INTRODUCTION

It is a well known fact that the diaphragm of mammals is an organ, playing an important role in the respiration. It is a muscular organ having an intermediate position between constantly working heart muscle and intermittently contracting appendicular muscle. It starts its activity just after parturition and stops at death, rather it works from the first breath till the last (Smith, 1963). Wade (1954) was able to measure vital capacity volume of the thorax by tracking the X-ray shadows of each dome of the diaphragm. He observed that one fourth of the vital capacity was attributable to the chest expansion while three fourth of the volume was attributed to the diaphragm. Jones et al. (1953) made electromyograph recordings of the respiratory muscles. Their general conclusions are that the intercostals supply only the tension to keep the ribs at constant distances from each other while the major chest expansion is due to this diaphragm. Davis and Morris (1953) could demonstrate a tremendous reduction in the minute volume by phrenectomy in the rats. All the above workers emphasize the importance of diaphragm in the respiration.

The diaphragm is not a homogeneous structure but consists of three distinct regions (Fig. 1). Morphologically these three different regions namely dorsal (vertebral), lateral (costal) and ventral (sternal) have been described in the human

being by Johnston and Whillis (1949). Recently the significance of the regional differences in the physiology of the diaphragm was emphasized by George and Susheela (1961).

It is a mixed muscle having two basic types of fibres the red and the white. On suitable staining intermediate forms designated as "intermediate fibres" could be distinguished.

The three different types of fibres were distinguished by the treatment for the histochemical demonstration of succinate dehydrogenase (SDH). The tissue sections stained for the histochemical demonstration of lipase and fat also exhibit the presence of three types of fibres in the diaphragm. The distribution pattern of these fibres does not show any definite arrangement. The three types of fibres could be distinguished in all the regions of all the mammals investigated. With further staining these fibres were characterised as red, narrow fat loaded fibres; the white, broad glycogen loaded fibres and the intermediate ones which are mid-way in these characteristics. A comparative study of the mammalian diaphragm with special reference to the cellular organization was made. The diaphragm of mammals of various sizes was investigated, with a view to find out this correlation between the size of a particular mammal and cellular organization of its diaphragm.

Further the comparative study was extended with a view to study the histochemical nature of the diaphragm of the mammals of various sizes. The comparative histochemical study was made with a special reference to the enzymes like lipase, SDH (succinate dehydrogenase) and the metabolite like fat. The distribution pattern of lipase, SDH and fat exhibited the difference in the three types of fibres. The tissue sections stained for all the above substances showed greater intensity in the narrow fibres while the white fibres exhibited the least intensity of staining, the intermediate fibres were really having the intensity which can justify them being considered as intermediate among the three types of fibres. The comparative histochemical study could also give an additional support to the view that the diaphragm of the small mammals has to work more as compared to the mammals bigger in size. The correlation between the size of the mammal and the histochemical nature of its diaphragm was clearly noticed.

Alkaline phosphatase was reported to be absent in the skeletal muscles of the various animals. Even in the human foetal and the adult human muscle it was said to be absent. A positive staining reaction was obtained in the pigeon breast muscle after prolonged incubation (George, Nair and Scaria; 1958). In the light of the above reports, it was thought desirable to investigate the possibility of demonstrating this enzyme in the mammalian diaphragm. During the present investigation it was found that the histochemical

demonstration of alkaline phosphatase is possible only after prolonged incubation. Secondly alkaline phosphatase activity was found to be localized in the sarcoplasmic reticulum. No difference in the distribution pattern of enzyme activity was seen between the different types of fibres.

Same as alkaline phosphatase the acid phosphatase was claimed to be absent in the skeletal muscle. Goerge and Pishawikar (1961) successfully demonstrated the histochemical localization of it in the pigeon pectoralis by using Glick's method. Recently Ogata and Mori (1963) failed to demonstrate this hydrolytic enzyme in the muscles of various animals by employing the azo-dye coupling technique. As against this Greenstein (1942) demonstrated its presence in the mouse skeletal muscle while Vallyathan and George (1965) demonstrated its presence in the pectoralis of pigeon. In the light of the above reports it was thought desirable to investigate the possibility of demonstrating this enzyme in the diaphragm. During this investigation it was found that the enzyme can be localized in the diaphragm by employing Glick's technique. The enzyme was found to be present in the sarcoplasm, and no difference in the distribution pattern of enzyme activity was seen between different fibres.

Histochemical investigations of the cholinesterase and the morphology of the neuro-muscular junctions were carried out in the diaphragm of various mammals. No correlation was

found between the type of the nerve-ending and the type of the muscle fibre. In all the muscle fibres the nerve-endings were of "en plaque" type only. Acetyl cholinesterase (AChE) and Butyryl cholinesterase (BuChE) were found to be present at the same nerve-endings. AChE was found to be more than BuChE in all the three regions of the diaphragm of all the mammals investigated. The area of contact surface was judged from the diameter of the nerve-endings. In this investigation the existance of a correlation between the body size of the animal and the enzyme activity could be clearly observed. From the study of the area of contact surface (on the basis of the diameter of nerve-endings) it could be clearly noticed that the contact surface is large in smaller mammals having higher respiratory rate.

The histochemical investigation on cholinesterases was conducted in the diaphragm of human foetus and the adult human. This study was carried out with a view to study their enzymic levels and the morphology of the nerve-endings in the pre-natal, post-natal and the adult human beings. As it is a well known fact that the cholinesterase activity develops at an early stage in the development of muscle (Gerebtzoff, 1959). It was observed that by the end of intra uterine life (pre-natal life) and during the first few weeks of extra uterine life (post-natal life) the activity of cholinesterases of skeletal muscle was considerably higher than in the adult skeletal muscle (Nachmansohn, 1938; Liebson, 1939). High concentrations

of cholinesterase and myosin cholinesterase were noticed in the development of the rabbit gastrocnemius muscle by Varga et al. (1957). Khera and Laham (1965) demonstrated high myristoyl cholinesterase activity in the thigh muscles in the duck embryos at the 19th day of incubation, but end-plates reacting with acetylthiocholine were not observed, such end plates were however many in three day old ducklings. The butyryl cholinesterase first appeared in 19 day old embryos and the number of butyrylthiocholine reacting end-plates increased during the following two days. Chinoy and George (1966) studied the cholinesterase activity of embryonic and post-embryonic pigeon pectoralis muscle. They studied Acetyl- and Butyryl cholinesterase activity from 9th day of pre-natal stage to hatching time. Acetyl cholinesterase activity was found to be higher than the butyryl cholinesterase activity in all the stages studied. The activity of these enzymes was found to be high towards the end of pre-natal development and during the early post-natal stages. In the light of the above findings the present histochemical investigation was carried out to collect some information about the level of cholinesterase activity and the morphology of neuro-muscular junctions during pre-natal, post-natal and the adult human.

The effect of exercise on the succinate dehydrogenase (SDH) and the glycogen content in the rat diaphragm was

studied. This study was conducted with a view to study the changes in the level of SDH activity and glycogen content during the different durations of exercise. These observations showed that the important role in the prolonged respiratory activity is played by the lateral region of the diaphragm. This also aided to conclude that for the long sustained and strenuous activity this organ uses fat as the fuel with the help of the oxidative enzyme like SDH.

