so as to update all the information regarding the spread, and prevention of HIV/AIDS.

Furthermore, the people should be told the specific modes of transmission of the AIDS virus and be made aware of the fact that an AIDS patient should not be shunned but encouraged by the society to live in and share his/her feelings regarding the disease and educate the youth on the preventive measures that should be exercised. In addition the various high-risk groups should be targeted with different approaches. The campaign has to be shaped in such a way that it reaches out to the general public. For example the radio is the most far reaching electronic media in the country. Hence, Radio Pakistan should devise an AIDS theme, be it in the form of a song, poem, or documentary and have a few famous celebrities present it on the air so as to attract even more listeners.

The largest hurdle or resistance that such a comprehensive campaign can encounter is that from the nation's clergy. Well if there is a reaction against the candidness of the campaign by the religious leaders, then they should be made to understand that such a campaign is absolutely necessary to combat this deadly disease and any deliberate misinformation about HIV/AIDS will result in ignorance about the dangerous consequences of not only the disease, but also the fact that if it is not dealt with immediately the whole nation could be at great risk. We, as a nation need to take the clergy into confidence and need to work together to stop the spread of AIDS, by promoting the use of condoms, screening all blood products and, at the same time fight against the population explosion.

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# PLAGUE; A MEDIEVAL KILLER IN THE PRESENT WORLD: A REVIEW

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CONTENTS



**Abstsract**: Plague as a disease was known in the sixth century, but it was not till the fourteenth century that it became pandemic resulting in the ove 14 million deaths in Europe between 1348 and 1400. Unlike smallpox, plague can never be eradicated, as is evident from -the recent outbreak of the disease in India. Plague is a disease of the earth, of creatures that run and burrow and of the fleas that live on them. It is found mainly in the wild, often far from man. Plague bacterium need not attack man in order to persist, for it finds all that it needs in wild places. In India, between 1898 and 1919 there may have been more than ten million deaths due to plague. According to WHO between 1961 and 1970 a total of 26,262 cases and 1,887 deaths have been reported worldwide and from 1971-80 there were 16,480 cases and 932 deaths due to plague. During the recent outbreak of plague in India an estimated 55 individuals died of the disease. The plague pathogen is known as *Yersinia pestis* and its host is black rat, its vector being the flea *Xenopsylla cheopis* which lives on the rat. Plague is perpetuated by 3 cycles, (1) natural foci among commensal rodents with transmission by fleas (wild plague), (2) urban rat plague, which is transmitted by the rat flea (domestic plague), and (3) human plague, which may be acquired by contact with either of the former cycles and which may be transmitted by pneumonic spread, or, rarely by the bite of a human flea. Plague is now recognized as a well marked disease caused by a Gram-negative facultative anaerobic bacterium. It comes in three forms, all of which are fatal if not properly treated, a) bubonic plague, producing bubos/swellings of the lymph glands, b) pneumonic plague, attacking primarily the lungs; and c)septicemic plague, killing the patient rapidly by poisoning the blood. In bubonic plague the incubation period is 2-8 days. If left untreated the infection spreads to other parts of the body through the blood stream eventually infecting the lungs- leading to pneumonic plague. This type is highly contagious as the patient's sputum contains the bacillus and droplets of the sputum can spread the disease from person to person, resulting in localized outbreaks, or devastating epidemics. Incubation period is 1-6 days after exposure and the patient experiences fever, headache, vomiting and a marked clouding of consciousness. Pneumonic plague is the most fatal, as well as the most directly infectious form of the disease. Treatment of plague involves a variety of antibiotics, namely streptomycin, tertracycline, chloramphenicol and sulfonamides-dosages also vary. A vaccine has also been developed using the killed or attenuated pathogen, but is useless in rapidly developing localized outbreaks-such as the recent outbreak in India. Control measures are the key methods for curbing the outbreaks of plague and they include those required during an outbreak to bring it to an end and long term action to prevent the spread of infection from the wild to human population.

## INTRODUCTION

Plague is now recognized as a well-marked disease caused by bacillus, *Yersinia pestis*. There are three forms of the disease: a) bubonic plague, producing buboes, or swellings of the lymph glands, b) pneumatic plague, attacking primarily the lungs, and c) septicemic plague, killing the patient rapidly by poisoning the blood.

