THE LIPID AND GLYCOGEN CONTENTS AND THEIR REDUCTION IN THE FLIGHT MUSCLE AND LIVER OF SOME BIRDS AND A BAT DURING THE SUSTAINED ACTIVITY OF THE FORMER

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The importance of glycogen as the chief storage form of carbohydrates in animals was recognised as far back as 1885 by Claude Bernard. Ever since, the role of glycogen in tissue metabolism has attracted the attention of physiologists and biochemists. The role of glycogen in the tissue as fuel for energy in muscular activity is now a matter of common knowledge. With this great emphasis laid on carbohydrates for muscular activity resulted in a neglect of other energy producing fuels particularly fat. It is an accepted fact now that the fuel for muscular activity is not always carbohydrate. That a considerable amount of protein is utilised during strenuous muscular exercise in man was shown by Cathcart and Brunett (1926) and that fat too may be utilised, by Krogh and Lindhard (1920) and Gemill (1940 and 1942). There seems to be some indirect evidence to show that fat is the chief fuel for energy during long and sustained muscular activity. Niemierko (1929) using spring frogs showed that prolonged stimulation of the muscle on one leg caused a decrease in its fat content up to 31 percent. Buchwald and Cori (1931) using summer frogs found that the fatigued muscle of one leg contained 19.7 percent less fatty acids than the resting muscle of the other side. Bloor and Snider (1934) showed that the phospholipids content of the muscle of a wild rabbit was higher than that of a laboratory one. Later, Bloor (1937) established that the phospholipids content of muscle increased as a result of muscular exercise and he (1940) also claimed that there exists an inheritance effect of exercise in the phospholipids and cholesterol content of muscle. Cruickshank and Kosterlitz (1941) have shown that rat heart can utilise fatty acids. Recently Weis-Fogh(1952) in his excellent paper observed that flying locusts utilise fat for muscular activity and as for the humming bird he has put a question mark. Fontaine and Hatey (1953) believed that fat is the chief fuel in migrating fishes (Salmon).

George (1947 and 1952) has shown the presence of a large amount of fat in the flight muscle of the pigeon (Columba livia). Nair (1952) showed that the total solids in the pectoralis major muscle of the bird was higher than that in the pectoralis minor, thereby suggesting that the former is metabolically a more evolved muscle than the latter. The fat content of the pectoralis major has also been found to be greater and so also the iron content. He also observed that good fliers show a remarkably higher percentage of these substances in the muscle. Menon (1954) has shown that the glucose and fat in the blood of birds are the highest in the vertebrate series. George and Menon (1954) have suggested a physiological lag in the domestic fowl as the fat content in the blood is low and to which facts they ascribed its inability to fly.

Having been convinced on circumstantial evidence of the possibility of the utilization of fat by birds during flight, I tried to ascertain if a reduction of fat occurs either in the flight muscle or in the liver or in both in the pigeon when the flight muscles are stimulated. The results of the preliminary work published in 1953, 1955a, 1955b showed that the fat content in the breast muscle as well as liver of the pigeon gets reduced when the flight muscle is subjected to prolonged stimulation or forced flight.

These studies were extended to other birds and also to a bat. It was also considered desirable to ascertain in each case the amounts of free and bound lipids in the breast muscle and if a reduction of either or both of them occurs as a result of prolonged stimulation. For comparative purposes the treatment was also extended to the leg muscles.

Materials and Methods:

The animals experimented with, were the local parakeet, (Psittacula krameri), kite, (Milvaus migrans), pigeon, (Columba livia), domestic fowl, (Gallus domesticus) and a bat (Rousettus lechinaulti). The animal was pithed and a small piece of about 1 gm. for lipid estimation and another tiny bit of about 5 to 10 mg. for glycogen estimation of the breast muscle, Pectoralis major on one side and a similar piece of the liver - both to be used as control, were cut out for the estimation of their lipid and glycogen contents, while the muscle on the other side was electrically stimulated by a series of make and break shocks (about 50 per minute) with the current passed through the Du Bois induction coil from a battery cell of 4.0 volts, and adjusting the current at a minimum for a visible contraction and then gradually increasing its intensity until the muscle was thoroughly fatigued. Then a small piece was cut out from the fatigued muscle, as well as from the liver as before for estimations. The glycogen content of the breast muscle and liver of the parakeet was not estimated.

Similarly for the estimation of the lipid content a piece of the calf muscle of one leg was removed after the animal was pithed and then the same muscle of the other leg was stimulated as was done in the case of the breast muscle. A small piece of the fatigued muscle was also cut out.

The lipid and glycogen contents of the controls as well as of the stimulated muscle and of the liver before and after stimulation of the muscle were separately estimated. Another set of experiments were conducted as follows: A pigeon was made to fly in a large hall till it was more or less fatigued and was immediately pithed, the liver and the muscle samples were cut out for the estimations. Another pigeon after such forced flight was pithed and the breast muscle was electrically stimulated till it was completely fatigued and then the muscle and liver samples were cut out for the estimations as before. These experiments were repeated several times and results obtained.

It is found that after extraction with ether when the material is further treated with a mixture of ethanol and ether, a considerable amount of lipid is still obtained. It is assumed that ethereal extracts normally consists mostly of free lipids as neutral fat and free fatty acids, and a combustion of these should be mainly responsible for the reduction in the amount of lipid due to exercise of the muscle. I have ventured to call the first ethereal extract therefore as free lipid and the ether-soluble part of the ethanol-ether extract as total lipid. So, by total lipid I mean the total ether-soluble lipid which will consist~~s~~ of mostly of combustible fat.

