hexose 6-phosphate. This enzyme catalyses the rate-limiting step of glycolysis by the free parasite and may be 6-phosphofructokinase.

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REFERENCES

Bandurski, R. S. & Axelrod, B. (1951). *J. biol. Chem.* **193**, 405.
Bovarnick, M. R., Lindsay, A. & Hellerman, L. (1946). *J. biol. Chem.* **163**, 523.
Bowman, I. B. R., Grant, P. T. & Kermack, W. O. (1960). *Exp. Parasit.* **9**, 131.
Bowman, I. B. R., Grant, P. T., Kermack, W. O. & Ogston, D. (1959). *Biochem. J.* **73**, 42P.
Bueding, E. (1959). *J. Pharm., Lond.*, **11**, 392.
Carles, J., Schneider, A. & Lacoste, A. (1958). *Bull. Soc. Chim. biol., Paris*, **40**, 221.
Claycomb, C. K., Hutchens, T. T. & van Bruggen, J. T. (1950). *Nucleonics*, **7**, 38.
Crane, R. K. & Sols, A. (1953). *J. biol. Chem.* **203**, 273.
Fraser, D. M. & Kermack, W. O. (1957). *Brit. J. Pharmacol.* **12**, 16.
Fulton, J. D. & Grant, P. T. (1956). *Biochem. J.* **63**, 274.
Fulton, J. D. & Spooner, D. F. (1956). *Exp. Parasit.* **5**, 59.
Jones, A. R., Dowling, E. J. & Skraba, W. J. (1953). *Analyt. Chem.* **25**, 394.
Jones, E. S., Maegraith, B. G. & Gibson, Q. H. (1953). *Ann. trop. Med. Parasit.* **47**, 431.
Khym, J. K. & Cohn, W. E. (1953). *J. Amer. chem. Soc.* **75**, 1153.
Krebs, H. A. & Eggleston, L. V. (1940). *Biochem. J.* **34**, 442.
Lohmann, K. (1933). *Biochem. Z.* **262**, 137.
McKee, R. W. (1951). In *Biochemistry and Physiology of Protozoa*, vol. 1, p. 252. Ed. by Lwoff, A. New York: Academic Press Inc.
Mansour, T. E. & Bueding, E. (1954). *Brit. J. Pharmacol.* **9**, 459.
Marshall, P. B. (1948). *Brit. J. Pharmacol.* **3**, 1.
Moulder, J. W. (1948). *J. infect. Dis.* **83**, 262.
Moulder, J. W. (1949). *J. infect. Dis.* **85**, 195.
Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
Phares, E. F., Mosbach, E. H., Denison, F. W. & Carson, S. F. (1952). *Analyt. Chem.* **24**, 660.
Pirie, N. W. (1946). *Biochem. J.* **40**, 100.
Popják, G. (1950). *Biochem. J.* **46**, 560.
Sakami, W. (1955). *Handbook of Isotopic Tracer Methods.* Cleveland: Western Reserve University.
Shreeve, W. W., Leaver, F. & Siegel, I. (1952). *J. Amer. chem. Soc.* **74**, 2404.
Somogyi, M. (1952). *J. biol. Chem.* **195**, 19.
Speck, J. F. & Evans, E. A. (1945*a*). *J. biol. Chem.* **159**, 71.
Speck, J. F. & Evans, E. A. (1945*b*). *J. biol. Chem.* **159**, 83.
Speck, J. F., Moulder, J. W. & Evans, E. A. (1946). *J. biol. Chem.* **164**, 119.
van Slyke, D. D. & Folch, J. (1940). *J. biol. Chem.* **136**, 509.
Weil-Malherbe, J. & Bone, A. D. (1951). *Biochem. J.* **49**, 339.

Biochem. J. (1961) **78**, 478

Influence of Exercise and Restricted Activity on the Protein Composition of Skeletal Muscle

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It is well known that when a muscle is exercised it will increase its strength by undergoing hypertrophy. In other words, its volume rises, and this might be accompanied by thickening of individual myofibrils (Mölbert & Jijima, 1959). Conversely, inactivity gives rise to muscular hypotrophy. As the myofilaments are alone responsible for the contraction, whereas the sarcoplasm has the more passive function of supplying nutrients, it would be interesting to establish whether the ratio of myofilaments to sarcoplasm undergoes any changes during exercise and inactivity, or whether these remain constant so that contractile strength and muscular diameter would be directly proportional (Pratt & Eisenberger, 1919; Sassa & Sherrington, 1921; Spector, 1956).