All types are fatal, if not properly treated. Plague is transmitted to man by fleas from black rats and other rodents' such as wild squirrels, gophers and gerbils. It produces high fever, agonizing pain and prostration (complete physical and mental exhaustion). Plague was frequently accompanied by outbreaks of typhus and `English fever', which was a deadly form of influenza.

Plague was known as a disease in the sixth century Roman Empire and even earlier in North Africa, but it was not till the 14th century that plague became pandemic-- the European epidemic.

It was first thought that the black rat was brought to Europe by the Crusaders returning from the Middle East, but this seems unlikely as prehistoric sites of rats have been found in Switzerland.

The 14th century pandemic started in 1348 from the Italian ports, apparently from merchant ships from the Middle East region. During the next two years, plague swept across Spain, France, England, Central Europe and Scandinavia.

It was a slow yet unrelenting advancement of the disease striking with deadliest effect in crowded, unsanitary towns. Each year the epidemic rose to a peak in late summer and subsided in the winter months only to return in spring. The 1348-50 A.D. pandemic was followed by long series of recurrent outbreaks all over Europe at intervals of ten years or less. For example, in London at least 20 attacks of plague were reported in 15th century. In Venice the black death struck 23 times between 1348 and 1576. It has been generally accepted that 25% of Europe's population was wiped out in the first epidemic between 1348-50. Over the next 50 years, mortality rose to more than 33% of the population-totalling over 44 million deaths, although the rate and occurence varied in various regions.

In 1665 A.D., England was afflicted with bubonic plague epidemic which killed over 40,000 people in London alone. Afterwards plague mysteriously disappeared. Some scientists thought that the larger brown rat had killed off the smaller black rats but this was not the case. Something must have happened to the fleas, the bacillus, or the living conditions of the human host. Probably the better living conditions and personal hygiene had something to do with the subsiding of the plague epidemic. In Florence, Italy, 45,000 died out of a population of 90,000, people, in Sienna, France, 27,000 died out of a population of 42,000 people, while in Hamburg, Germany, 66% of the population perished.

In Venice the local Board of Health kept accurate counts of the deaths due to plague outbreak of 1576-77 A.D. totalling 46,721 out of 160,000 people. In Marseilles, (1720 A.D.) 40,000 people died out of a population of 90,000 and Messina lost 58% of its population in the 1743 A.D outbreak of plague. In India, between 1898 and 1919, there may have been more that 10 million deaths from plague, but the numbers have fallen greatly since then. According to WHO, between 1961 and 1970 a total of 26,262 cases and 1887 deaths have been reported worldwide; the corresponding figures for 1971-80 were 16,480 with 932 deaths.

Eighty percent of all those who came in contact with this disease died within 2-3

days usually in agonizing pain. Prevention and cure was not known at that time. It was interpreted as being God's way of punishing humans for their sins.

Plague epidemic of the 14th and 16th century frightened many people to migration from towns. Emperors, kings, princes, clergy, merchants, lawyer, professors, judges, students, even physicians rushed away, leaving behind the sick to die. In the 1563 plague epidemic, Queen Elizabeth I took refuge in Windsor Castle and errected gallows to hang anyone who had the gall to enter Windsor from plague ridden London. Entire streets were closed off by chains and the sick quarantined. French surgeon Pane, in 1568 wrote that husbands and wives deserted each other and parents abandoned their children. People went mad with terror and often committed suicide.

*The disease and causative agent*

Unlike smallpox, plague can never be eradicated, for it is a disease of the earth, of creatures that run and burrow, and of the fleas that live on them. It is found mainly in wild regions, often far from man -- in dry deserts or in wet rice fields, in treeless steppes or dense forests, on foothills, on the higher slopes of mountains, or across wide lowland plains. Plague needs not attack man in order to persist, for it finds all it needs in wild places. If man steps into the wild, as a hunter, trapper, or even tourist, he can catch palgue and then carry it far away from the source before the disease takes hold.

The host of plague bacillus is commonly a rodent and a vector, commonly the flea *Xenopsylla cheopis*. They interact and, if conditions are favorable, all three persist together in nature. Their numbers rise and fall over the years under thre influence of both internal and external factors. The internal factors include differences in the susceptibility of the host to the plague bacillus, or in the efficiency of the vector in transmitting it. External factors are the conditions in the environment in which the interactions take place, such as temperature, humidity, season, the availibility of food and shelter for hosts etc.

