# CHAPTER 1

## EFFECT OF DISUSE ATROPHY ON PECTORALIS MUSCLE AND BLOOD IN THE PIGEON

It is well known that when a muscle is under disuse or subjected to motor denervation, atrophic and degenerative changes are produced. The histological and physiological changes have been reported by several authors, Tower (1939), Reid (1941), Eccles (1941), Fischer and Ramsey (1946), Fischer (1948), Chor et al, (1937) and Thimann and Padykula (1955). However, the changes in muscle undergoing atrophy as well as changes in the blood have not received sufficient attention. Though numerous studies on induced atrophy have been conducted, most of them have been on laboratory mammals and hardly any of importance on birds. An exploratory study of histological changes occurring in the partially atrophied pigeon breast muscle was reported earlier by George and Naik (1959), Naik (1960). The pigeon breast muscle, which is one of the most metabolically active vertebrate skeletal muscles, has two distinctly different types of fibres, one highly specialized for an anaerobic metabolism, the broad, glycogen-loaded fibres with few mitochondria and little oxidative enzyme activity; and another for aerobic metabolism, mostly using fat as the chief fuel, the narrow red, fat-loaded fibres with numerous mitochondria and large concentrations of oxidative enzymes. Since these two types of fibres with contrasting effect exist side by side in the same system they make the pigeon breast muscle all the more interesting

for studies on muscular atrophy.

It was shown by several workers that there is an increase in the lipid content of the muscle during atrophy, Humoller et al, (1952), Friedlander et al, (1941), Helander (1960). It was also reported by Oppenheimer et al, (1958) that there is a severe disturbance in the metabolism of lipids in muscular dystrophy produced by vitamin E deficiency.

In the present investigation experiments were conducted to see whether there is any corresponding changes in the lipase activity of the muscle and blood with respect to the changes in the fat content of the muscle. Here in this chapter is reported the results of a quantitative study of the lipase activity in the muscle and blood and fat and water content in the muscle of the pigeon under disuse muscular atrophy.

## Material and Methods

The experiments were conducted on fully grown, healthy pigeons (Columba livia) of both sexes weighing between 290 to 330 gm. A plaster cast was applied on the middle of the upper wings after putting them in a back-toback position (Fig. 1). By employing this device in the pigeon, George and Naik (1959) from histological observations, have shown that disuse atrophic changes could be induced in the breast muscle. Sufficient care was taken not to block the blood supply to the wings by making sure the cast was not too tight. In the beginning the pigeons had a little difficulty in balancing themselves, but within a day or two, they adjusted and behaved like normal pigeons in

feeding and sexual behaviour. They were fed on a normal diet along with other pigeons, but their intake of water was found to be greater than usual in the first few days. They were sacrificed after varying lengths of time from 1 day to 60 days.

At the end of each experimental period the pigeons were sacrificed, and the blood was collected from the heart in clean centrifuge tubes. A piece of the breast muscle in its entire depth was excised always from one particular region, since it is known the superficial and deep layers not only differ in the number of their component broad and narrow fibres (George and Naik, 1959a) but also in their biochemical constituents (George and Talesara, 1961). Lipase activity in the superficial and deepest layers was found to be 19.08 and 51.18 µl $CO_2$ / mg. dry muscle / hour respectively (George and Talesara, 1961).

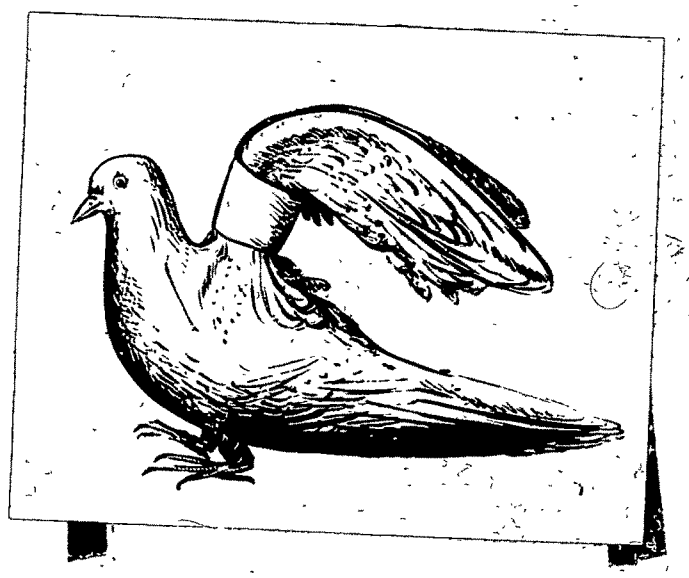

Fig. 1. Pigeon showing position of wings with plaster cast.