For obtaining the free lipid I have used water-free ethyl ether. Since it was found that the amount of lipid obtained after dehydration by heat was slightly greater than when dehydrated under vacuum, It was suspected that heat does release some bound lipid. The material was therefore dehydrated ~~first~~ under vacuum. For obtaining the total lipid the material was first completely dried by heating first to 100° C. till a constant weight was obtained. Then a one to one mixture of water-free ethyl ether and ethanol was used to extract the lipid. In either case the material was transferred to a fat-extraction thimble which was inturn placed in the extractor part of the soxhlet apparatus.

The percentages of lipid and glycogen were calculated on the weight of the fresh material, but for muscle after fatigue the percentages of lipid and glycogen were calculated first on the dry weight and then converted for fresh weight, because there is considerable loss of water when muscle is subjected to continuous contraction. The same procedure was adopted in the case of liver after the muscle was fatigued.

The method used for the estimation of glycogen was a Colorimetric micro-method according to Kemp, et al (1954) using the Bechman's Spectrophotometer (DU Model). The tissue was crushed in methanol (80 %) in a graduated centrifuge tube. Being insoluble in 80 % methanol, glycogen was left in the residue and was then extracted by heating the residue with a deproteinising solution at 100°C for fifteen minutes. The colour reaction was carried out with 1 ml. of the above fluid with 3 ml. of concentrated sulphuric acid and intensity of the colour produced was measured spectrophotometrically at 520 mμ.

It is assumed that the reduction in the fat and glycogen contents of the breast muscle and liver noted at the end of the continuous muscular exercise denotes the amount of the substances utilized for energy. Reduction if any in the protein was very slight and it was not taken into account for the present study. The respective energy values were obtained by multiplying the fat reduction figure by 9.5 and the glycogen reduction figure by 4.2 (Krogh and Lindhard, 1920).

Results:

The normal lipid and glycogen content of the muscle and liver was found to vary within limits depending on the state of the animal. Such were also fat and glycogen reduction values, since some specimens got fatigued sooner than others. It was therefore thought advisable to show the range in variation instead of mean values. The results are given in the following tables.1 to 8 and illustrated in histograms (Figs.7 to 12).

Table I

The lipid content and its reduction in the pectoralis muscle

Animal.	Percentage of free lipid before stimulation.	Percentage of free lipid after stimulation.	Actual reduction of free lipid in gm. per 100 gm. of muscle.	Percentage of reduction of free lipid.	Percentage of total lipid before stimulation.	Percentage of total lipid after stimulation.	Actual reduction of total lipid in gm. per 100gm. of muscle.
	1.	2.	3.	4.	5.	6.	7.
Pigeon.	2.50-3.00	2.00-2.10	0.50-0.90	20.00-30.00	4.21-4.73	3.72-3.80	0.49-0.93
Parakeet.	3.30-3.80	2.46-2.48	0.82-1.34	24.85-35.26	4.90-5.76	4.05-4.56	0.85-1.20
Kite.	2.83-3.20	2.56-2.85	0.27-0.35	9.50-10.94	4.50-5.00	4.25-4.61	0.25-0.39
Fowl.	0.50-0.60	0.45-0.50	0.05-0.10	10.00-16.66	0.90-1.10	0.77-0.88	0.13-0.22
Bat.	9.00-10.00	7.81-8.60	1.19-1.40	13.22-14.00	11.38-13.98	10.21-12.56	1.17-1.42

Table 2

The lipid content and its reduction in the liver

Animal.	Percentage of free lipid before stimulation.	Percentage of free lipid after stimulation.	Actual reduction of free lipid in gm. per 100 gm. of liver.	Percentage of reduction of free lipid.	Percentage of total lipid before stimulation.	Percentage of total lipid after stimulation.
	1.	2.	3.	4.	5.	6.
Pigeon.	2.50-3.50	2.25-3.00	0.25-0.50	10.00-14.29	3.31-4.50	3.05-3.97
Parakeet.	4.20-4.70	3.49-3.80	0.71-0.90	16.90-19.14	5.20-5.90	4.50-5.02
Kite.	3.20-3.90	2.80-3.42	0.40-0.48	12.30-12.50	4.10-4.90	3.71-4.40
Fowl.	2.90-3.20	2.59-2.81	0.31-0.39	10.69-12.19	2.98-3.40	2.68-3.00
Bat.	5.60-6.00	5.20	0.40-0.80	7.14-13.33	6.53-7.24	6.11-6.41

Table 3

The lipid content and its reduction in the leg muscle

Animal.	Percentage of free lipid before stimulation. 1.	Percentage of free lipid after stimulation. 2.	Actual reduction of free lipid in gm. per 100gm. of muscle. 3.	Percentage of reduction of free lipid. 4.	Percentage of total lipid before stimulation. 5.	Percentage of total lipid after stimulation. 6.	Actual reduction of total lipid in gm. per 100 gm. of muscle. 7.
Pigeon.	1.70-2.00	1.44-1.70	0.26-0.30	15.00-15.30	1.94-2.25	1.69-1.95	0.25-0.30
Parakeet.	2.00-2.60	1.60-2.03	0.40-0.57	20.00-21.11	2.30-2.90	1.94-2.34	0.36-0.56
Kite.	1.40-1.57	1.21-1.35	0.19-0.22	13.57-14.02	1.90-1.97	1.69-1.74	0.21-0.23
Fowl.	1.10-1.30	0.96-1.12	0.14-0.18	12.73-13.85	1.81-2.43	1.38-1.90	0.43-0.53
Bat.	4.60-5.30	4.20-4.60	0.40-0.70	8.70-13.21	5.64-6.90	5.23-6.23	0.41-0.67

Table 4

Comparison of free and bound lipids per 100 gm. of pectoralis muscle, liver and leg muscle

	Pigeon	Parakeet	Kite	Fowl	Bat
Free lipid	2.50-3.00	3.30-3.80	2.80-3.20	0.50-0.60	9.00-10.00
Bound lipid	1.71-1.73	1.60-1.96	1.63-1.80	0.40-0.50	2.38-3.98
Free lipid	2.50-3.50	4.20-4.70	3.20-3.90	2.90-3.20	5.60-6.00
Bound lipid	0.81-1.00	1.00-1.20	0.90-1.00	0.08-0.20	0.93-1.24
Free lipid	1.70-2.00	2.00-2.60	1.40-1.57	1.10-1.30	4.60-5.30
Bound lipid	0.24-0.25	0.30	0.40-0.50	0.71-1.13	1.04-1.60

Table 5

Total lipid and glycogen contents and their reduction in the pectoral muscle and liver after electrical stimulation of the former in some birds and a bat.