There are not just one, or, two hosts to consider, *i.e. Rattus rattus* and *Rattus norvegicus*, but over three hundred rodents and other species are known hosts of the plague bacillus, and not just one or two fleas, such as *X. cheopis* and *Pulex irritans*, but at least 30 of the 3000 or more known species are capable of transmitting the organism. The plague bacillus may vary in virulence under the influence of slight environmental changes.

Three species of the genus *Yersinia* are primarily animal pathogens, but also produce human disease. *Y. pestis* is the cause of plague, while in humans *Y. pseudotuberculosis* and *Y. enterocolitica* are most commonly associated with G.I. tract diseases. Previously this organism was classified in the genus *Pasteurella*, now the pathogen has been placed in the family *Enterobacteriaceae*. It expresses the enterobacterial antigen and its physiological characteristics and lipid composition is similar to the other species of this family.

The causative agent, *Y. pestis* is a Gram-negative, non-motile coccobacillus. It shows marked bipolar staining, especially in tissue impressions, bubo aspirates and pus

stained with Giemsa's stain. The cells have a safety-pin appearance with the polar bodies staining blue and the remainder staining light blue to reddish. Freshly isolated virulent organisms are enveloped. *Yersinia* are facultative anaerobes or anaerogenic and usually do not ferment lactose. They are oxidase negative and produce catalase.

*Culture characteristics and strain identification*

*Y. pestis* can grow over a wide temperature range; from 0°C to 43°C, the optimal temperature of growth being 28°C. Several phenotypic characteristics are best expressed at room temperature. It can grow on ordinary laboratory media even from small inoculate. On nutrient agar plates, small mucoid colonies apperar in 1-2 days.

Three bio-types have been detected on the basis of their ability to reduce nitrate to nitrites and to ferment glycerol. These bio-types have been designated *orientalis*, *mediaevalis*, and *antigua* and are characterized by the differences in their geographical distribution. *Orientalis* is the usual bio-type of western North America.

*Y. pestis* strains are also characterized by quantitative differences in their antigens. At least twenty different antigens have been identified on the basis of gel diffusion and biochemical analysis, many of which are shared with *Y. pseudotuberculosis* and *Y. enterocolitica*.

*Determination of virulence*

*Yersinia* are facultative intracellular parasites. Virulence is thus assumed to reflect an ability of the organisms to proliferate within mammalian cells. Most of the virulence factors that have been defined are largely associated with resistance to mechanism of intracellular killing and with invasive abilities to gain access to favored sites of replication in fixed phages.

Among the factors associated with virulence are (1) $Ca^{++}$ ions; (2) V and W antigens; (3) F-I (envelope antigen); (4) Pesticin, coagulase and fibrinolysin production; (5) Pigment absorption.

The V and W antigens develop early in infection and appear to confer on *Y. pestis* the ability of small numbers of bacilli to establish infection in animals. Once the infection is established the F-I (envelope) antigen, pesticin, coagulase and fibrinolysin contribute to the rapid extension of the disease process.

*V and W antigen:* The V and W antigens are always produced together. *In vivo*, the V and W antigen correlate with pathogenicity and with the ability of *Y. pestis* to rapidly proliferate and to cause overwhelming septicemia.

*Envelope antigen*: F-I antigen,.or envelope antigen is a soluble antigen contained within the bacterjal envelope. It consists of two immunologically identical complexes; (a protein complexed with polysaccharides).

Envelope antigen appears to be antiphagocytic and prevents pagocytosis by

professional phagocytes, thus potentiating the rapid evolution of septicemia that characterized the clinical disease. It is highly immunogenic and may constitute as much as the 7% of the dry weight of the organism. Antibody to F-I appears to be protective in both humans and experimental animals.

*Pesticin I, coagulase and fibrinolysin:* The production of pesticin-I, coagulase and fibrinolysin is always correlated. Pesticin I is a bacteriocin produced by *Y. pestis* that inhibit the growth of *Y. pseudotuberculosis*, as well as some strains of *E. coli* and *Y. enterocolitica.*

Strains of *Y. pestis* lacking these enzymes are infectious for the mouse, or guinea pig. But, lethality is significantly attenuated. Augmentation and persistence of the infectious process in organs correlate with these properties.