It would be feasible to carry out such an investigation with the aid of histological methods, but they have two major disadvantages. First, fixation and staining are accompanied by shrinkage of sections, rendering impossible sufficiently precise determinations of the proportions of sarcoplasm and of myofilaments in the total cell volume. Secondly, the myofilaments are forming myofibrils which are not clearly separated from the sarcoplasm in sections studied by histological methods. Electron microscopical examination has disclosed that myofibrils have no envelope; moreover, it is

known that various chemical constituents of sarcoplasm, such as creatine phosphate, adenosine triphosphate and mitochondrial products, are capable of freely passing into and out of the myofibrils. Also, larger molecules, for example inulin with a molecular weight of 6000, can penetrate into the myofibril (Perry, 1956). It is difficult to tell whether the larger sarcoplasm-protein molecules actually pass into the myofibrils; but, at least theoretically, there is room for them between the myofilaments, which in the region of the I bands are separated by wide spaces as judged by the lack of staining with osmium.

Hence methods other than histological seem to be preferable for studying the relationsip between sarcoplasmic volume and myofilamental volume. Such a method is the quantitative biochemical technique described by Helander (1957) for the separate determination of myofilamental proteins and of sarcoplasmic proteins.

MATERIAL AND METHODS

Animal experiments

Guinea pigs. Male guinea pigs (weight 800–1100 g.) were used. After delivery from the breeder these guinea pigs were reared for 4–6 weeks and kept under observation. They received a normal diet and showed no signs of disease. All the animals were capable of undergoing the experimental exercise. They were divided into three groups each of similar weight distribution.

Group I. Controls were kept in a roofless cage measuring 300 cm. by 75 cm.

Group II. Exercised animals were kept in the same kind of cage as group I. During exercise they were transferred to a box measuring about 150 cm. × 55 cm. subdivided into six compartments; one guinea pig was made to run in each compartment. The floor of this box was replaced by a motorized endless belt moving at a constant velocity of 30 m./min. Six days per week all the animals were made to run a distance of 1000 m. As some of the guinea pigs tended to become rather exhausted when they ran this distance without interruption, two 10 min. pauses were interposed. This programme was maintained for about 4 months, the number of working days per animal averaging 100 and ranging from 95 to 105.

Group III. Animals with restricted activity were kept in groups of three for approximately 4 months in 22 cm. high cages with a floor area of 35 cm. by 24 cm., which were just large enough for three animals.

Throughout the duration of the experiment the animals' state of health was checked closely. In addition they were weighed each week, and any animal showing weight deviations above 10% was removed from the experiment. At the end of the experimental period each group consisted of sixteen guinea pigs, which were weighed (Table 2).

Rabbits. Healthy albino rabbits between 3 and 5 months (weight 1200–1800 g.) were used. They were given normal fodder, and water *ad libitum*. These trials were carried out on twenty-five rabbits, which were divided into four groups.

Group I. Ten controls whose activity had not been restricted were killed at the beginning of the experiment.

Group II. Six rabbits were kept for 6 months without restriction of activity.

Group III. Five rabbits were kept for 6 months in 35 cm. high cages with a floor area of 35 cm. by 70 cm. About one-third of the floor was occupied by food and containers.

Group IV. Four rabbits were kept for 3 years in the same type of cages as those of group II.

Biochemical analysis

At the end of the experiments all animals were killed and exsanguinated. In the guinea pig series the calf-muscles were excised and weighed, whereas in the rabbit series the quadriceps femoris were removed and weighed. A representative portion of each specimen was set aside for determination of water content. The dehydrated muscle specimen was then used for estimation of total N content.

The protein composition of the muscular tissue was analysed in accordance with the procedure described by Helander (1957). In that paper the preparation and extraction procedures have been discussed in detail. Means of exhaustive extraction of sarcoplasmic and myofilamental proteins were devised. Histological controls and re-extraction procedures showed that the extraction was complete. In that paper it is shown that after 3–4 hr. the extraction of both sarcoplasmic and myofilamental proteins is complete. The proteins can be exhaustively washed out by repeated extractions. The earlier devised method included such repeated extractions, which aimed to recover the proteins quantitatively. However, as it is not necessary to wash out the muscle proteins repeatedly to determine their quantities, the analytical procedure has been modified in the present investigation. A brief description follows.