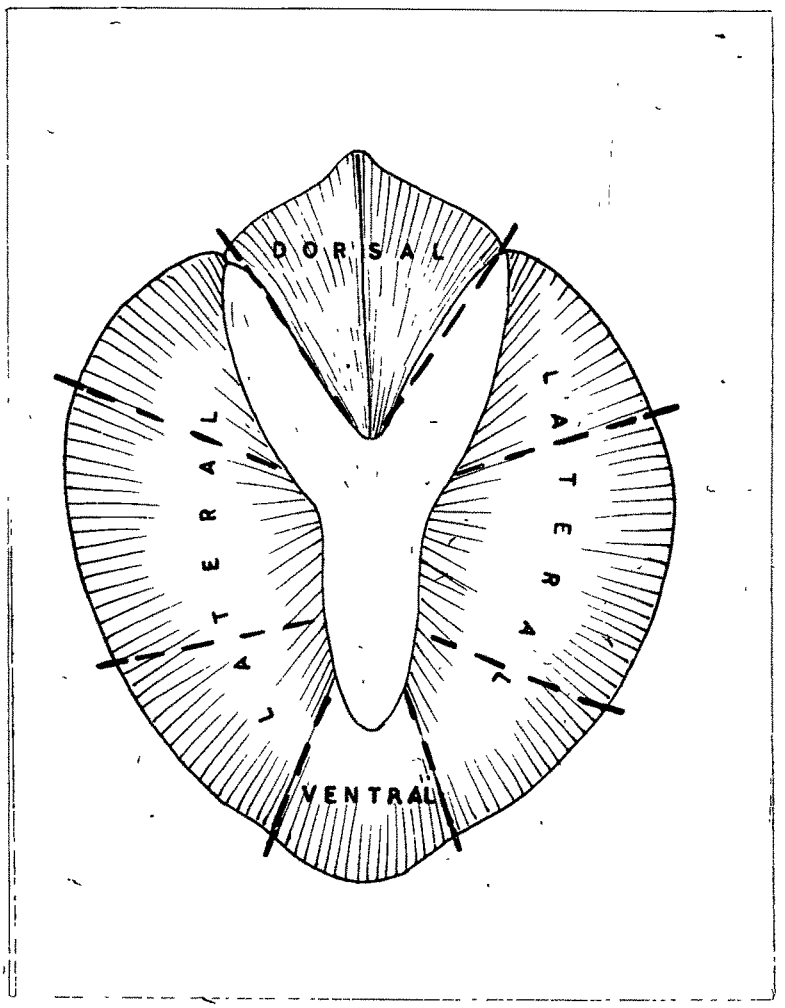

Fig. 1

Diagrametic representation of the mammalian diaphragm showing the three different regions namely '_D'- Dorsal (vertebral), '_L' - Lateral (costal) and '_V' - Ventral (sternal).

CHAPTER 1

THE CELLULAR ORGANIZATION OF THE MAMMALIAN DIAPHRAGM

A comparative study of the skeletal muscles like pectoralis of birds (Chandra-Bose and George, 1965), the muscles of the arm and fore-arm (Nene, 1966) and the muscles of the diaphragm (George and Susheela, 1961; Gauthier and Padykula, 1966) have revealed the fact that there is a definite correlation between the histochemical nature of the muscle and its functional activity. Studies on the structure and the physiology of the flight muscles of birds, particularly the avian pectoralis have shown the existance of relationship between structure and function and that an identical organization of fibres may be met within the pectoralis of birds belonging to different orders but having the same mode of flight (Chandra-Bose and George, 1965). The study of the diaphragm by previous workers has demonstrated a relationship between the cellular organization of the diaphragm and its histochemical nature and the body size of the mammal (George and Susheela, 1961; Susheela, 1963; Gauthier and Padykula, 1966).

It is now an established fact that the diaphragm is a mixed muscle having two basic types of fibres as described by George and Susheela (1961), but the use of certain histochemical techniques differentiates three types of fibres taking part in the formation of this mixed muscle.

These three types of fibres are namely red fat loaded, white glycogen loaded and the intermediate ones which are mid-way in these characteristics. A tissue section stained for succinate dehydrogenase (SDH) localization clearly shows the above mentioned types of fibres. Ogata (1958) demonstrated these three types of fibres in the diaphragm of cat; and Padykula and Gauthier (1963) in the diaphragm of albino rats. Susheela (1963) showed the presence of these three types of fibres in the goat diaphragm. Most recently Gauthier and Padykula (1966) made a comparative study of the mammalian diaphragms in order to demonstrate a relationship between the cytological characteristics of diaphragm and the body size. Their investigation was limited to the right costal region only, in all the species studied by them. As it is a well accepted fact that the histochemical pattern and the cellular organization varies in the different regions (George and Susheela, 1961) the present investigation was made with a view to compare the three different regions namely the dorsal, lateral and ventral in the mammals of various sizes.

MATERIALS AND METHODS

In the present investigation the three regions of the diaphragm of the mammals like rat, rabbit, cat, civet cat, dog and fox were studied with special reference to the nature, number, distribution pattern and diameter. The three different regions of the diaphragm of the mammals like rat, rabbit, cat, civet cat,

dog, fox, hedge hog and monkey were studied with special reference to the distribution pattern of SDH, lipase and fat. The animals were killed either by decapitating them or by cutting the jugal vein, and allowing them to bleed till death, depending upon the size of the animal. Smaller animals were decapitated while the larger ones were killed by the later method. After killing the animals the diaphragm was quickly removed and then it was blotted well to remove the blood. It was then spread on the clean and dry filter paper. The regions were separated as described by George and Susheela (1961). The three regions are the dorsal (vertebral),lateral (costal) and the ventral (sternal). From each of the three regions the strips of the tissue were taken into the line with the orientation of the muscle fibres. From the muscle strips transverse sections were cut for the present investigation. For differentiating the different types of fibres, method for the histochemical localization of SDH is used. The tissue sections were first stained for SDH and the fibre diameter as well as the number of fibres per unit area were counted by the following methods. For histochemical studies transverse tissue sections were stained for the histochemical localization of SDH, lipase and fat by the methods described below.

Measurement of the fibre diameter and fibre count

The tissue sections stained for SDH localization were used for these purposes. With this staining the three different types

of fibres can be distinugished showing the difference in the intensity of staining. The respective number of red, intermediate and white fibres within a certain field were counted with the help of a fibroscope. The diameter of the respective fibres was determined with the help of an eye-piece and a micrometer scale.

Histochemical:

Succinate Dehydrogenase (SDH)

Thin frozen transverse sections were stained for the histochemical localization of SDH by an improved method of George and Talesara (1961a) using Neo-tetrazolium chloride as hydrogen acceptor. The sections were first placed in ice cold 0.1M. Phosphate buffer (pH 7.4 to 7.6) for 10 minutes to destroy the endogenous substrate. They were then placed in the incubation medium at 37°C for 5-10 minutes. The purple granules of the diformazan formed indicated the sites of enzyme activity. The sections stained for SDH were fixed by a short treatment of dil. neutralized formalin, and were mounted in glycerine jelly after rinsing them with distilled water. The enzyme activity was judged by the duration of the incubation required for the clear appearance of the enzyme activity and the intensity of staining.

Lipase

The lipase activity was studied by an improved method of George and Iype (1960a). Thin frozen transverse sections were placed on the clean slides previously coated with 2.0% gelatin

and allowed to dry at room temperature. To fix the tissue sections the slides were placed in 6.0% neutral formalin for 3-4 hours. The sections were then coated with 2.0% gelatin and again fixed in 6.0% neutral formalin. The slides along with the tissue sections were washed thoroughly under running tap water and rinsed well with distilled water to remove formalin. The sections were incubated for 16-24 hours at 37°C in an incubation medium containing 'Tween 85' as the substrate. After incubation, the sections were washed well with distilled water. The sections were then treated with lead nitrate for half an hour and then with dilute yellow ammonium sulphide. Finally the sections were rinsed with 1-2 % acetic acid for clearing them. The black-brown precipitates of lead sulphide were formed at the sites of enzyme activity.

Fat

Thin frozen sections (transverse) were cut from the fresh tissue and were spread on the clean and dry slides. The sections were allowed to dry at room temperature for some time. These tissue sections were then fixed in 10.0% calcium formol for 3-4 hours, after which they were washed well under running tap water and rinsed well with distilled water to remove the fixative completely. The sections were stained with the 'sudan black B' stain and mounted in glycerine-jelly.

RESULTS

The relative distribution of the three types of fibres in the different regions of the diaphragm of the various mammals is given in Table 1.

The following facts can be seen from Table 1.

(1) In all the regions of the diaphragm of all the mammals studied the three different types of fibres can be clearly distinguished with the histochemical technique to localize SDH (Figs. 1 to 11).

(2) The red fibres are smaller in diameter in the lateral region amongst the three regions of the diaphragm. The red fibres are more in number in the lateral region as compared to the other two regions.

(3) The white fibres are usually bigger in diameter and are less in number in the lateral region as compared to the other two regions.

(4) The intermediate fibres with intermediate characteristics (such as the diameter and the staining intensity) are mostly broader than the red fibres but are narrower than the white fibres. They are less in number in the lateral region as compared to the other two regions.