*Pigment absorption:* In virulent strains unidentified surface components are present that result in the absorption of hemin and basic aromatic dyes to form colored colonies.

*Other virulence-associated factors:* Two additional factors that have been proposed as virulence factors are murine toxin and endotoxin. For rats and mice the lethal dose of murine toxin is less than 1 $\mu$g, but for other animals it is relatively a toxic-hence the name murine toxin.

*Epidemiology*

Plague was introduced into the United States from China in 1900, when the first human case of the disease was reported in San Fransisco. In 1907-08, a major epidemic of 167 cases occured in San Fransisco. Permanent foci of plague now exist that involve at least 57 wild rodent species and their fleas. These extend as far east as Kansas, Oklahoma, and Texas and to approximal areas of Canada and Mexico.

Plague is perpetuated by three cycles, (1) natural foci among commensal rodents with transmission by fleas (sylvatic plague, wild plague, (2) urban rat plague weich is transmitted by the rat flea (domestic plague, urban plague), and (3) human plague, whicy may be acquired by contact with either of the former cycles and which may be transmitted by pneumonic spread or, rarely, by the bite of a human flea.

*Flea related factors*

In nature, the flea is essential for perpetuation of plague. At least four flea-related factors influence the epidemic potential of plague;

1) Fleas vary greatly in their vector efficiency. Most wild rodent fleas are relatively inefficient in the transmission of disease to humans. However, the Oriental rat flea, *Xenopsylla cheopis*, is highly efficient and has been the classic vector in urban rat-borne epidemics. 2) The restricted feeding habits of most wild rodent fleas limit their threat to humans. Human occasionally have been infected when wild rodent death during an epizootic left hungry fleas in search of a new host. Dog and cat fleas are very poor vectors and have been associated with individual cases of human plague, but not with

outbreaks. 3) Some infective wild rodent fleas survive in burrows for long periods of time, even after the rodent hosts have died. Survival of fleas for as long as 15 months has been shown. 4) The development of dichloro-diphenyl-trichloromethane (DDT)-resistant fleas in some areaas may influence the epidemic potential of plague. This occured in some instances with widespread DDT spraying during malarial control programs.

Transmission of plague by flea may occur in several ways. The most efficient involves ingestion of the organism by the flea during a blood meal from a bacterimic host. In the flea stomach, the infected blood is coagulated by coagulase that is produced by *Y. pestis* in the presence of an enzyme from the flea stomach. Bacteria are thus trapped in a matrix of fibrin, which fixes them to the spines of the flea proventriculus. As the bacteria multiply, the proventriculus is occluded, which causes blockage. The time between ingestion and blockade is the extrinsic incubation time, and usually for *X. cheopis* it is about 2 weeks. During subsequent attempts to obtain a blood meal, regurgitation of the infected material results in the infection of the new host. The hungry flea also becomes less fastidious about its host and will readily attack humans. Hot, dry weather adversely affects all stages in the life cycle of the flea, which explains the subsidence of many epidemics at the beginning of the hot and dry season. Blockage is enhanced at temperatures below $26^{0}$C. Above $27^{0}$C the fibrinolytic factor of *Y. pestis* and the trypsin-like activity of the flea stomach enzyme are activated, thereby destroying the fibrin meshwork needed for blockage. Decreased blockage results in decreased vector efficiency.

Mechanical transmission by contaminated mouth parts of the flea are important in the transmission of plague, especially in wild plague.

*The host*

The facts that were established about the spread of plague in rats are as follows:

1). Unless fleas are present, plague is not spread from infected rats, even from those that die of palgue, to susceptible rats in close contact with them; it does not spread from infected females to their sucklings; 2). Plague does not spread through the air from infected rats, nor from soil contaminated with infected feces, urine or food; 3) A rat can be infected by being fed contaminated food of pieces of a rat that died of plague, but the pathological findings -- mesenteric lymphadenitis and intestinal lesions -- are not encountered even once in necropsies of 5000 rats that died from the natural disease; auch animals showed cervical lymphadenitis and lung congestion, but no intestinal lesions; 4) Plague spreads from one infected rat to another, or from contaminated soil, if fleas are present, and the spread from such sources to a new host is limited by the distance, or the height that a flea can jump. Without fleas, infection does not spread.