The muscular tissue is chilled as rapidly as possible with solid CO_2, and then stored in well-stoppered glass jars (to prevent loss by evaporation) in a deep freezer for not more than 24 hr. Three representative portions each weighing about 1 g. are sectioned in a freezing-microtome, the 30 μ thick sections being weighed in the frozen state. The first portion is used for determination of water content (because a minor discrepancy compared with the directly found water content may occur). The second portion is extracted for 4 hr. at 2° with 10 ml. of 1·1 M-KI + 0·1 M-phosphate buffer at pH 7·4, and, after centrifuging, the total protein N and non-protein N contents of the supernatant are determined. The third portion is likewise extracted for 4 hr. at 2° but with 10 ml. of 0·025 M-phosphate buffer at pH 7·4, and, after centrifuging, the total protein N and non-protein N contents of the supernatant are similarly determined. The total protein N quantities extracted with KI, and with the weak buffer, can then be calculated from a knowledge of the total extraction volume, including the volume of muscular tissue whose specific gravity is assumed to be 1·06 (Spector, 1956).

Figures for water content, total N and non-protein N are provided directly by the analysis. The amount of sarcoplasmic protein N is equivalent to the protein-N amount extracted with the weak buffer, whereas the amount of myofilamental protein N is equivalent to the protein N amount extracted with KI less the amount of sarcoplasmic

protein N. The amount of stroma protein N, lastly, is calculated from the difference between the amount of KI-extractable total N and the amount of total N in fresh muscular tissue.

The reproducibility of the method was evaluated by making a series of eighteen duplicate determinations of rabbit calf-muscles. The statistical treatment was performed according to Snedecor (1956). The numerical values have been treated by variance analysis. Statistically significant differences are said to occur when $P < 0.05$.

RESULTS

The data establishing the error of the biochemical method are given in Table 1.

Guinea pigs. The data derived from the experiments with guinea pigs were treated statistically, the resulting means and standard error of the means being given in Tables 2 and 3.

Table 2 reveals that the groups were entirely comparable with respect to the body weights. Nor did the weights of the calf-muscles on the two sides differ appreciably. A slight tendency can be discerned for muscular weight to increase with rising degrees of functional activity. The ratio of calf-muscle weight to body weight did not exhibit any statistically significant differences between the three groups.

Table 1. *Nitrogen components in muscular tissue*

Values are the mean of 18 duplicate determinations ±S.D. of their differences.

Nitrogen component	Nitrogen content (mg./g. of wet tissue)
Total	33·11±0·5
Myofilamental	18·14±0·7
Sarcoplasmic	6·13±0·5
Stroma	4·78±0·5
Non-protein	4·18±0·4

Table 3 makes it clear that there was no difference in water content between the controls and the animals in the restricted activity group, and, also, that it was significantly higher in both these groups than among the exercised guinea pigs. In the exercised animals the total nitrogen content of wet muscular tissue tended to be a little higher than in the controls and the restricted activity group, but there was no significant difference. The three groups exhibited no appreciable differences with respect to sarcoplasmic proteins, stroma proteins and non-protein nitrogen. However, the exercised group showed a significantly higher content of myofilamental protein nitrogen than the two other groups.

Rabbits. The protein fractions determined for individual rabbits are listed in Table 4.

Owing to the small number of animals, no conclusions based on statistical evaluation can be drawn. It is seen that there is a complete agreement in the protein composition of the muscles from the animals of groups 1 and 2. In all groups the total nitrogen contents and the proportions of stroma nitrogen and of non-protein nitrogen remained unaltered. The animals in groups 3 and 4 showed changes of the amounts of myofilamental and sarcoplasmic nitrogen. Thus the rabbits of groups 1 and 2 exhibited a myofilamental nitrogen content more than twice as large as the sarcoplasmic, whereas in group 4 these two muscle constituents were present in approximately equal proportions; group 3 occupied an intermediate position.

DISCUSSION

The experiments with guinea pigs demonstrated that exercise increases the proportion of myofilamental nitrogen in skeletal muscle. On the other hand, the protein composition of the muscles in the

Table 2. *Weight of body and calf-muscles of guinea pigs after various physical activity*

Values are given as means±S.E.M.