The total lipid content of the muscle was estimated by the conventional Soxhlet extraction method using a 1:1 mixture of ethanol and ether. Muscle for fat extraction was dried in an air oven at 95°C for 24 hours and kept in a vacuum descicator until constant weight was obtained. Then the muscle was powdered and extracted for 18 hours. The extracted material was dried, weighed and the percentage fat content was calculated on dry weight basis, since the water content of the muscle in the first two weeks of atrophy showed sharp changes. The water content of the muscle was also calculated from the same material dried for fat extraction.

The lipase activity in the muscle and blood serum was determined at different intervals of atrophy from one day to sixty days. The muscle was homogenized in ice cold distilled water in a mortar chilled in the freezer of a refrigerator and the homogenate was used as the enzyme material. In the case of serum, the blood samples collected in the centrifuge tubes were allowed to clot at room temperature for 5 to 7 minutes and then centrifuged at a speed of 2500 r.p.m. for 5 minutes. Since the lipase activity of the serum is very high, a dilution of 50 times was always found convenient for the assay of the enzyme in the manometric system.

The method employed for the estimation of lipase activity was one adopted from Martin and Peers (1953) with a bicarbonate carbon dioxide buffer system of pH 7.4 at 37°C using the Warburg apparatus as followed in this laboratory

(George, Vallyathan and Scaria, 1958). The side arm contained 0.5 ml. of 4% (v/v) tributyrin in 0.0148 M bicarbonate, emulsified by shaking with a drop of ' Tween 80'. Each reaction flask contained 1.5 ml. of 0.025 M bicarbonate buffer and 1 ml. of the enzyme solution being tested in the main chamber. The flasks and the manometers were gassed for three minutes with a mixture of 95% nitrogen and 5% carbon dioxide. After an equilibration of 10 minutes in the Warburg constant temperature water bath, the substrate was tipped in and allowed to equilibrate for another three minutes to ensure complete mixing of the contents. The manometers were shaken at about 120 oscillations / minute allowing an amplitude of 4 to 5 cm per oscillation. The readings were taken at regular intervals for one hour. The enzyme activity in the muscle is expressed as microlitres of $CO_2$ / milligram of protein / hour. The protein content of the enzyme solution was determined by the micro-Kjeldahl steam distillation method as outlined by Hawk et al, (1954). The enzyme activity in the serum is expressed as microlitres of $CO_2$ / millilitre of serum / hour.

## Results

Body weight:

The pigeons in which atrophy was experimentally induced showed a great reduction in body weight by the end of one month. The maximum weight loss (about 12%) was, however, noticed after a period of 10 days. By the end of 2 months the pigeons have regained nearly 50% of the lost weight.

Water content and weight of muscle:

There was a great increase of water content from the normal 73.34% in the muscle soon after the wings were put in the plaster cast. This increase in the water content was maximum during the first two weeks and then gradually decreased. By the 30th day of atrophy the water content of the muscle was almost equal to that of the normal pigeons (Fig. 2 and Table 1).