*Plague infection and spread*

*In man*: Infection may be maintained for years in such a focus without the overt appearance of plague, unless some outside host intrudes. A man may stray into the focus as a hunter and be bitten by a flea searching for a host, or by one that jumps from

a dead rodent; or he may skin a small animal for its pelt or for food. The hunter usually catches plague by direct contact with the infected flesh of an aimal. So also does the cook who handles refrigerated meat in which *Y. pestis* has survived. Man may unknowingly enter a focus as a tourist, be bitten, and become ill a few days later after having traveled far from the source of infection. Much more important, however, are massive human migrations into untrodden areas, such as occur durig a war. These migrations bring man close to the earth, to fleas and rodents, and to unsuspected enzootic plague.

*In dogs:* As man can intrude into a wild focus so too can domestic animals. Serological surveys in rural areas have shown that dogs are often infected, though usually they remain well and do not spread infection. They can be regarded as sentinel animals, their seropositivity indicating that plague is in the area and may spread to man. There were 19 cases of human plague in western US in 1982, at a time when serotesting of dogs and other animals had demonstrated the presence of the disease i the area.

*In cats:* Cats too may be infected, but they tend to become ill and some die. They may have buboes, or abscesses in the thigh, and some develop respiratory symptoms and die of pneumonic plague.

*In goats, sheep and camels*: Some of man's other domestic animals, sheep and goats for example, may stray into wild focus, become infected, and then carry the infection to man. In one outbreak in Lybia, six shepherds died, five of them having slaughtered ailing or morbid sheep a day or two earlier. Again in Libya, a peasant farmer developed plague meningitis two days after killing a goat; the illness spread in his family, and goats of the area proved to be seropositive.

Perhaps more impórtant is the camel, which travels over vast areas yet is constantly close to man. Camels have been infected experimentally with *Y. pestis*, albeit with some difficulty. In one study, two of the four camels inoculated subcutaneously died, as also did each of the six infected by aerosol; but the feeding of contaminated hay produced only a mild illness, with buboes in the neck, from which the animals recovered. In libya, four men who killed and skinned an ailing camel with swellings in its neck all died within three days; plague also affected a further ten persons, two fatally, who handled or ate the camel meat. In another outbreak in Libya, sudden unexplained deaths in children were followed by three sudden deaths in adults, one of whom had slaughtered a sick camel two days earlier, sharing the meat with the other two. Human plague related to contact with camels has also occured in Saudi Arabia in the form of septicemic and meningitic infections, but nothing has yet been published on these occurence.

*Local escape:* Infections resulting from the intrusion of man or his domestic animals into a plague focus are in a sense accidental. They are not the result of any change within the focus. True active spread, however, occurs when infected hosts leave the focus to forage or explore outside it. They carry their infected fleas with them, and if they come near a dwelling place of man they encounter `peridomestic' rodents such as *R. norvegicus*, and infected fleas then pass from one to the other. *R. norvegicus* comes into contact with the `commensal' rat *R. rattus*, and fleas again pass from one to the

other. *R. rattus* and flea again pass between the species. *R. rattus* lives close to man, in the walls or roof-spaces of his house or hut. Being highly susceptible, the rat dies of plague, and its fleas must then find another host. The new host may be man, infected in his home. Thus a new focus is established within an area of human habitation.

The type of outbreak varies according to local circumstances. For example the commensal host may be *Bandicota bengalensis* (mole-like burrowing rat of south and south-east Asia) instead or *R. rattus*; the flea may be a poor transmitter, such as *X. astia* instead of an effective one such as *X. cheopis*. Temperature, humidity and other environmental circumstances may also play a role. In a newly formed focus the interaction between host, flea and organism is once again delicately balanced.

*Worldwide escape:* Man may interfere with nature and disrupt a wild plague focus. He may, for example, harvest a field, driving out the rats and their fleas to seek new shelter, perhaps nearer to human habitations. He may empty a grain store, carting loads of grain to some distant village or town; rats in the grain may then mingle with the local rats, passing on infected fleas. Many rats die, and soon plague breaks out among the human inhabitants. There is no limit to the scale of man's interference. He may send his cargo, together with rats, fleas and plague, by train into a continent, or by ship across the ocean. Thus plague can leap from one part of the world to another.