(5) The diameter of the red fibres is smaller in the smaller mammals as compared to the mammals bigger in size. The diameter of white fibres is more in smaller mammals in

Cellular Organization of the mammalian diaphragm in different mammals

No. & Animal	Region of the Diaphragm	No. of Fibres in per cent per unit area			Ratio of the fibres per Unit area R : I : W	The Diameter of the fibres in μ			Diameter Ratio R : I : W
		RED	INTERMEDIATE	WHITE		RED	INTERMEDIATE	WHITE	
1. RAT (5)	Dorsal	54.33	14.37	31.29	3.8:1:2.2	56.33	70.50	93.16	1:1.25:1.6
	Lateral	58.35	16.26	25.13	3.6:1:1.5	42.13	67.33	95.13	1:1.6 :2.2
	Ventral	49.89	16.34	33.82	2.8:1:1.9	45.83	67.81	84.66	1:1.8 :1.8
2. RABBIT(5)	Dorsal	43.02	23.71	33.28	1.8:1:1.4	49.50	49.60	61.20	1:1 :1.2
	Lateral	52.05	16.99	30.97	3.2:1:1.8	49.00	54.50	71.20	1:1.1 :1.4
	Ventral	49.67	16.10	34.05	3.1:1:2.1	50.00	53.60	33.30	1:1 :1.2
3. CAT (5)	Dorsal	35.02	23.92	41.05	1.5:1:1.7	60.40	65.60	107.20	1:1.1 :1.8
	Lateral	43.54	20.75	35.71	2.1:1:1.7	58.00	65.60	93.20	1:1.1 :1.6
	Ventral	41.34	27.94	36.62	1.7:1:1.3	60.50	78.50	107.75	1:1.3 :1.8
4. CIVET CAT (2)	Dorsal	34.11	15.55	50.34	2.2:1:3.2	61.33	66.16	75.66	1:1.1 :1.2
	Lateral	50.00	12.96	37.04	3.8:1:2.8	45.16	49.80	70.66	1:1.1 :1.5
	Ventral	42.98	13.78	43.25	3.1:1:3.1	41.50	54.00	73.16	1:1.3 :1.8
5. DOG (5)	Dorsal	43.16	23.22	33.62	1.9:1:1.5	56.00	73.90	79.60	1:1.3 :1.4
	Lateral	49.06	22.64	28.30	2.1:1:1.2	51.75	77.50	79.75	1:1.5 :1.5
	Ventral	50.81	23.52	25.68	2.3:1:1.1	52.50	72.00	68.75	1:1.4 :1.3
6. FOX (1)	Dorsal	38.23	28.68	33.11	1.3:1:1.1	45.33	55.50	71.66	1:1.3 :1.6
	Lateral	47.76	22.79	30.14	2.1:1:1.3	44.50	66.50	69.83	1:1.5 :1.5
	Ventral	33.62	30.75	35.63	1.1:1:1.2	44.33	59.66	89.33	1:1.3 :2.0

Note: The number in the (...) denotes the number of specimens used.

comparison with that of larger mammals. The intermediate fibres also appear to be following the same pattern as the white fibres.

(6) The red fibres are more in number in the smaller mammals while in the larger animals they are less. The white fibres are less in number in the smaller mammals as compared to the mammals larger in size. The intermediate fibres follow the same pattern as the white fibres with respect to the number of fibres per unit area.

Histochemical:

SDH

The SDH localization distinguished three types of fibres in the mammalian diaphragm. The red narrow fibres showed the highest intensity, the intermediate fibres showed less intensity of staining than the red fibres but more intensity than the white fibres, while the white fibres exhibited the least activity. The above mentioned pattern of distribution was observed in all the mammals investigated. The staining intensity was more in the lateral region and the dorsal region, while the ventral region exhibited the least activity in all the mammals studied. The enzyme activity was found to be the highest in the rat and Hedge hog and lowest in the dog, fox and monkey, while in rabbit and cat it was comparatively less than rat but more than dog and monkey. As the period of incubation required for the clear appearance of the enzyme activity is very short the intensity is determined

by visual observations.

Lipase

The distribution pattern of lipase is also the same as SDH. Lipase is found to be more in the red narrow fibres, least in the white broad fibres while in the intermediate fibres it was found to be more than the white fibres but less than the red fibres (Figs. 12 to 20). The lipase is found to be more in the dorsal and the lateral regions, it is least in the ventral region. Amongst the dorsal and the lateral regions the lipase activity was found to be slightly more in the dorsal region in some cases. From the table given below it can be observed that the lipase activity is higher in the smaller mammals than the bigger ones.

Table 2

Showing the level of lipase activity as determined on the basis of the period of incubation in terms of hours.

Animal	Dorsal region	Lateral region	Ventral region
Rat	16	17	20
Rabbit	18	20	21
Cat	18	20	23
Dog	22	22	24
Monkey	24	24	24
Fox	22	21	22

Fat

The fat was also found to follow the similar pattern as that of SDH and lipase. The 'sudan black B' staining differentiates the three types of fibres constituting this mixed muscle. The red fibres were found to be loaded with fat. The white and the intermediate fibres are less sudanofilic than the red fibres (Figs. 21 to 30). Amongst the white and the intermediate fibres, the intermediate fibres are comparatively more sudanofilic. Amongst the three regions the dorsal region showed higher fat contents while the intensity of staining was found to be the least in the lateral region. The smaller mammals showed higher fat content in the diaphragm as compared to the larger ones. The observations with regard to fat are visual observations.

DISCUSSION

The diaphragm is a muscular organ which has an important position next to the heart in the mammals, having a continuous and similar function in all the mammals. Its function is to assist in respiration. Its activity amongst the different mammals varies with the body size of the animal. In general its activity is found to be inversely proportional to the body size of the mammal. The respiratory rate is higher in the smaller mammals as compared to the larger ones, for example the respiratory rate in a mouse is much higher than in man

(Crosfill and Widdicombe, 1961). The metabolic rate of an animal is also inversely proportional to the body size of the animal (Benedict, 1938; Krebs, 1950; Zeuthen, 1955). Krebs (1950) also suggested that the variation in the metabolic rate according to the body size also involves the variation in the cellular organization and the histochemical nature of the muscle fibres constituting the diaphragm.

Gauthier and Padykula (1966) have shown that the histochemical localization of enzymes like succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) confirms the pattern of mitochondrial distribution observed after staining with 'sudan black B', which means that the enzyme SDH and the metabolite fat are localized in the same fibres and they are found to be localized in the mitochondria. The lipase is also found to be localized in the same fibres in which SDH as well as fat are localized. Moreover the lipase is found to be localized in the mitochondria. The mitochondrial content of the muscle fibre within an individual is said to be inversely proportional to the diameter of that fibre (Porter and Palade, 1957; Nachmias and Padykula, 1958; Dubovitz and Pearse, 1960; George and Susheela, 1961; Padykula and Gauthier, 1963). Thus the red fibres which are smaller in diameter in all the smaller mammals contain more mitochondria than the white or the intermediate, of the diaphragm of the same mammal. This is because of the fact that the red fibres are narrow, the white fibres are broad and the intermediate

fibres are broader than the red fibres and narrower than the white fibres.

The histochemical localization of SDH was studied and it was observed that the enzyme is found to be more in the red fibres which are more in the lateral region as compared to the other two regions in all the mammals. Its activity in the lateral region is more. In smaller mammals its activity is found to be more which decreases in comparatively larger forms. Thus its level is proved to be inversely proportional to the body size.

With the histochemical study of lipase localization it can be observed that its activity is more in the red fibres than the intermediate fibres, in the white fibres it is found to be the least, rather it gave negative reaction. Its level is also higher in the diaphragm of smaller forms as compared with the same in the larger forms. Susheela (1963) obtained similar results with the rat and the goat diaphragm during her studies of the above mentioned enzymes namely SDH and lipase.

The red fibres are loaded with fat and have higher fat content as compared with the intermediate fibres. The white fibres did not take any stain. The fat content also varies in quantitative manner according to the body size of the mammal. The higher fat content in the dorsal and the ventral regions of the diaphragm in all the mammals studied suggest

that the dorsal and the ventral regions are acting as the storage organ of fat which can be broken down with the aid of lipase and can be brought to the lateral region in the form of fatty acids (George and Susheela, 1961).