Animal.	Percentage of total lipid content of muscle before stimulation.	Percentage of total lipid content of muscle after stimulation.	Actual reduction of total lipid in 100 gm. of muscles.	Percentage of total lipid content of liver before stimulation.	Percentage of total lipid content of liver after stimulation.
	1.	2.	3.	4.	5.
Pigeon.	4.21-4.73	3.72-3.80	0.49-0.93	3.31-4.50	3.05-3.97
Kite.	4.50-5.00	4.25-4.61	0.25-0.39	4.10-4.90	3.71-4.40
Fowl.	0.90-1.10	0.77-0.88	0.13-0.22	2.98-3.40	2.68-3.00
Bat.	11.38-13.98	10.21-12.56	1.17-1.42	6.53-7.24	6.11-6.41

Table 6

Actual reduction after electrical stimulation of the pectoral muscle, in the total lipid and glycogen contents of the pectoral muscle and liver, and with the respective and total energy values.

Animal.	Reduction of the total lipid per 100gm. of muscle during exercise (electrical stimulation)	Energy value in Caloris for total lipid reduced per 100 gm. of muscle during exercise (electric stimulation)	Reduction of total lipid per 100 gm. of liver during exercise (electric stimulation)	Energy value in Calories for total lipid reduced per 100 gm.of liver during exercise (electric stimulation)	Total reduction of lipid per 100 gm. of muscle and 100 gm. of liver during exercise (electric stimulation)	Total energy value in Calories for total lipid reduced in 100 gm. of muscle and 100 gm. of liver during exercise (electric stimulation) (L)	Reduction of glycogen per 100gm. of muscle during exercise. (electric stimulation)	Energy value in Calories for glycogen reduced per 100gm. of muscle during exercise (electric stimulation)
	1.	2.	3.	4.	5.	6.	7.	8.
Pigeon.	0.49-0.93	4.66-8.83	0.26-0.53	2.47-5.04	0.75-1.46	7.13-13.87	0.02-0.05	0.08-0.21
Kite.	0.25-0.39	2.37-3.71	0.39-0.50	3.71-4.75	0.64-0.89	6.08-8.46	0.34-0.37	1.43-1.55
Fowl.	0.13-0.22	1.24-2.09	0.30-0.40	2.85-3.80	0.43-0.62	4.09-5.89	0.05-0.07	0.21-0.29
Bat.	1.17-1.42	11.11-13.49	0.42-0.83	3.99-7.88	1.59-2.25	15.10-21.37	0.03-0.16	0.13-0.67

Table 7

Total lipid and glycogen contents of the pectoral muscle and liver and their reduction after (1) forced flight (2) forced flight and electrical stimulation in the pigeon.

Animal.	Percentage of total lipid content~~s~~ in muscle before flying.	Percentage of total lipid content in muscle after flying.	Actual reduction of total lipid per 100 gm. of muscle after flying.	Percentage of total lipid content in muscle after flying and stimulation.	Actual reduction of total lipid per 100 gm. of muscle after flying and stimulation.
	1.	2.	3.	4.	5.
Pigeon.	4.21-4.73	2.98-3.00	1.21-1.75	2.81-2.90	1.40-1.83

Table 8

Actual reduction in the total lipid and glycogen contents of the pectoral muscle and liver with the respective and total energy values.

Animal.	Reduction of total lipid per 100 gm. of muscle after flying.	Energy value in calories for total lipid reduced per 100 gm. of muscle after flying.	Reduction of total lipid per 100 gm. of liver after flying.	Energy value in calories for total lipid reduced per 100 gm. of liver after flying.	Total reduction of lipid per 100 gm. of muscle and 100 gm. of liver after flying.	Total energy value in calories for total lipid reduced per 100gm. of muscle and 100gm. of liver after flying. (L_1)	Reduction of total lipid per 100 gm. of muscle after flying and stimulating.	Energy value in calories for total lipid reduced per 100 gm. of muscle after flying and stimulating.

Discussion:

Ninteen years ago Heilbrunn(1936) wrote " Although it is now generally realised that protoplasm is more than a solution of proteins, and that non-aqueous lipids are essential to its life, biologists and physiologists have scarcely given thought to the problem of how the lipids are related to the rest of the protoplasm. Most students of the cell are scarcely aware of the existance of the problem. And yet the pathologists have known for many years that the fats and fat-like substances of protoplasm are so bound or united to the protein as to be for the most part non-recognizable in the living or stained cell. It is only when degeneration occurs that the lipids are freed from their union with protein. In such degenerated cells, fats previously concealed may occupy a large part of the cell volume. Obviously, such a freeing of lipids results in an impairment or loss of protoplasmic activity, and this bears witness to the importance of the lipids for the vital machinary." Even today our knowledge of the relationship of lipids to proteins remains obscure. In the present work, however, no attempts have been made to make this picture clearer. But I have found that for the muscle tissue three values of lipid are obtainable. The ether extractable lipid portion consisting of neutral fats and free fatty acids, constitutes one value and this has been assumed to be the free lipid portion. The lipid portion extractable with a mixture of ether and ethanol has been taken as the total lipid. The difference between the two values is regarded as that of the bound lipid. What I have tried to understand is whether the lipid affected during continued muscular contraction in the muscle itself and also in the liver, is free lipid, bound lipid, or total lipid.