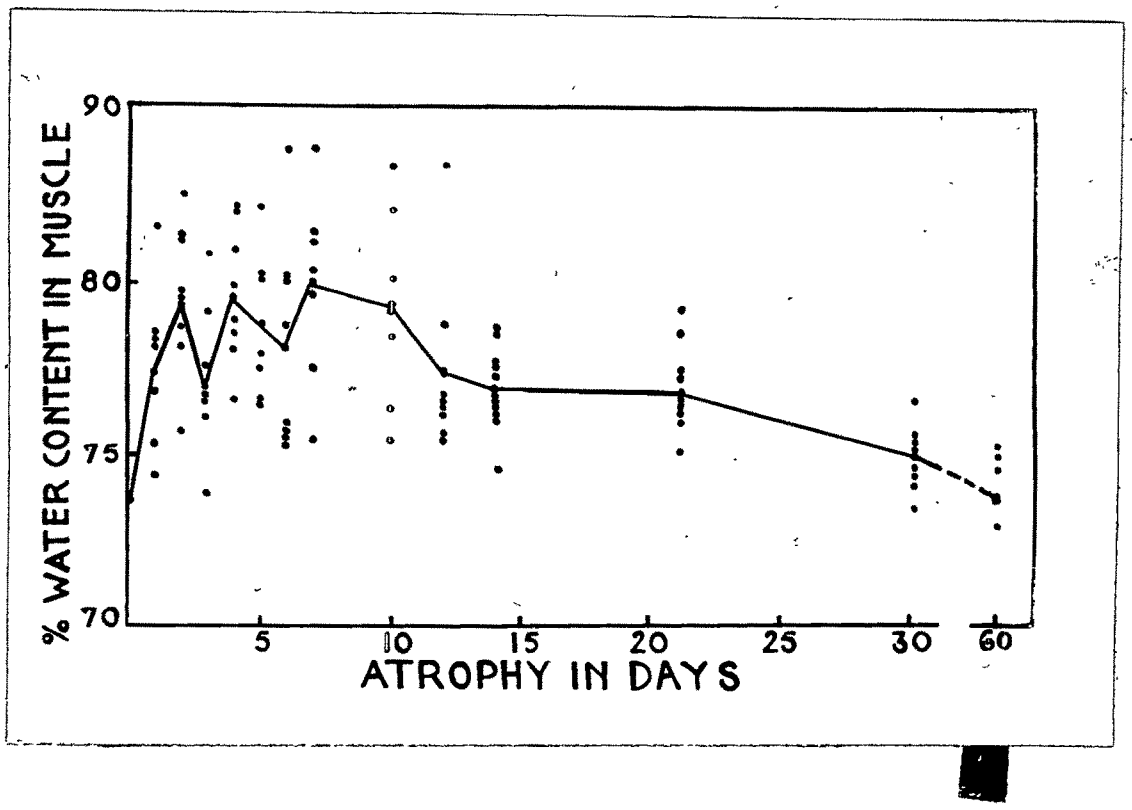


Fig. 2. Showing the quantitative changes in water content in pigeon breast muscle during induced muscular atrophy. Dots indicate the individual readings, and the line denotes the average of the readings.

During the early stages and particularly in the first week of atrophy the muscle appeared flascid and vascular. The bulk of the muscles of both sides was considerably reduced after a period of two weeks, and at the end of 30 days the muscle weight came down to nearly one eighth of the body weight, which is usually one fifth of the weight of the body in normal pigeons. But by the end of two months there was a slight increase in the size of the muscle. The muscle appeared pale in colour and on examining hand-cut sections of frozen muscle, connective tissue was found to be increasing after two weeks of atrophy. After a period of 30 days there was a great increase in the connective tissue. By the end of two months the muscle had almost acquired its orginal colour and nearly regained 50% of the lost weight. Throughout the experimental period, expept for the first day of putting the wings in plaster, the birds ate and drank. There was no possibility of the plaster cast causing any venous occlusion since it was sufficiently loose.

Fat content:

Fat content increased from the normal value of 13.3% of dry muscle from the first day but started to decrease by 4 to 6 days, and by the end of one month the total lipid content again rose above that of the normal pigeons. However, it was observed in all the experiments on two months of atrophy that the total fat content in the muscle was remarkably lower than that of the values (11.32%) for the normal pigeons ( Fig. 3 and Table 1 ).