In the mammals studied, it was found that the red fibres are more in the diaphragm of smaller mammals while in the large mammals they are found to be fewer in number. The red fibres in the smaller mammals are smaller in diameter. Thus it can be concluded that the number of red fibres is inversely proportional to the body size while their diameter is directly proportional to the body size of the animal. As the red fibres are specialized for a long and sustained activity it can be expected that they are more in number in the smaller mammals with higher respiratory rate as compared with that of the larger mammals. The red fibres are more and with smaller diameter in the smaller mammals present a greater surface area for the exchange of gases, ions and metabolites than does an equivalent total mass of red fibres with larger diameter in the larger mammals. The red fibres are more in number and having less diameter suggests that they have more number of mitochondria. As there are numerous mitochondria the enzymes like SDH and lipase are also more, the fat is also more. SDH, lipase and fat are found to be more in the smaller mammals because they are localized in the mitochondria.

The intermediate fibres are smaller in number in the smaller mammals but their percentage is found to be considerably higher in the bigger mammals. The number of intermediate fibres is directly related to body size. The diameter of the intermediate fibres is smaller in smaller mammals while that is bigger in bigger mammals.

The white fibres are less in number in smaller mammals while they are more in the bigger mammals. Thus their number is directly proportional to the body size of the animal. The fibre diameter of the white fibres is found to be inversely proportional to the body size of the animal. From these facts it can be concluded that the diaphragm in the smaller mammal has more activity than that of a larger one. The presence of more and predominating white fibres in larger mammals can safely be correlated with the slow respiratory rate or breathing rate.

From the above mentioned facts it can be clearly seen that the fibre diameter of the various fibres of the diaphragm, bears a definite relationship with the body size of the mammal. Recently it has also been shown by Gauthier and Padykula (1966) that there exists a relationship between the fibre diameter and the body size of the animal by their studies on the right costal region of the diaphragm in different mammals.

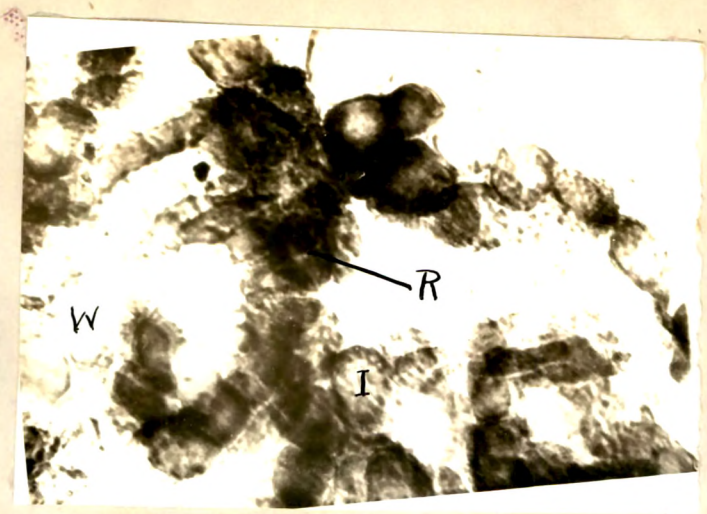

Fig.1

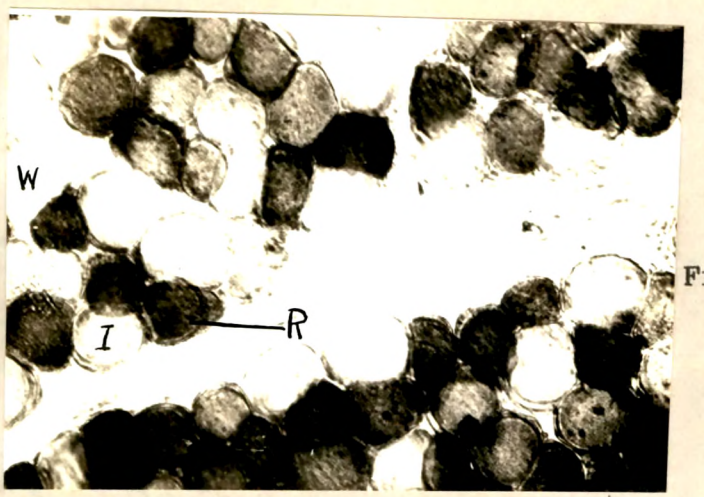

Fig.2

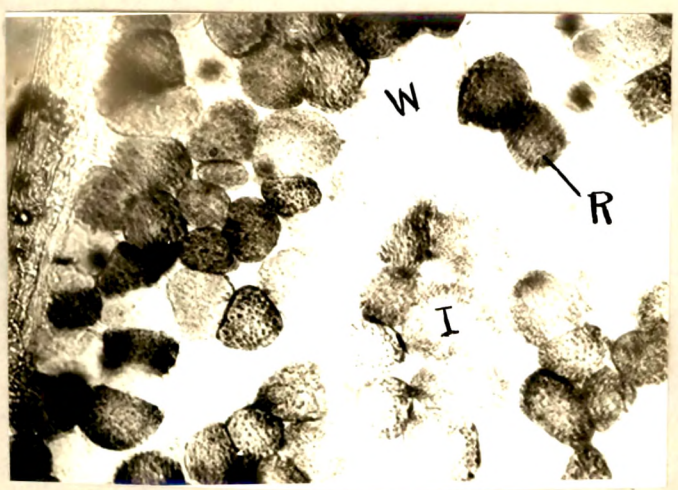

Fig.3

Figs. 1, 2 & 3 Histochemical demonstration of SDH in the diaphragm of Fox. The three types of fibres are seen. 'R' Red, 'I' Intermediate and 'W' White in all the regions of the organ. Figs. 1, 2 & 3 are showing the activity in the dorsal, lateral and ventral regions respectively.

All 128 x.