From the results obtained it is seen that the <u>pectoralis major</u> muscle of the domestic fowl contains the least amount of lipid both as free as well as bound, while the <u>pectoralis major</u> of the bat the highest, and those of the parakeet, kite and pigeon come near to that of the bat in that order. The free and bound lipid figures are compared in table 4. The bound and free lipid values are more or less equal in the fowl, while in the other animals the free lipid values are definately higher.

In the leg muscle the free lipid values are very much higher than those for the bound lipid in all the animals except the fowl, in which the corresponding value is only moderately high.

In the liver the free lipid values are very much higher than those for the bound lipid in all the animals.

In my published paper (1953) it was shown that there is a reduction in the fat content of the <u>pectoralis major</u> of the pigeon as a result of continuous muscular contraction, and it has been suggested that fat gets transported to the muscle from the liver as it gets reduced in the former. The present investigation confirms the previous one and such a reduction has been noted in the case of the leg muscle also as a result of continuous stimulation.

It must be mentioned that the time taken for fatigue of the pectoralis muscle in the pigeon, parakeet and bat is from 15 to 25 minutes, whereas the kite muscle got fatigued in 8 to 10 minutes. Correspondingly the reduction of lipid was also less in the kite. This peculiarity may be due to the fact that the kite alone amongst these is adapted for soaring and the mechanism of energy utilization in soaring is different form that of flapping flight.

In the domestic fowl which is a poor flier the actual reduction of free and total lipid is little, but the total lipid redution is greater, which is not so in the other animals. This suggests that in the cse case of the fowl, the bound lipid is utilized also on stimulation. This is the case both in the breast and leg muscle. This release of bound lipid is in some way connected with the acidity of the muscle. J.F.Manery et al (1935) have shown that the pectoral muscles of hens and ducks form unusually large quantities of lactic acid post-mortally. Heilbrunn (1928) inferred from protoplasmic particles migrating to the cathode on passing an electric current in the cell, that some protoplasmic proteins are on the acid side of the isoelectric point. He (1936) further explained that an increase in the pH or alkalization of the protoplasm could affect some of the proteins and thereby bring about the release of the bound lipid. It seems possible that there is a lipo-protein binding on either side, the acid as well as the alkaline side of the isoelectric point and changes in the pH could bring about the release of the lipid. In the case of the fowl muscle the lactic acid formed on stimulation perhaps helps in the release of bound lipid which is also utilised.

The liver also contains significant quantities of lipid. On electrically stimulating the breast muscle, the liver lipid also gets reduced. Since the actual reduction in total lipid is more or less same as in free lipid, it is evident that no bound lipid was released from the liver on stimulation of the breast or leg muscle even in the fowl. That the liver is a depot for fat is well-known. It is also known that this fat is utilized during the breeding period, especially in the female for depositing it in the eggs. The present study has shown that liver fat may be utilized for locomotion also.

It is understandable that the leg muscle of the fowl takes 15 to 20 minutes to get fatigued, since some bound lipid is released and which seems to have been utilized. Considering the large amount of free lipid present in the leg muscle of parakeet and bat, the time taken to get fatigued by these two muscles is relatively less than that taken by the leg muscle of the fowl. The domestic fowl is a confirmed walker using its legs constantly, whereas the other two use their leg muscles in tonic contraction for perching. As was stated in the case of the breast muscle of the kite, the mechanism for energy utilization in the leg muscle in tonic contraction while perching might be different from that in walking.

In respect to the glycogen content of the pectoral muscle (Table 5), the kite shows the highest with the mean value nearly more than double that of the pigeon and bat separately and nearly as much as that of the pigeon and bat put together. This is to be expected because the kite muscle consists of only the broad glycogen loaded variety and the kite being a more active bird its breast muscle contains more glycogen than that of the domestic fowl whose muscle also consists of the large variety of fibres. As for the glycogen content of the liver, it is more or less the same in all the four animals.

After electrical stimulation of the pectoral muscle till it was fatigued, a reduction in the lipid and glycogen contents of the muscle as well as liver was noted and the respective energy values calculated (Table 6). It is seen that in the particular type of exercise, the pigeon, kite,fowl and bat utilized a total amount of energy of about 15, 12, 8 and 26 Calories respectively (mean of the respective figures in column 13 of table 6) obtained from the sum total of fat and glycogen reduced in both muscle and liver together.

Of these energy values again, in the pigeon, kite, fowl and bat, 70 %, 62 %, 63 %, and 70 % respectively (mean of the respective figures in column 14 of table 6) of the energy utilized was obtained from lipid alone while the remaining only (column 15 of table 6) was from glycogen.

In the second experiment (table 7) in which a pigeon was forced to fly continuously till it was fatigued, about 77 % (mean of the figures in column 26 of table 8) of the total energy expended was obtained from fat and only the remaining from glycogen. In the third one in which a pigeon was forced to fly till fatigued and then electrically stimulated, it was found that about 78 % (mean of the figures in column 29 of table 8) of the total energy expended was obtained from fat and the rest from glycogen.

The general conclusion that can be drawn from this study is that the fuel in the flight muscles of birds and bat is to a great extent fat and that fat is the chief fuel in long and sustained muscular activity in these animals. It is also brought out that when the fat supply in the muscle is depleted, that of the liver is drawn upon. In the light of these studies, the long - distance flight of migrating birds like the golden plover becomes more easily understandable. Weis-Fogh (1952) concluded that the long-range migrations of small flying animals could only be possible if fat were to be used as the fuel. The present study supports the conclusions of Weis-Fogh.