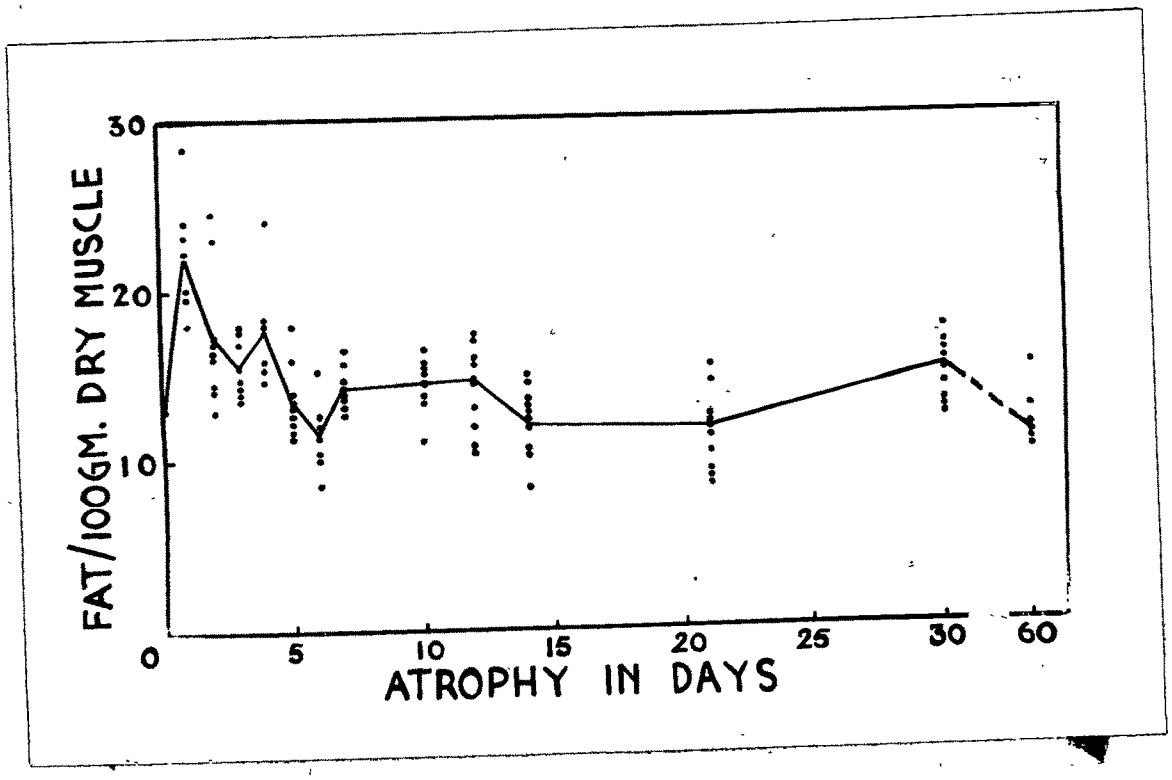


Fig. 3. Showing the quantitative changes in fat content in the pigeon breast muscle during induced muscular atrophy. Dots indicate the individual readings, and the line denotes the average of the readings.

Muscle lipase activity:

An increase in the lipolytic activity from the normal value of 40 µl $CO_2$ / mg. protein / hour in the muscle was observed soon after the muscle was subjected to the experimental conditions. This increase in the enzyme activity was at its peak from the 3rd to the 7th day. After the high enzyme activity in the first week it was found to be decreasing slowly, and after 30 days it

came to half of the normal. The enzyme activity was again found to be increasing slightly by the end of two months. The lipase activity in the muscles of the females under experimentation was always found to be a little higher, as it also was in the normal pigeons. The differences in the enzyme activity of the two sexes was very high during the 3rd to the 7th day. Figure 4 and table 2 represents the lipase values in the breast muscle of the pigeon for the different periods