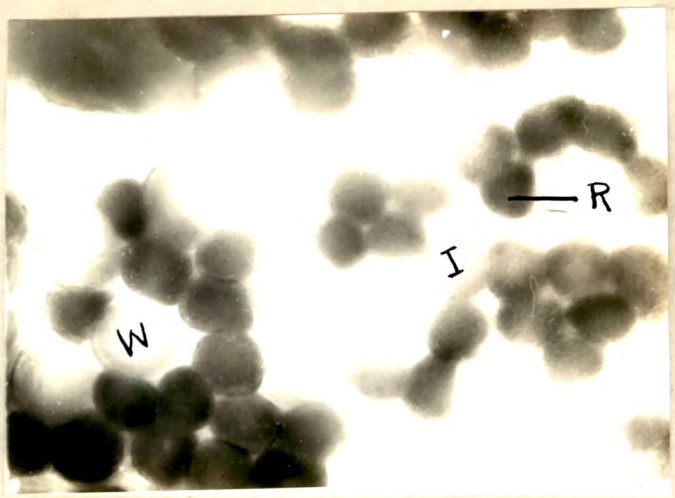

Fig.4

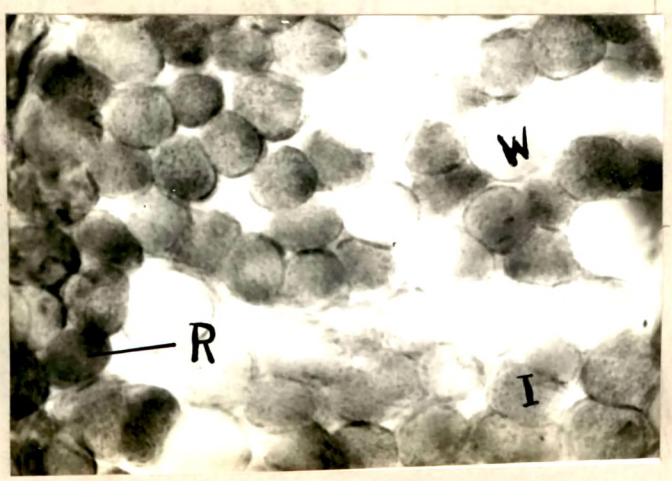

Fig.5

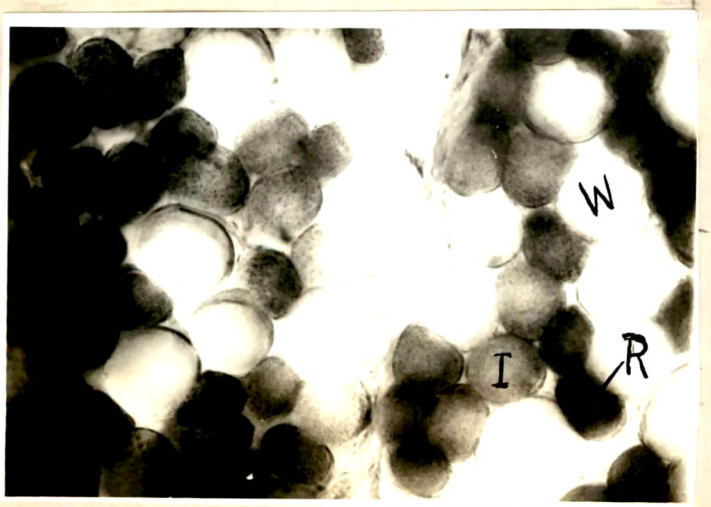

Fig.6

Figs. 4, 5 & 6 Histochemical demonstration of SDH activity in the diaphragm of Monkey. The transverse muscle sections show the three types of fibres after staining sections for the histochemical demonstration of SDH activity. The three types of fibres: 'R'-Red, 'I'-Intermediate and 'W'-White fibres. The Figs. 4, 5 & 6 show the localization of enzyme activity in the Dorsal, Lateral and Ventral regions respectively

All. 128 x

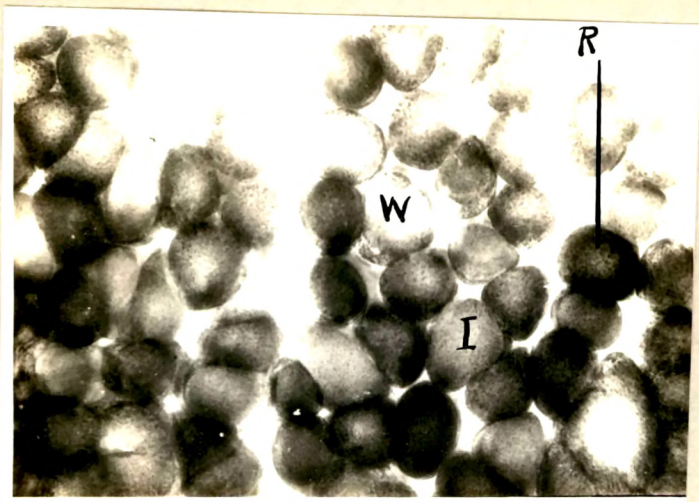

Fig.7

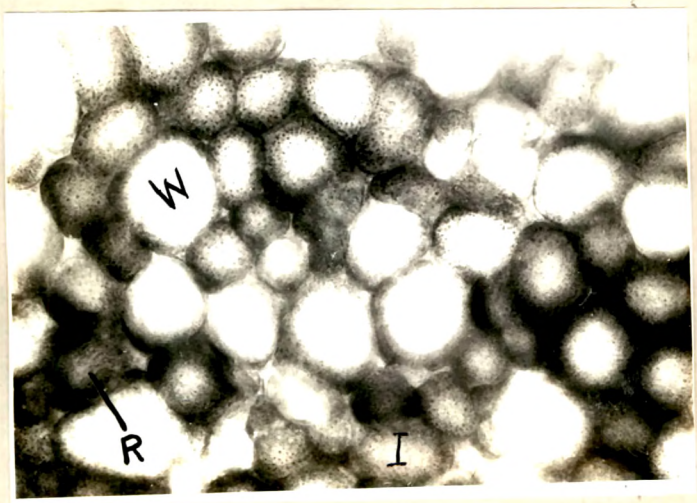

Fig.8

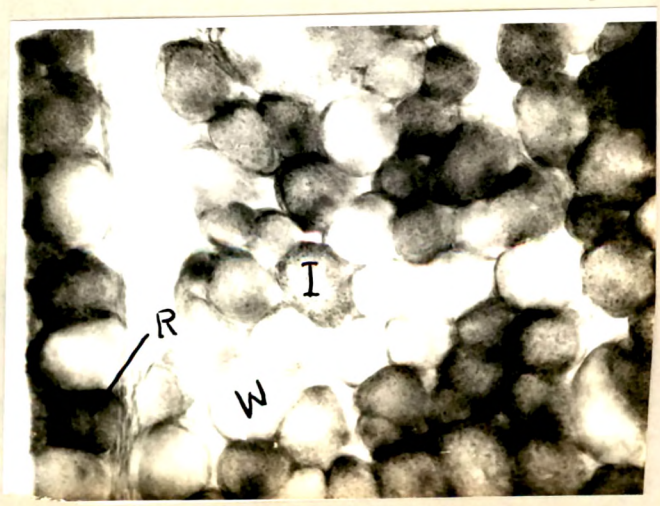

Fig.9

Figs. 7, 8 & 9 showing the three types of fibres: 'R'-Red, 'I'-Intermediate and 'W'-White in the Dog diaphragm after treating the tissue sections (Transverse) for the histochemical demonstration of SDH. Figs.7,8 & 9 show the histochemical demonstration of SDH in the Dorsal, Lateral and Ventral regions respectively

All 128x

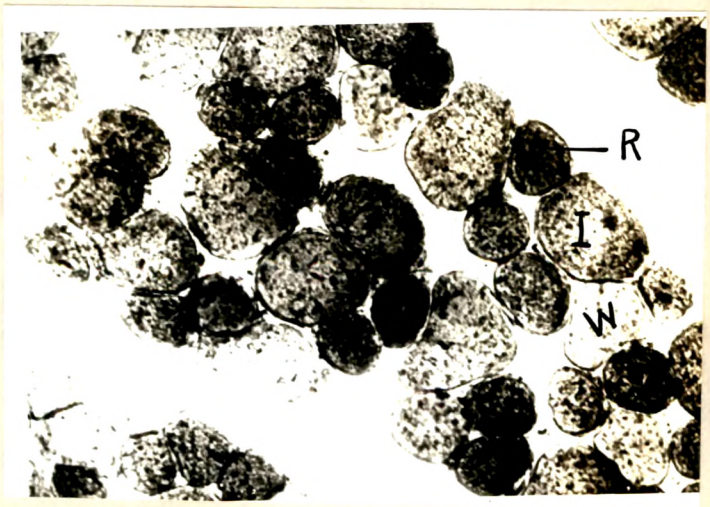

Fig. 10

Histochemical localization of SDH in the diaphragm of Hedge hog, showing the red, intermediate and white types of muscle fibres in transverse muscle sections. 128X

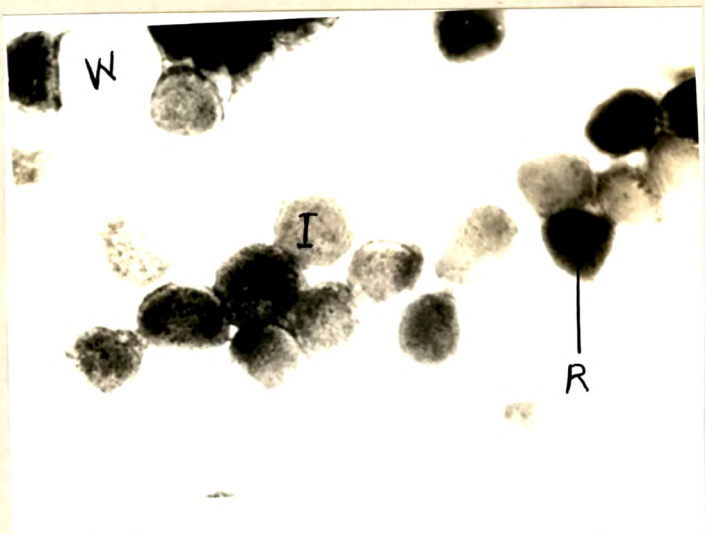

Fig. 11

Histochemical localization of SDH in the diaphragm of Cat, showing the red, intermediate and white types of fibres, in the transverse muscle sections. 128 x