The mechanism of fat utilization remains to be explained. While much work has been done on the metabolism of carbohydrates which finally led to the Embden - Parnas - Meyerhof scheme of glycolysis, the metabolism of fatty acids has not been receiving so much attention except in the last ten years or so. Thanks to the works of Munoz, Lehninger, Green, Ochoa and Lipmann, their collaborators and others. We have now a fairly clear picture of the mitochondrion as a centre of the oxidation of fatty acids. The mitochondria of the heart, liver and kidney cells are now established centres of such oxidation. The fatty acids are converted into acetic acid and on further oxidation converted into carbon dioxide and water through the citric acid cycle thus liberating energy. The first step in the oxidation of fatty acid is its conversion to the corresponding Coenzyme A derivative triggered by ATP generated by oxidative phosphorylation. In the second step the fatty acyl derivatives of Co A enter the β-oxidation cycle liberating at each stage acetyl Co A . The acetyl Co A with oxalacetate condenses to form citrate liberating Co A. The citrate thus formed can undergo oxidation back to oxalacetate through -Ketoglutarate, Succinate and Fumarate (Malate) as intermediates which is essentially the pathway of the Kreb's cycle.

It is most likely that the oxidation of fat takes place in the mitochondria of the muscle cells. With the oxidation of acetic acid, ketone bodies can be formed. Increase in ketone bodies in the blood should also denote fat utilization . Preliminary observations in this department has indicated such an increase of ketone bodies during sustained muscular activity in the pigeon (George and Naik, unpublished,1956).

The total fat content and its reduction in the breast muscle during sustained muscular exercise (electrical stimulation).

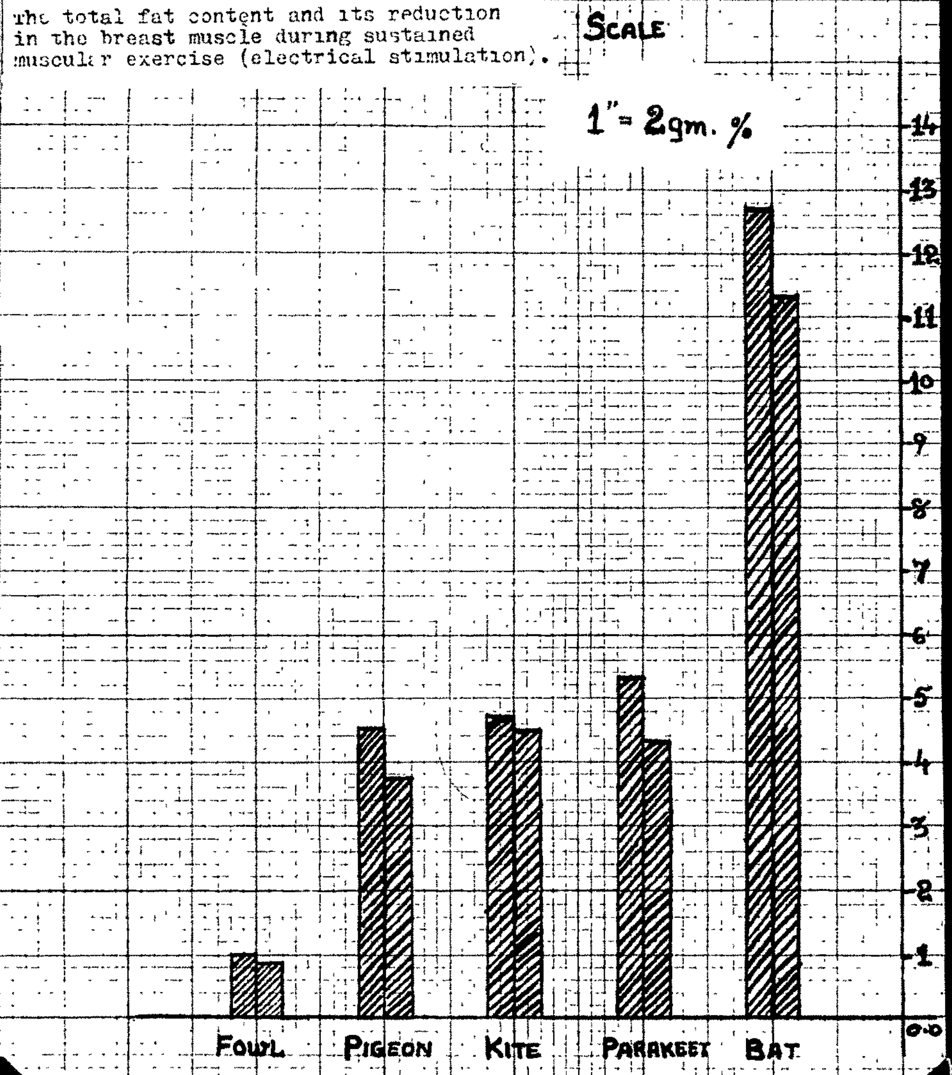


FIG. 7

The total fat content and its reduction in the liver during sustained muscular exercise (electrical stimulation).