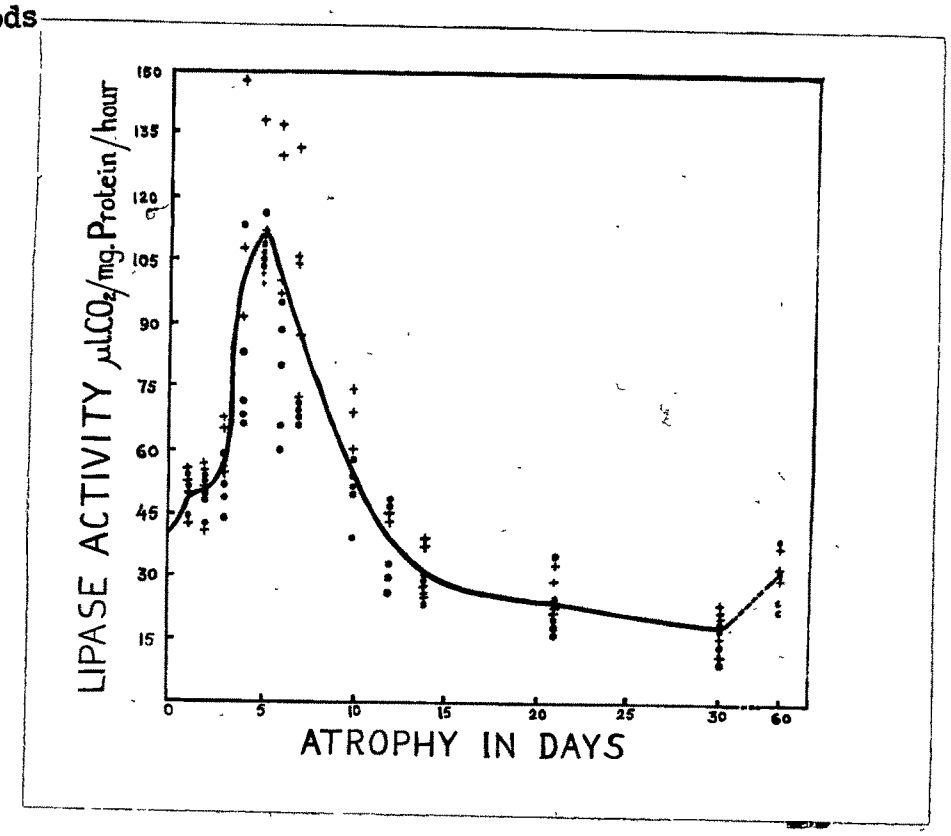


Fig. 4. Changes in lipase activity in pigeon breast muscle during induced muscular atrophy. Dots indicate individual readings for males, plus signs for females, and curve is average of both.

Serum lipase activity:

During the first few days, when there was an increase in the water content in the muscle, a decrease in the serum concentration of the blood was noted. The total serum obtained during these days was less compared to the yield of serum from the normal pigeons and from those in the latter stages of atrophy. A fall in the lipolytic activity of the serum was very obvious after 2 days immobilization of the wings. The enzyme activity

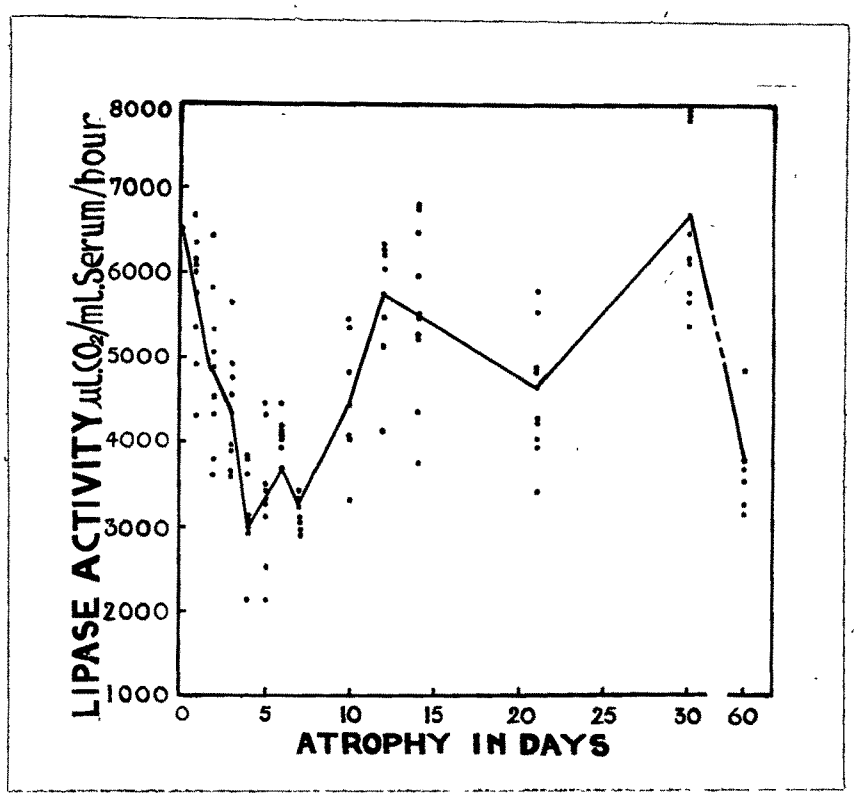


Fig. 5. Changes in the serum lipase activity in pigeon during induced atrophy of the breast muscle. Dots indicate the individual readings, whereas the line denotes the average of the readings.