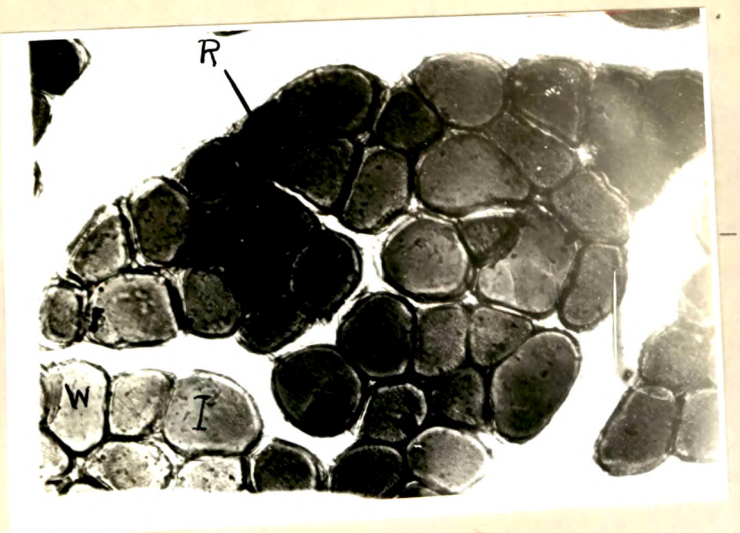

Fig.12

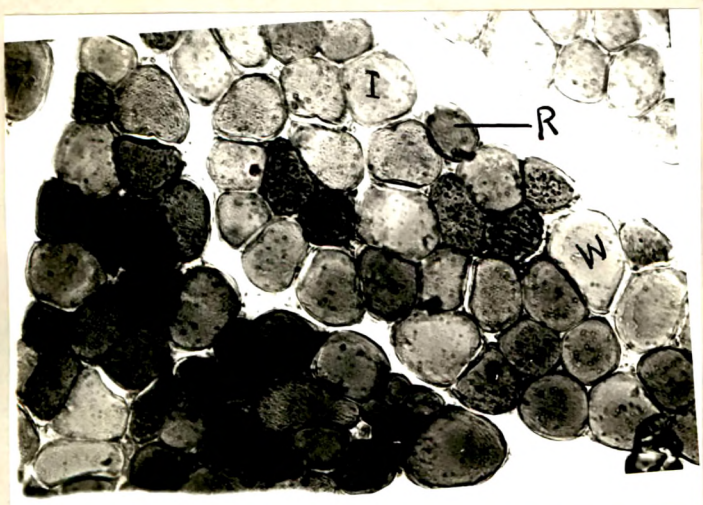

Fig.13

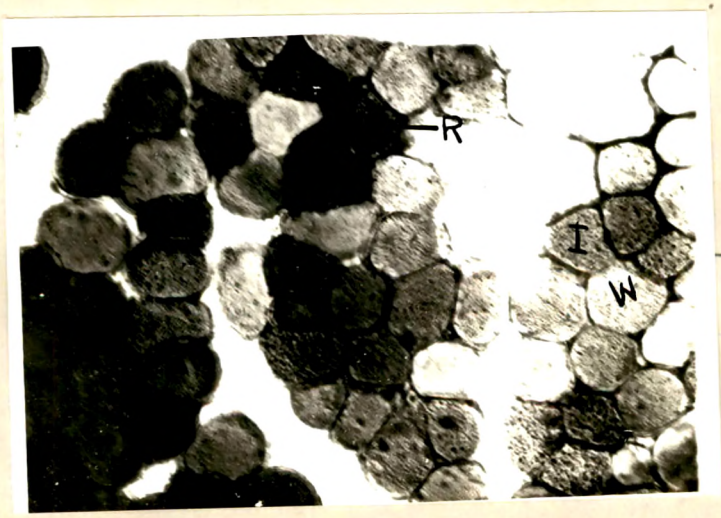

Fig.14

Figs.12,13 & 14 Histochemical demonstration of Lipase activity in the diaphragm of Fox. The activity is seen different in the different types of fibres. Lipase activity follow the similar pattern of distribution as SDH and Fat. The Figs. 12,13 & 14 represent the enzyme activity in the dorsal, lateral and ventral regions respectively.

All 128x.

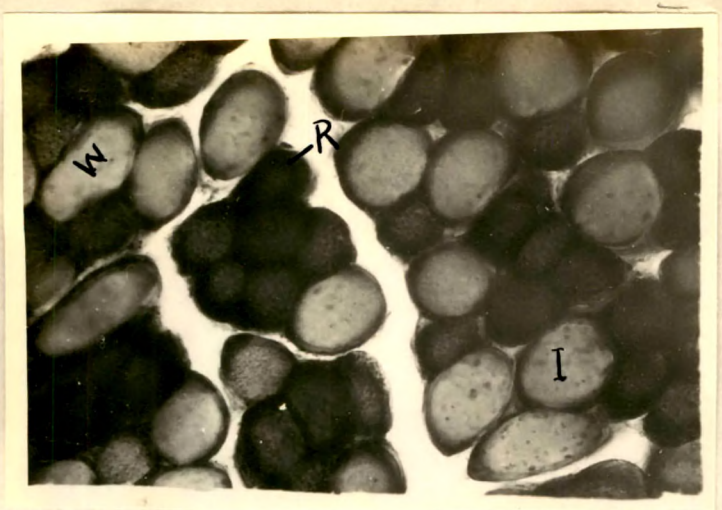

Fig.15

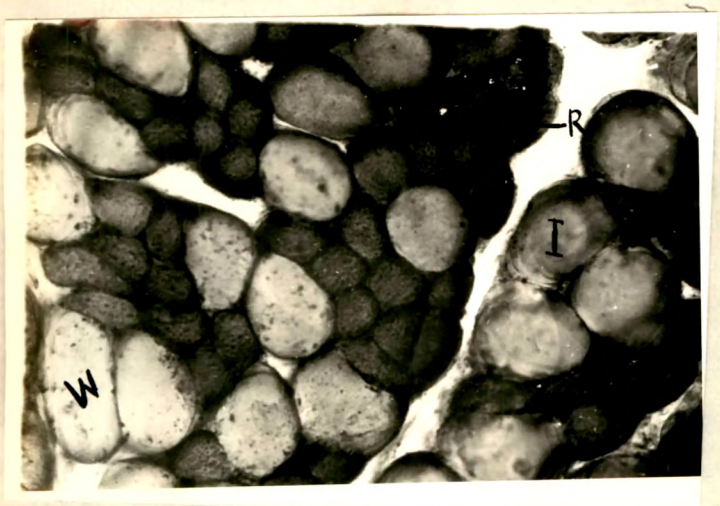

Fig.16

Figs.15,16 & 17 Histochemical demonstration of Lipase activity in the diaphragm of Rat. The activity is seen different in different types of fibres. Lipase activity is highest in the narrow fibres (Red fibres).