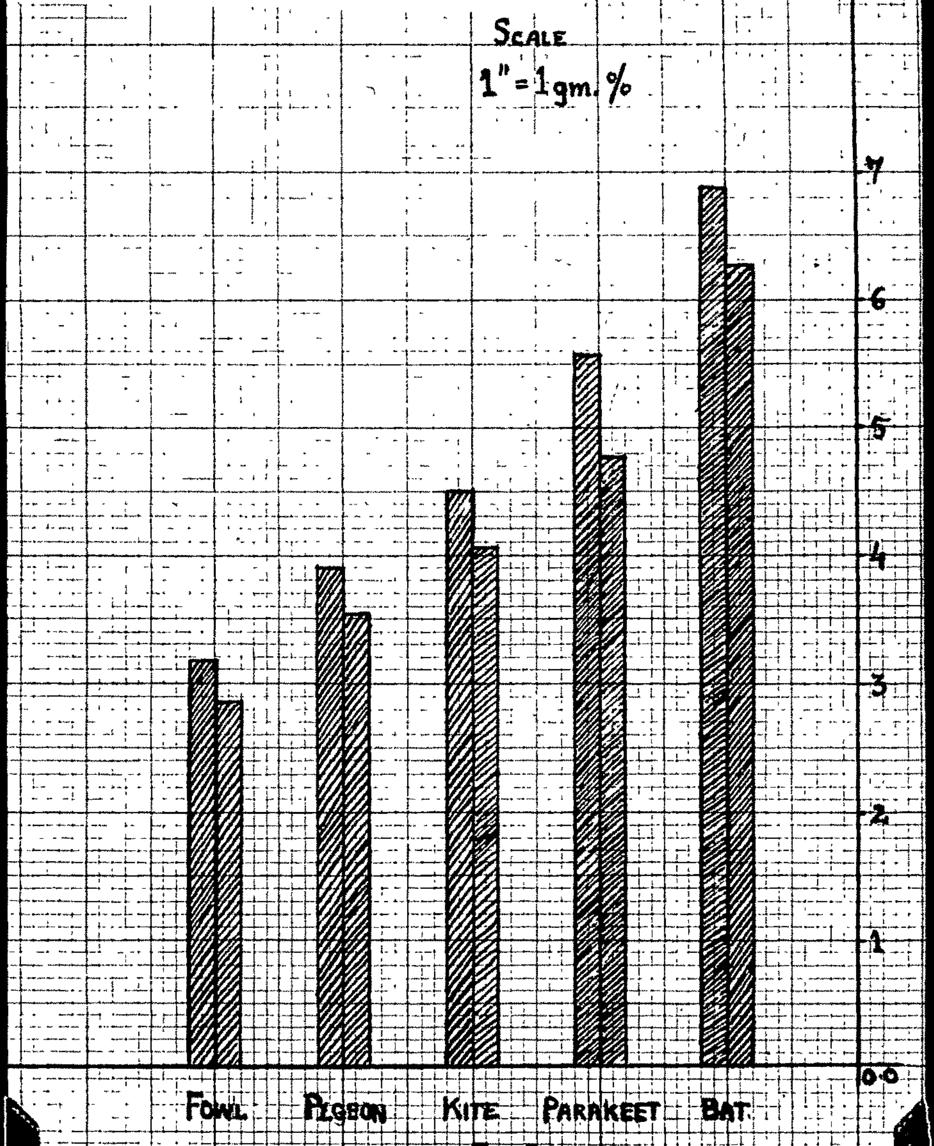
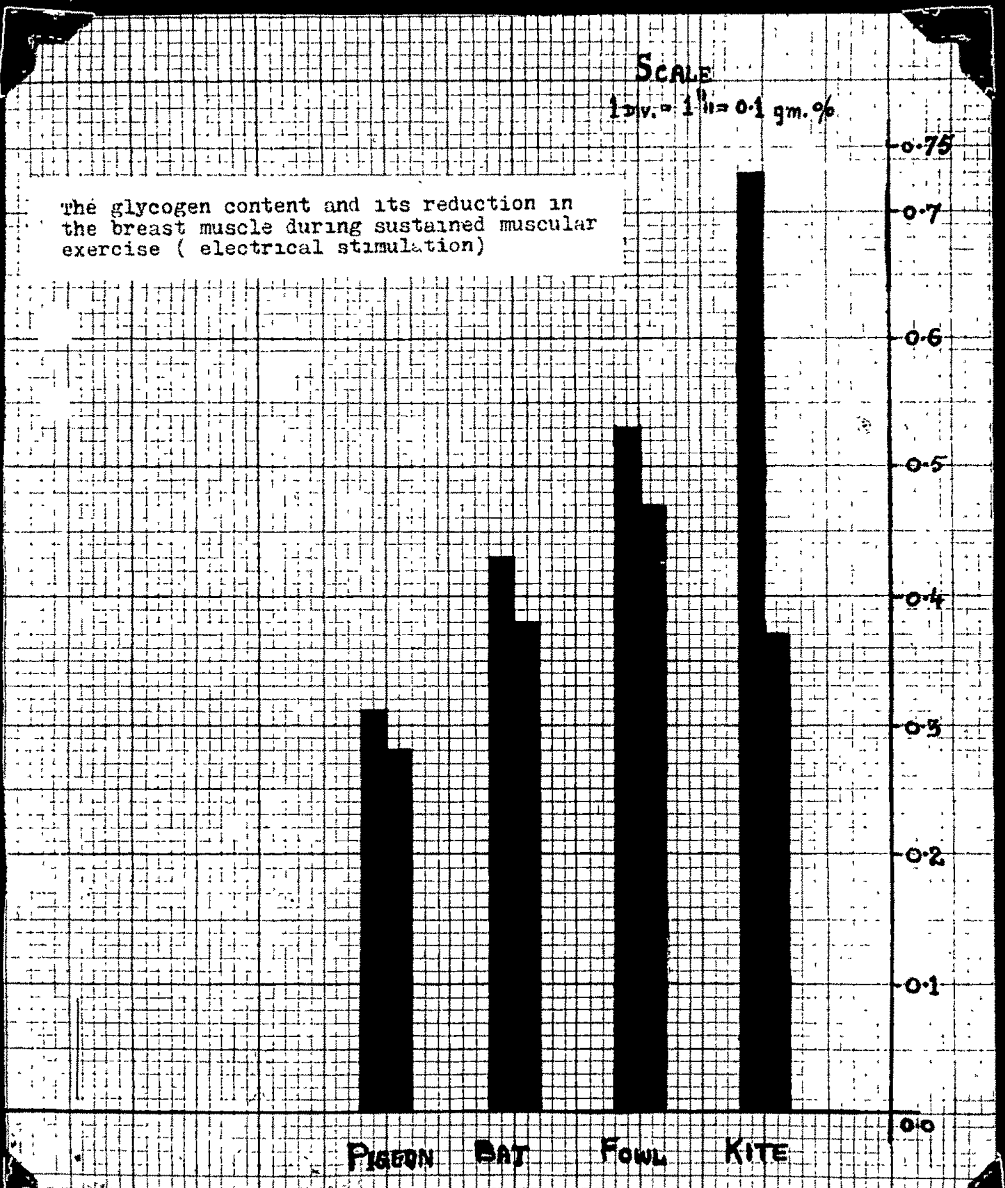