Table 1

Changes in the fat and water content in the muscle of the pigeon during induced muscular atrophy.

| Atrophy in days | Sex | Fat content % gm. on dry muscle | | | Water content % | | | No. of Expts. |
|---|---|---|---|---|---|---|---|---|
| 1 | Male | 22.67 | ± | 1.56 | 78.77 | ± | 1.81 | 4 |
| 1 | Female | 22.14 | ± | 3.91 | 76.41 | ± | 1.53 | 4 |
| 2 | Male | 17.14 | ± | 3.19 | 79.97 | ± | 1.07 | 5 |
| 2 | Female | 18.22 | ± | 4.21 | 79.02 | ± | 2.62 | 4 |
| 3 | Male | 16.00 | ± | 1.66 | 78.33 | ± | 1.80 | 4 |
| 3 | Female | 15.72 | ± | 1.42 | 76.01 | ± | 1.52 | 4 |
| 4 | Male | 16.10 | ± | 1.11 | 79.88 | ± | 2.05 | 4 |
| 4 | Female | 19.68 | ± | 2.77 | 79.41 | ± | 1.59 | 5 |
| 5 | Male | 13.36 | ± | 1.64 | 78.33 | ± | 1.80 | 4 |
| 5 | Female | 14.02 | ± | 2.56 | 79.24 | ± | 1.81 | 4 |
| 6 | Male | 10.89 | ± | 1.32 | 76.75 | ± | 2.00 | 4 |
| 6 | Female | 12.48 | ± | 1.89 | 79.65 | ± | 3.00 | 4 |
| 7 | Male | 15.22 | ± | 1.05 | 79.87 | ± | 1.48 | 5 |
| 7 | Female | 13.72 | ± | 0.76 | 80.13 | ± | 3.09 | 4 |
| 10 | Male | 15.35 | ± | 1.02 | 81.22 | ± | 1.82 | 4 |
| 10 | Female | 13.58 | ± | 1.62 | 77.39 | ± | 1.79 | 4 |
| 12 | Male | 13.58 | ± | 1.96 | 76.62 | ± | 1.11 | 4 |
| 12 | Female | 15.56 | ± | 1.62 | 78.26 | ± | 2.99 | 4 |
| 14 | Male | 11.38 | ± | 1.86 | 77.71 | ± | 1.01 | 5 |
| 14 | Female | 13.03 | ± | 1.92 | 76.27 | ± | 1.08 | 6 |
| 21 | Male | 11.50 | ± | 3.04 | 77.37 | ± | 1.06 | 6 |
| 21 | Female | 11.70 | ± | 0.83 | 76.42 | ± | 0.88 | 4 |
| 30 | Male | 14.36 | ± | 1.54 | 75.00 | ± | 1.15 | 4 |
| 30 | Female | 15.57 | ± | 1.11 | 75.08 | ± | 0.95 | 5 |
| 60 | Male & Female | 11.32 | ± | 0.97 | 74.63 | ± | 0.87 | 6 |
| Normal (Control) | Male & Female | 13.30 | ± | 1.66 | 73.34 | ± | 0.45 | 7 |

Table 2
Changes in the lipolytic activity of the breast muscle and blood serum of the pigeon during induced muscular atrophy.

| Atrophy in days | Sex | Lipase activity in muscle µl $CO_2$/mg. protein/hr. | | | Lipase activity in serum µl $CO_2$/ml./hr. | | | No. of Expts. |
|---|---|---|---|---|---|---|---|---|
| 1 | Male | 50.04 | ± | 3.43 | 5598.75 | ± | 859.95 | 4 |
| 1 | Female | 50.51 | ± | 4.55 | 5865.50 | ± | 569.59 | 4 |
| 2 | Male | 49.79 | ± | 3.82 | 4876.00 | ± | 670.73 | 5 |
| 2 | Female | 50.62 | ± | 6.26 | 4868.oo | ± | 1049.08 | 4 |
| 3 | Male | 51.16 | ± | 4.96 | 4818.00 | ± | 581.47 | 4 |
| 3 | Female | 61.64 | ± | 5.46 | 3925.25 | ± | 378.54 | 4 |
| 4 | Male | 80.36 | ± | 19.57 | 3579.50 | ± | 341.18 | 4 |
| 4 | Female | 119.09 | ± | 31.23 | 2609.40 | ± | 569.65 | 5 |
| 5 | Male | 108.55 | ± | 4.92 | 3563.00 | ± | 439.32 | 4 |
| 5 | Female | 112.62 | ± | 15.52 | 3185.75 | ± | 837.85 | 4 |
| 6 | Male | 74.52 | ± | 10.94 | 3768.75 | ± | 820.37 | 4 |
| 6 | Female | 116.98 | ± | 17.07 | 3776.50 | ± | 542.22 | 4 |
| 7 | Male | 68,89 | ± | 1.88 | 3239.75 | ± | 131.57 | 5 |
| 7 | Female | 104.58 | ± | 21.09 | 3345.50 | ± | 557.85 | 5 |
| 10 | Male | 49.21 | ± | 5.41 | 4473.75 | ± | 560.39 | 4 |
| 10 | Female | 69.05 | ± | 5.83 | 4524.29 | ± | 850.35 | 3 |
| 12 | Male | 39.26 | ± | 8.98 | 5229.50 | ± | 729.72 | 4 |
| 12 | Female | 40.66 | ± | 5.86 | 6202.67 | ± | 110.42 | 4 |
| 14 | Male | 30.01 | ± | 1.91 | 5822.50 | ± | 1215.30 | 4 |
| 14 | Female | 32.74 | ± | 5.31 | 5344.00 | ± | 661.90 | 5 |
| 21 | Male | 23.51 | ± | 5.81 | 5298.67 | ± | 743.51 | 6 |
| 21 | Female | 26.87 | ± | 4.87 | 3926.25 | ± | 311.88 | 4 |
| 30 | Male | 16.99 | ± | 4.54 | 6911.25 | ± | 994.28 | 4 |
| 30 | Female | 19.85 | ± | 4.67 | 6331.60 | ± | 854.81 | 5 |
| 60 | Male & Female | 30.67 | ± | 6.06 | 3698.00 | ± | 614.99 | 6 |
| Normal (Control) | Male | 38.19 | ± | 0.87 | 6653.00 | ± | 322.95 | 5 |
| | Female | 40.86 | ± | 6.21 | 6591.80 | ± | 706.83 | 5 |