AII 128 x

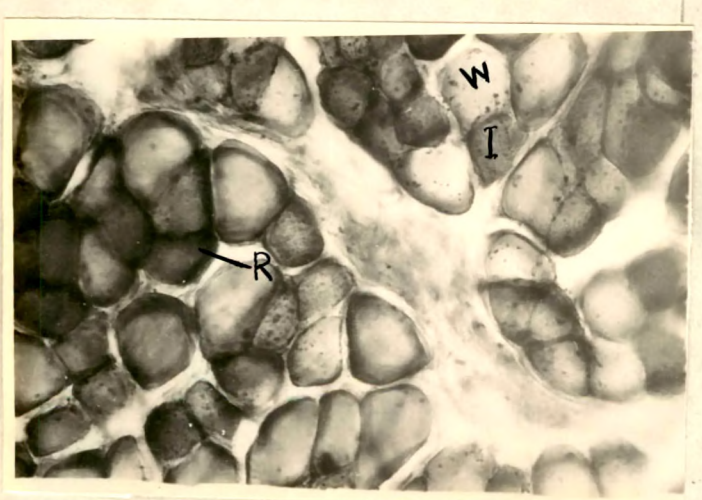

Fig.17

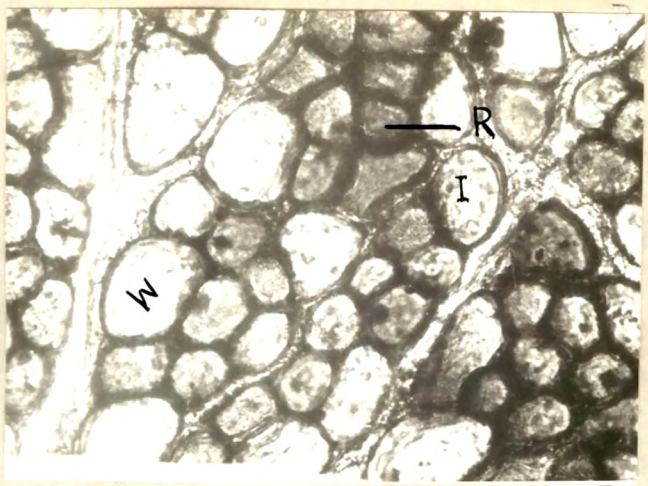

Fig.18

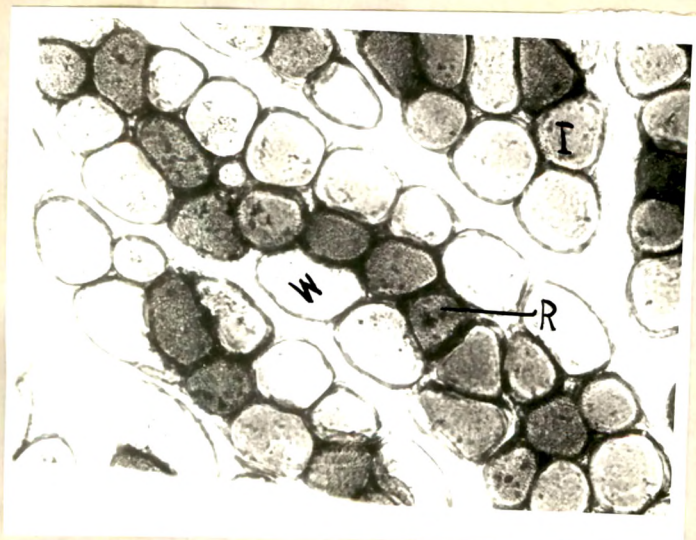

Fig.19

Figs. 18, 19 & 20 Histochemical demonstration of Lipase in the diaphragm of Civet cat. The localization of lipase activity also exhibits the three types of fibres having different intensity of staining. Figs. 18, 19 & 20 show the enzyme activity in dorsal, lateral and ventral regions respectively.

All 128 x.

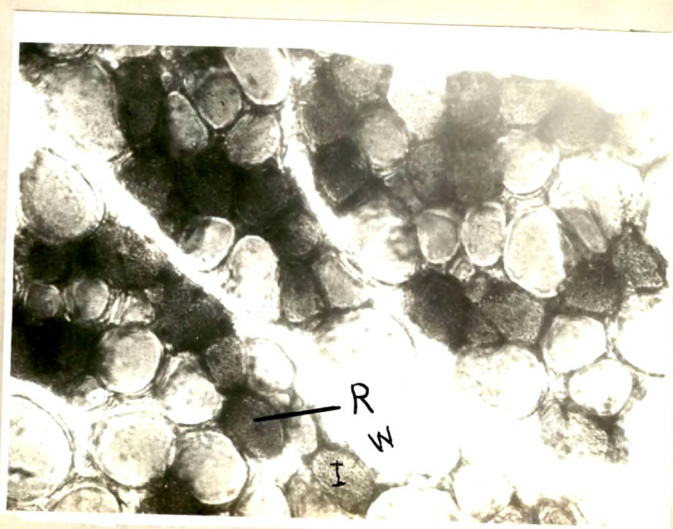

Fig.20

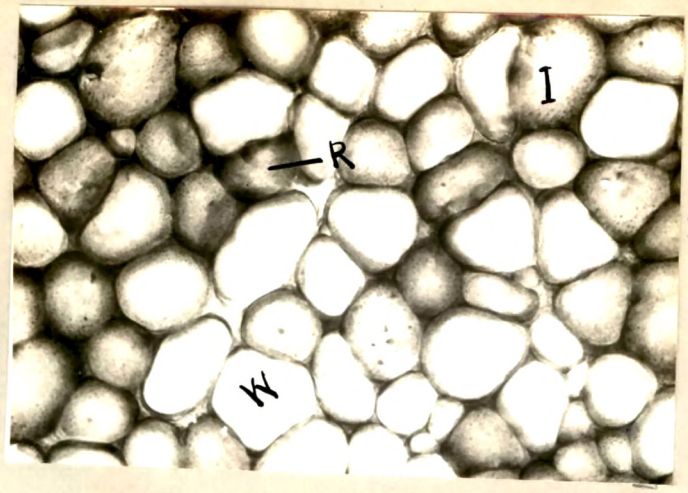

Fig.21

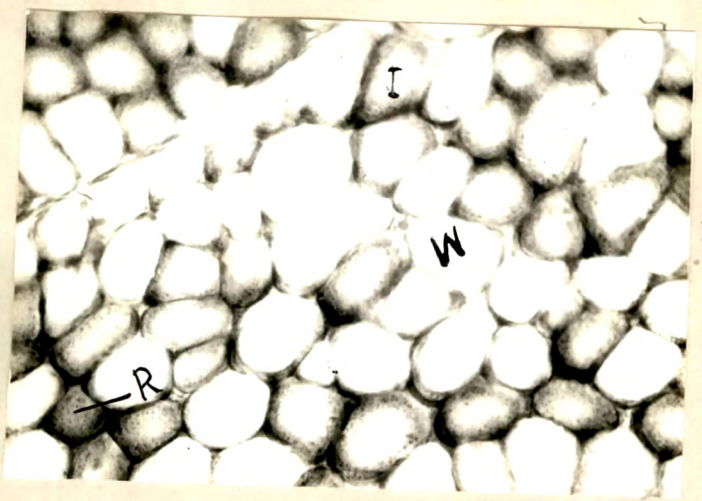

Fig.22

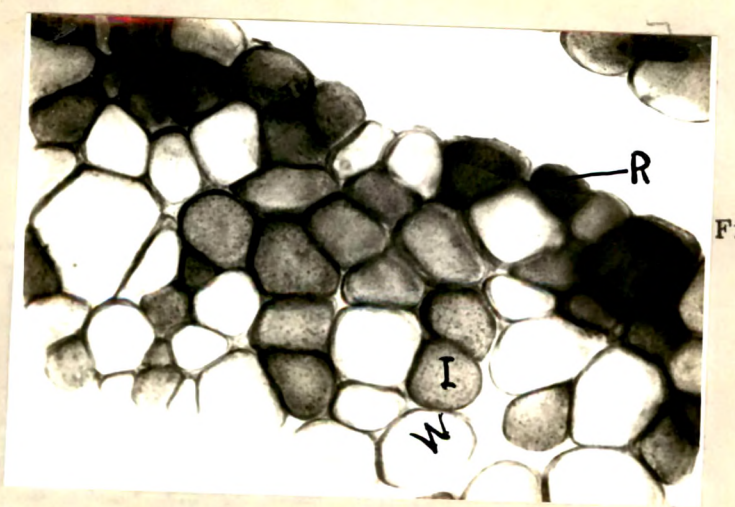

Fig.23

Figs.21,22 & 23 showing the distribution of fat in the different types of fibres in Dog diaphragm. The transverse tissue sections stained for the localization of fat distinguishes the 'R'-Red, 'I'-Intermediate and 'W'-White fibres. Figs. 21, 22 & 23 shows the localization of fat in the dorsal, lateral and ventral regions.