FIG. 8



The glycogen content and its reduction in the breast muscle during sustained muscular exercise (electrical stimulation)

FIG. 9

The glycogen content and its reduction in the liver during sustained muscular exercise (electrical stimulation).

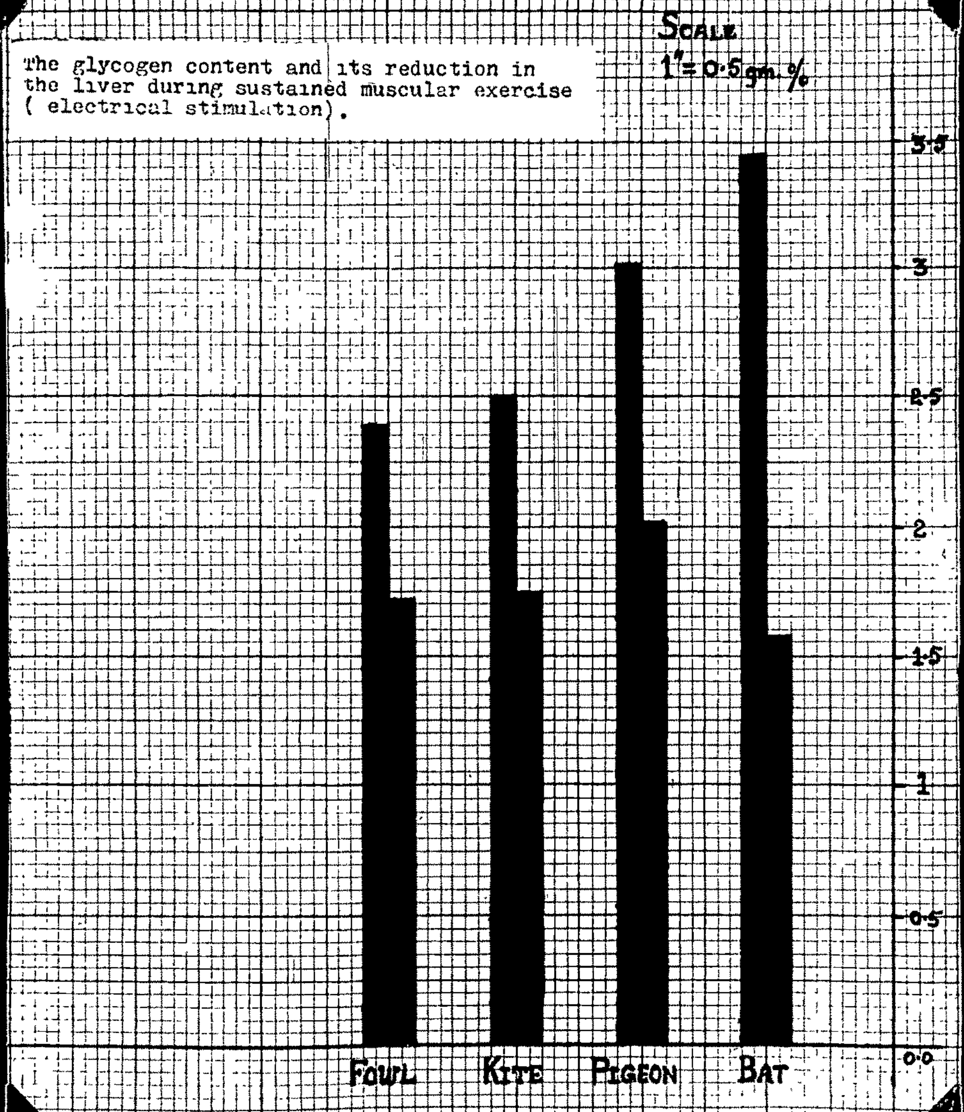


FIG. 10

The total fat content and its reduction in the breast muscle and liver of the pigeon during (1) forced flight and (2) forced flight and electrical stimulation of the breast muscle.