in the serum was almost half than that of normal pigeons from the 3rd to the 7th days. The enzyme activity gradually rose, after a few ups and downs and became normal after a period of 30 days. But by the end of two months the enzyme level again fell lower than in the normal pigeons. Figure 5 and table 2 represents the lipase values of the blood serum during varying lengths of atrophy.

## Discussion

It should be mentioned that the device improvised for keeping the muscle unused is not without defects. Complete inactivity of the muscle is perhaps unattainable in experimental conditions. Slight wing movements while righting itself or during sex play could cause some contractions of the muscle, which would undoubtedly reduce the extent of disuse and thereby the rate of atrophy. It would therefore be more accurate to say that the plaster cast reduced the activity of the muscle by a very considerable extent. Eventhough, sufficient care was taken to keep the blood supply normal it is probable that a slight pressure might be exerted on the heart and the arteries in pumping blood to the wings, which were tied together in a backwardly extended position.

In the present investigations a marked decrease in total body weight of the pigeon was observed. Eccles (1941) in inactivated leg muscles of the cat reported a loss of 40% in the bulk of the muscle. Chor and Dolkart (1936) recorded a

12.8% loss in six weeks in the atrophied leg muscle of monkeys. The loss in muscle weight observed in these experiments, therefore should be mainly due to the reduction in muscle sarcoplasm.

An increase in water content of the muscle during the early stages of atrophy has been noted by several workers, Fischer and Ramsey (1946), Hines and Knowlton (1937) and Helander (1960). In the present investigation too, an increase in the water content of the muscle during the first two weeks of immobilization has been recorded. Fischer and Ramsey (1946) also showed a loss of about one third of the total proteins in disuse atrophy. A high reduction in total proteins was also observed in these experiments on the atrophied pigeon breast muscle (preliminary observations). However, a decrease in the water content of the blood corresponding to an increase of water content in the muscle during the early days of atrophy was most striking. The increase in the water of the muscle could be result of increased osmotic extraction of water from the blood inorder to eliminate nitrogenous wastes formed by the breakdown of muscle proteins. The period of greatest increase in water content of the muscle (first 10 days) more or less coincides with the period of greatest decrease in protein content of the muscle. Another source of water could be a result of tissue breakdown and utilization of proteins, carbohydrates and fats (metabolic water). But this probably could not be considered significant and at the same time there is an increase of water taken in by the birds. In support of the first possibility it may be

mentioned that the serum concentration became extremely low and the bird drank more water during this period. This would also mean that the increase in water content in the muscle was not merely due to the production of metabolic water. Further, that the serum concentration remained low and that a considerable amount of water was passed along with the excreta tends to show that the quantity of ingested water was not sufficient to balance the amount excreted. This problem requires further study on blood and urine under similar experimental conditions. It should be mentioned here that it was observed in some preliminary investigations that the reduction in proteins in the early stages of atrophy was not due to any increase in proteolytic enzyme activity since such enzyme activity was found to be too low during this period. Proteolytic enzyme activity assayed in the atrophied breast muscle according to the method of Kunitz (1947) using casein as substrate at a pH of 8.6 was found to be very low or negligible during the early days of atrophy.