All 128 x

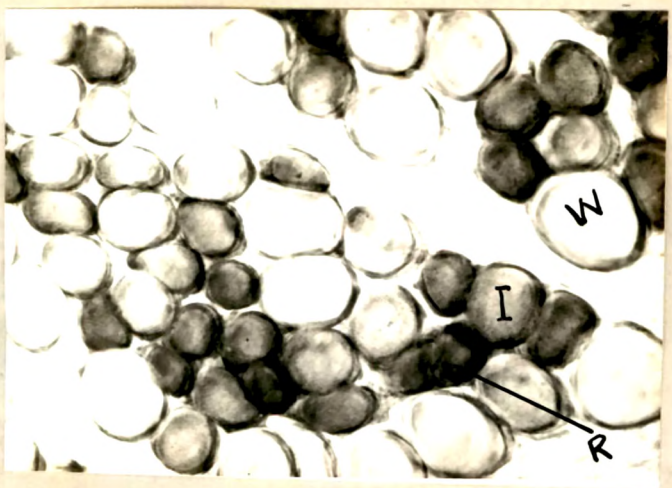

Fig.24

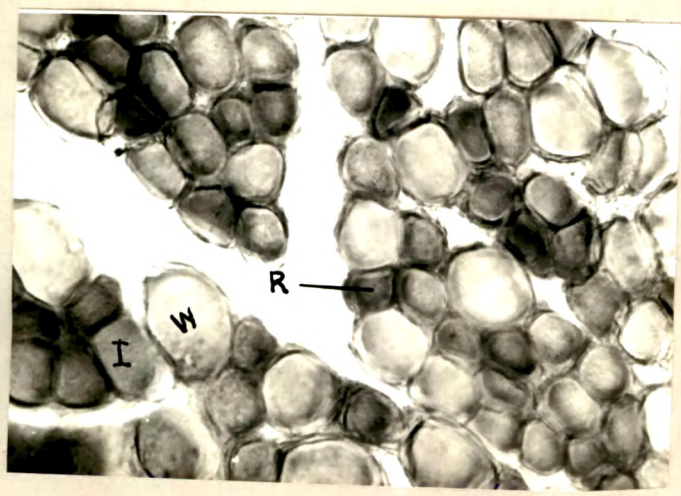

Fig.25

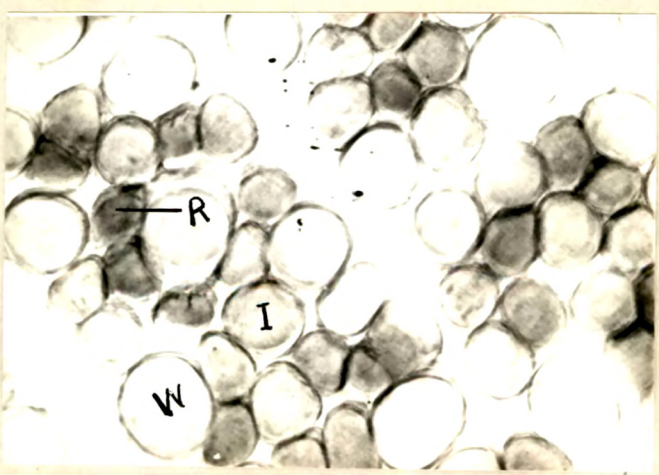

Fig.26

Figs.24, 25 & 26 showing the distribution of Fat in the different types of fibres in Monkey diaphragm. The localization of Fat distinguishes the 'R'-Red, 'I'-Intermediate and 'W'-White fibres. Figs.24, 25 & 26 show the localization of Fat in the dorsal, lateral and ventral regions respectively.

All 128 x

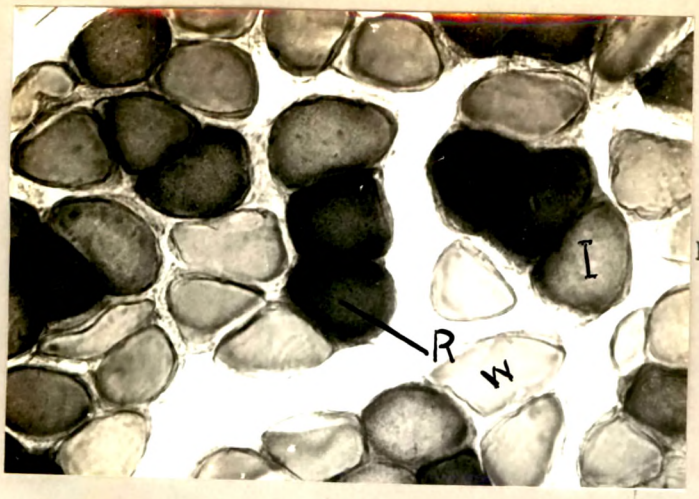

Fig.27

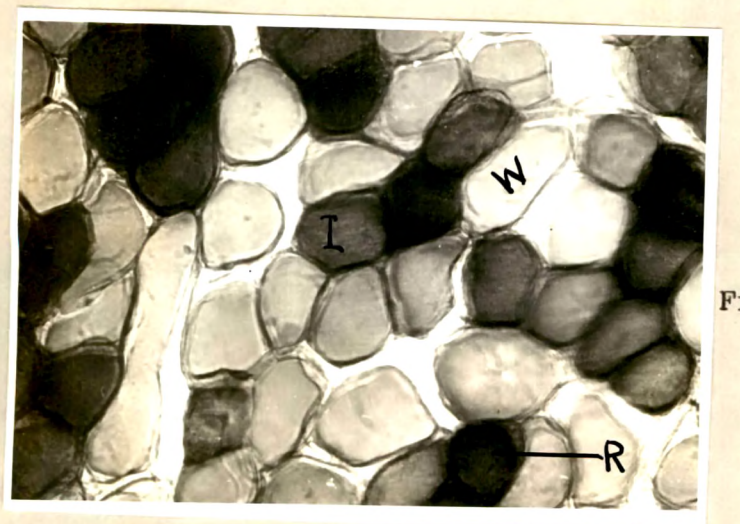

Fig.28

Figs.27, 28 & 29 showing the distribution of Fat in the different types of fibres in Rabbit diaphragm. The localization of fat also distinguishes the 'R'-Red, 'I'-Intermediate and 'W'-White fibres. Figs. 27,28 & 29 show the localization of fat in dorsal, lateral and ventral regions respectively.
All 128 x.

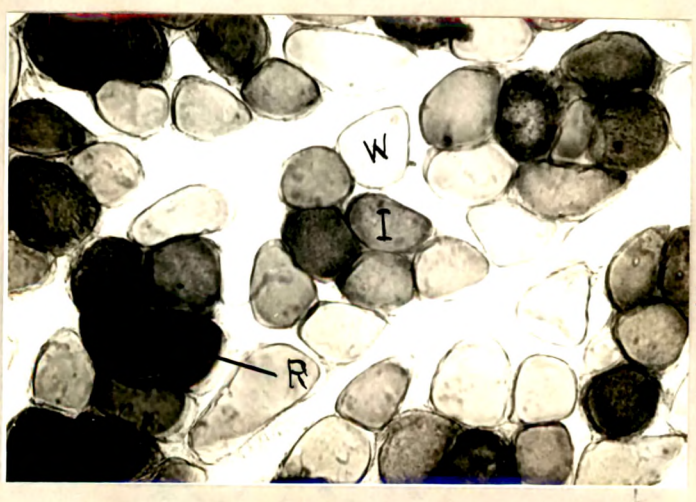

Fig.29

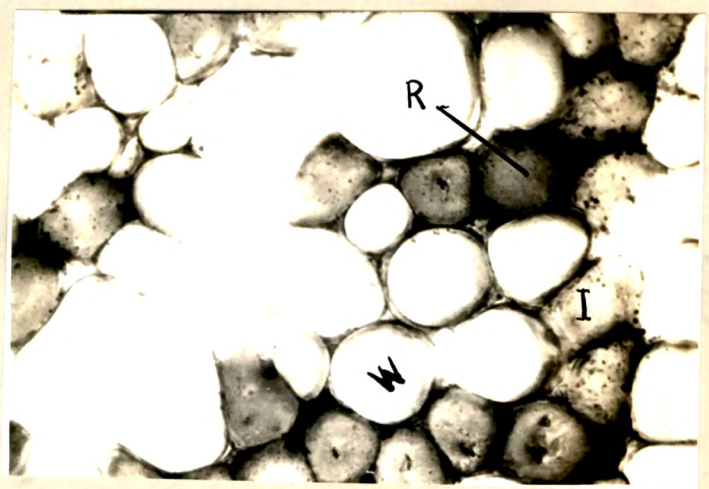

Fig. 30

Histochemical localization of Fat in the diaphragm of Cat, showing the red, intermediate and white types of fibres, in transverse muscle sections. 128 x.