SCALE 1" = 1 gm.%

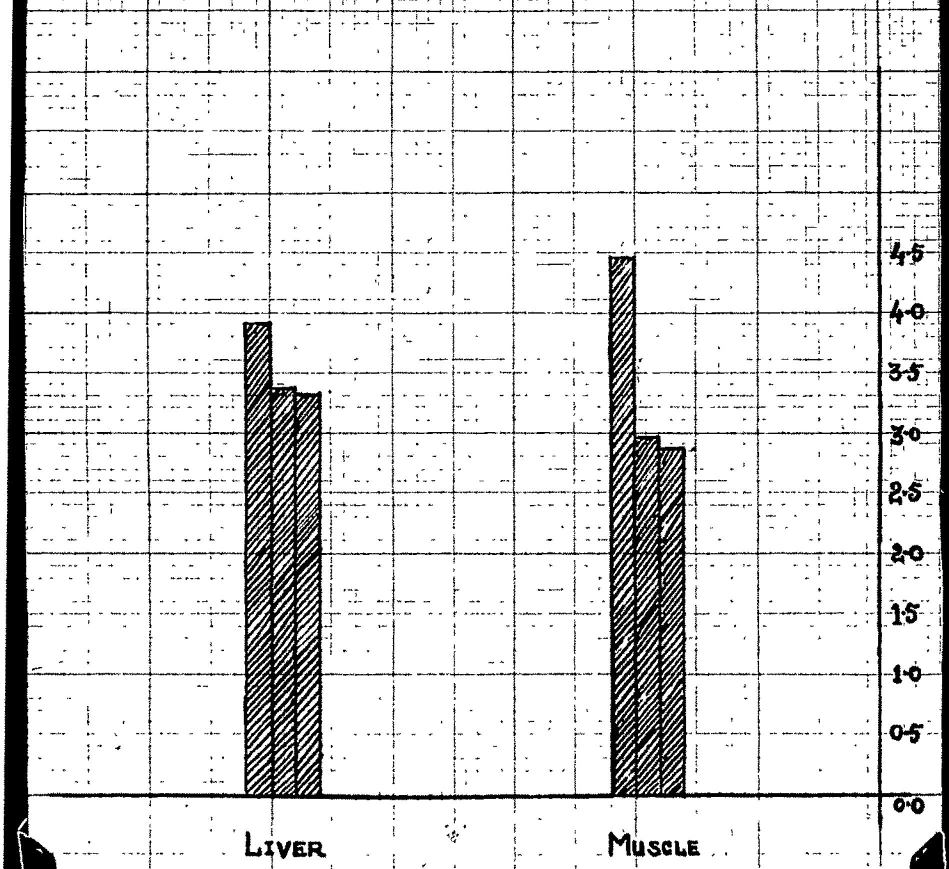
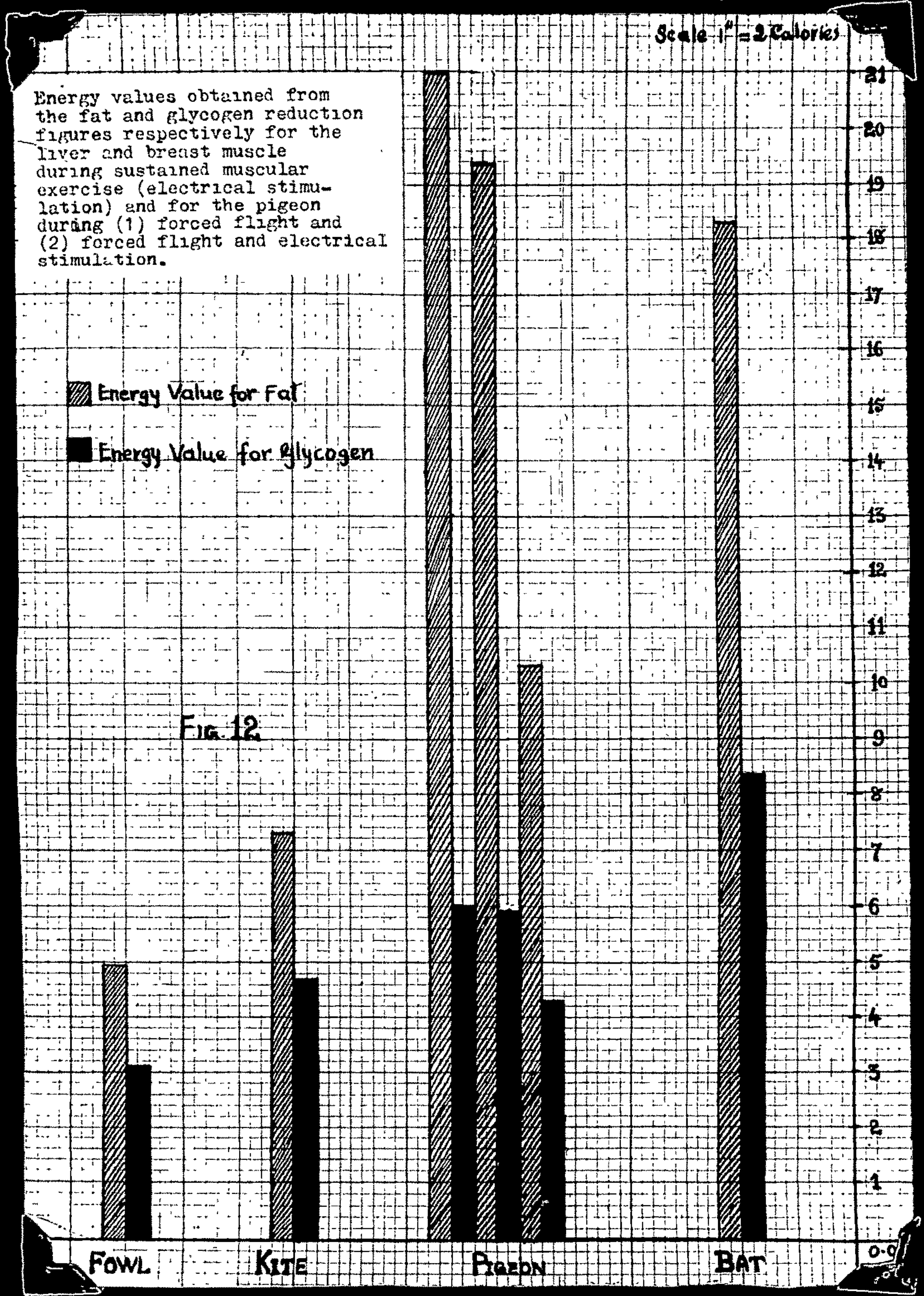


FIG. 11



Energy values obtained from the fat and glycogen reduction figures respectively for the liver and breast muscle during sustained muscular exercise (electrical stimulation) and for the pigeon during (1) forced flight and (2) forced flight and electrical stimulation.

FIG. 12