On the first day of atrophy the total lipid content of the muscle suddenly shot upto a phenomenal figure of over 22% compared to a value of 13% for normal pigeons. Thereafter a gradual reduction followed, reaching almost to normal values by the end of first week, which remained fluctuating within narrow limits. By the end of one month the total fat content was again found to be increasing to about 15%. By the end of two months, however, a fall to about 11% i.e. 2% below normal level, was recorded. An increase in the lipid content in the

later periods of atrophy was reported by Tower (1939), Adams et al, (1954), Humoller et al, (1952) and several other workers. It was also observed by Friedlander et al, (1941) in rats after neurotomy, that the capacity of the denervated muscle to deposit labelled phospholipid increased 200% after 60 hours. In Helander's (1960) observations on the atrophied leg muscles of the rabbit a steady increase in the lipid content in relation to the duration of immobilization was reported. However, in the present study the results obtained after two months of immobilization of the muscles are contrary to those just mentioned. This may be attributed to certain physiological adjustments taking place in pigeons. The sudden increase in the total lipid content was not observed by earlier workers in the field on other animals, which is reported in this study. Instead of a steady increase in the lipid content in the muscle here it was observed a number of ups and downs in the lipid level during the first three weeks and particularly more in the first 10 days of immobilization, which is indicative of severe disturbances in the lipid metabolism of the muscle.

An increase in the lipolytic activity in the muscle after immobilization was noted in the present study. The enzyme activity was at its peak from the third to the seventh day of atrophy. The lipase activity, which is calculated per milligram of protein, appears to be rather high, because it is known from the work of Fischer and Ramsey (1946) that there is a fall in the total protein content of the atrophied muscle. This fall

in the muscle protein was also observed on further investigations in the atrophied pigeon breast muscle. The calculation of lipase activity per milligram of protein content is therefore bound to give an apparently high value. Nevertheless, even when a statistical correction is made for the decrease in the protein content, the nature of the curve remains essentially the same and there is a still distinct increase in the enzyme activity. The gradual decrease in the total fat content after the sudden increase in the beginning and the increase in the lipolytic activity of the muscle during the same days (3 to 7) may be considered as an indication of fat catabolism. A loss of about one third of the total proteins was noted in disuse atrophy by Fischer and Ramsey (1946). A decrease in total carbohydrates during denervation was reported by Lazere et al, (1943). The sudden increase in lipid content and the high reduction in total proteins seen in the pigeon breast muscle are therefore of considerable interest. Whether this increase in the lipid content may be possibily due to the synthesis of fat from other metabolites which are brokendown at a greater speed in the beginning of atrophy. Nevertheless, it is evident that there is a great disturbance in the metabolism of the muscle during the early period of atrophy. A severe disturbance in the lipid metabolism was also reported by Oppenheimer et al, (1958) in muscular dystrophy produced by vitamin E deficiency.

In the study of the blood serum lipase in the pigeon during atrophy of the breast muscle, it was found that there

was a profound decrease in the activity of the enzyme corresponding to an increase in the enzyme activity of the muscle. This decrease in the serum lipase activity corresponding to the increase in the muscle was observed in every case, indicating that there is a relationship between the blood serum lipase and the muscle lipase. It is often inferred the changes in the serum enzyme activity is due to a rapid destruction of tissue, which will temporarily enhance the serum enzyme activity, particularly of those enzymes which are present abundant in that tissue. This hypothesis,however, fails to explain the fluctations in the lipolytic activity of the muscle and serum in the present studies, since there is a striking decrease in the enzyme activity of the blood serum during the early stages of atrophy when there is a greater tissue destruction.

A striking difference in the lipase activity between the two sexes (Fig. 4 and Table 2) is interesting. The enzyme activity in the muscle of females was always found to be higher than that of the males, particularly during the first week. The physiological significance of such a difference between the two sexes is difficult to explain.

It is concluded that the assay of serum enzyme activities particularly of lipase is of considerable diagnostic value. The decrease in the blood serum lipase activity at the onset of atrophy could be profitably employed as a diagnostic feature in humans, provided similar conditions occur in the human system also.