*Disease in man*

*Bubonic plague:* In this type of plague the incubataion period is two to eight days. A small lesion occurs at the site of the infected flea bite with the adjoining lymph glands becoming swollen and painful, followed by high fever, vomiting, intense thirst and some patients may have diarrhoea. The spleen and liver are usually enlarged. The lymph gland or bubo enlarges and becomes soft, bursts and discharges pus. If left untreated the infection progresses to other parts of the body through the blood stream and eventually infects the lung resulting in pneumonic plague.

*Pneumonic plague:*

Pneumonic plague is highly contagious as the patient's sputum contains the plague bacillus and droplets of this sputum can spread the disease from person to person thus resulting in localized outbreaks or devastating epidemics. The incubation period for pneumonic plague is one to six days after exposure. In pneumonic plague the patient is very ill, with fever, headache, vomiting and marked clouding of consciousness from the beginning. The sputum is thin, watery and bloodstained. If the disease is left untreated it can prove fatal by the fourth, or fifth day. Pneumonic plague is most fatal as well as the most directly infectious form of plague.

*Diagnosis and treatment*

In animals: Plague in rodents is strongly suggested by the presence of a bubo, and aspirates from this may reveal bacteria of characteristic morphology. Specimens, including rodents and fleas, must be sent to a laboratory recognized by WHO. Requirements for the final diagnosis of *Y. pestis* infection include confirmation of

typical microscopic and colonial morphology, lysis by specific bacteriophage, staining with flourescent-antibody conjugate for *Y. pestis* fraction 1, and production of characteristic lesions in laboratory mice or guinea pigs. During widespread outbreak, when the diagnosis of plague in an area has already been confirmed, the passive hemagglutination test, which is rapid and inexpensive, may be used for the examination of rodent sera.

*In man:* The initial diagnosis, which must be made on clinical grounds, is of great importance because, although mortality is high, early treatment is very successful. Swollen inguinal or axillary lymph nodes may suggest staphylococcal or other septic infection, lymphogranuloma, tularemia, filariasis and so on. But if the patient has contact with the wild the doctor should think of plague. If, on the other hand, the infection appears overwhelming, he must think of septicemia due to Meningiococci, or other Gram-negative bacteria, typhus, malaria and hemorrhagic fever as well as plague. If there are already cases of plague in the area, the treatment should include anti-plague drugs untill onother diagnosis is established. So-called peripatetic plague is particularly difficult to recognize because the patient may have traveled thousands of miles since becoming infected from an unsuspected source. A positive result on microscopic examination strengthens the clinical diagnosis and reinforces the urgency of the need of specific treatment. Should the bubo yield pus, rubbing this into the shaved abdominal wall of a guinea-pig will usually succeed in separating plague bacilli, which will penetrate the skin, from other pyogenic organisms, which will not do so.

For final diagnosis, bubo aspirat, blood, sputum and in meningitic cases, cerebrospinal fluid should be inoculated on to blood and MacConkey's agar, and into infusion broth. Material may also be injected into rats, guinea-pigs or mice. After their death *Y. pestis* is easily demonstrated in smears from lymph nodes, blood and other organs. After a patient's death, material for diagnosis is best obtained from buboes, or spleen. If opening of the body is not permitted, specimens can be obtained by means of viscerotomes, organ puncture or finger amputation. Alternatively, sternal bone marrow, venous or heart blood, blood-stained nasal discharge may be examined.

Hemagglutinating antibodies appear towards the end of the first week of illness. Serological testing is therefore useless in the early stages of the disease, when a diagnosis is urgently needed, but it is very useful for retrospective or confirmatory diagnosis. In the passive hemagglutinin test, *Y. pestis* fraction 1 is used as the antigen. It is virtually specific for *Y. pestis*. A titer of sixteen, or a four-fold rise in titer, is very suggestive of plague.

The persons who are in close contact with patients of plague should be given a course of antibiotics, not because the patient in infections, but because the contacts have probably also been exposed to the danger of flea-borne infection. Tetracycline in doses of up to 500mg four times a day for one week is suitable treatment for an adult. For children, doses of 25-40 mg/kg/day are suitable, the risk of dental damage being minute compared with the risk of plague. In a small isolated village, it may be advisable for everyone to have a course of antibiotics, but massive dosing in a widespread outbreak is useless.

Hospital staff will probably bot be familiar with plague and the risks must therefor be explained to them. A patient with bubonic plague is not infectious if there are no fleas on him, but a patient with pneumonic plague, or with septicemic plague and terminal respiratory symptoms, is very infectious until after several days of antibiotic treatment. If there are only one or two patients, hospital staff should take a course of antibiotics while looking after them, but if there is a succession of cases they should, instead, take their own temperature every six hours and have treatment at once if it rises. Laboratory staff unaccustomed to handling plague specimens should be made aware of the risks and precautions.

*Antibiotics for the patients:* Response to antibiotics is dramatic provided these are given early enough, and certainly before the patient is collapsed and moribund (near death); treatment must not await the outcome of laboratory tests. The specific drugs are streptomycin, tetracycline, chloramphenicol, and to a lesser extent, sulfonamides. Penicillin is useless and must not be given alone for any obscure febrile disease that might be plague.

Steptomycin - loading dose 1g intramuscularly; 0.5g/4h until the temperature falls, then 0.5g/6h for several days.

Tetracycline - loading dose 3g orally; then 3g daily in divided doses for one week; but in late or severe cases a first dose of 0.75-1.0g is given intramuscularly.

Chloramphenicol - similar doses.

Perhaps the best treatment is a combination of streptomycin and tetracycline. Sulfonamides, may be used if antibiotics are unavailable, in doses of up to 4-6g/day together with 2-4g of sodium bicarbonate to keep the urine alkaline; useless against pnrumonic plague.

*Vaccination:* Killed virulent, or live virulent (attenuated) vaccines both cause sharp reactions; only killed vaccines are in common use. Two doses at an interval of 1-3 months followed by a third after a further six months are recommended. Vaccine is useless in rapidly developing localized outbreaks, but should be given to those whose work exposes them to plague such as staff in plague laboratories, plague control teams, and hospital workers in known plague areas.

Protection is probably short-lived, and booster doses are therefore required every six months. Moreover, the degree of protection is limited and varies with the degree of exposure. Thus vaccination would not necessarily protect against exposure to pneumonic plague. A vaccinated person, even if known to have formed anti-plague antibodies, must be given antibiotics when exposed to a definite risk, especially of pneumonic plague.

*Prognosis*

Untreated bubonic plague has a mortality of about 50%, while untreated primary pneumonic plague is invariably fatal.

Plague can be cured, and lives saved, by informed and urgently applied treatment

Plague pandemics may now be a thing of the past, but in the wild the disease will continue and in developing countries, such as India and southeast Asia, epidemic can and sometimes do arise.

*Control of plague*

Measures to control plague includ,: 1) those required during an outbreak, to bring it to an end, 2) long-term action to prevent the spread of infection from the wild to human population.

*Control of outbreaks:* For control of the epidemic education is of great importance. The local people must be told: a) How plague spreads; b) The importance of personal hygiene in avoiding flea infestation; c) The need to keep their houses free of dirt and debris in which fleas can linger; d) The need to seek medical advice if they are bitten by fleas; e) Dogs and other pets should, if possible, be treated weekly with insecticide; f) A local control team should carefully spray the inside of dwellings and other buildings with an insecticide to which local fleas are sensitive; g) No attempt should be made to kill commensal rats unless insecticide spraying has been first done, otherwise the fleas will jump from the carcasses to man; h) Villagers should be warned not to hunt, or trap in dangerous areas.

*Long term control*: Anything that keeps rats away from man lessens the risk of human plague; a) Good sanitation and waste disposal; b) Houses made rat-proof, also warehouses, stores, airports and railway stations, trucks, containers, trains, ships and planes must also be made secure against rats; c) Rat escape routes must be blocked; d) Proper storage of food; e) Protection of population and health workers; f) Use of gloves, masks while handling bodies of persons dying of plague; g) Isolation and treatment of individuals showing symptoms of plague; h) Quarantine of persons coming from plague affected areas.

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