Group of animals	No. of animals	Body weight (g.)	Combined weight of both calf-muscles (g.)	Weight of calf-muscles as per cent of body weight	
				Right	Left
Normal	16	980±25	6·33±0·20	0·33±0·01	0·33±0·01
Exercised	16	980±17	6·53±0·19	0·34±0·01	0·33±0·01
Restricted activity	16	980±18	6·06±0·17	0·31±0·01	0·31±0·01

Table 3. *Protein composition of calf-muscles from guinea pigs after different degrees of physical activity*

Values are given as means±S.E.M.

Group of animals	Water content (%)	Total nitrogen content (mg./g. of wet tissue)	Nitrogen components (mg./g. of wet tissue)			
			Myofilamental	Sarcoplasmic	Stroma	Non-protein
Normal	76·7±0·25	29·1±0·46	15·0±0·34	7·2±0·25	3·5±0·43	3·1±0·03
Exercised	75·2±0·21	30·1±0·18	17·2±0·30	7·1±0·25	2·7±0·47	3·2±0·02
Restricted activity	76·2±0·22	28·6±0·49	15·0±0·42	6·9±0·30	3·0±0·89	3·1±0·04

Table 4. *Protein composition of thigh muscles from rabbits after various physical activity*

Group	Animal no.	Total nitrogen (mg./g. of muscle tissue)	Nitrogen components (% of total nitrogen)			
			Myofilamental	Sarcoplastic	Stroma	Non-protein
1	1	32·8	59	26	5	10
	2	32·1	58	25	7	10
	3	34·0	60	24	6	10
	4	32·6	62	23	6	10
	5	31·8	57	27	7	10
	6	33·1	56	27	6	11
	7	32·9	61	23	6	10
	8	32·4	60	24	6	11
	9	32·7	59	24	6	11
	10	33·4	62	22	7	9
	Mean	32·8	59	25	6	10
2	11	32·4	61	22	7	11
	12	34·0	59	23	7	11
	13	32·6	57	25	5	12
	14	33·5	59	24	7	10
	15	31·1	58	27	6	9
	16	33·3	56	26	6	11
	Mean	33·6	58	25	6	11
3	17	33·4	52	31	6	11
	18	32·9	52	33	4	10
	19	34·1	51	33	6	10
	20	32·0	49	34	6	11
	21	32·8	53	31	7	10
	Mean	33·0	51	32	6	10
4	22	34·3	40	44	6	11
	23	35·2	39	44	6	11
	24	32·6	44	41	5	10
	25	34·2	41	43	6	10
	Mean	34·1	41	43	6	10

restricted activity group did not deviate from that in the controls. This outcome must be due either to an insufficiently long experimental period or to the unsuitability of guinea pigs for experiments of this type: they manage to move about a good deal even when confined to a small space.

The experiments with rabbits disclosed that long periods of restricted activity are accompanied by a decrease of the myofilamental nitrogen amount, and at the same time an increase of the sarcoplasmic content of their skeletal muscles. Here, then, a change opposed to that found in the exercised guinea pigs has taken place.

In the experiments with guinea pigs the calf-muscle tended to be heavier in the exercised animals than in the controls. It seems likely that exercise had caused a slight hypertrophy at the same time that the myofilamental content rose. Both these changes enhance the combined contractile strength of the muscles. It is not easy to establish how high the myofilamental proportion can rise. But I have never seen either in these or other experiments any specimen of muscular tissue in which it exceeded 65% of the total nitrogen content.

In interpreting the experiments with rabbits the possibility of age influence and of muscular atrophy must be considered. Comparison is made between rabbits of approximately 4 months (group 1), 10 months (groups 2 and 3) and 40 months (group 4) in age. If the results of groups 2 and 3 are compared it is seen that restricted muscular activity seems to be accompanied by a diminished myofilamental nitrogen content and an increased sarcoplasmic nitrogen content. However, in animals in group 4 it is impossible to eliminate the effect of age. Aging might have had an effect itself on the protein composition of the muscle cell or it may be combined, as in humans (Ufland, 1932), by a diminished muscular activity, which gives rise to the reported alteration.

I have previously reported (Helander, 1957), and histological investigations by others have borne out, that muscular atrophy is accompanied by a rise in the amount of stroma in the muscular tissue. Most likely this increase of connective tissue in early stages is relative rather than absolute (Fischer & Ramsey, 1946; Helander, 1957). However, a true increase of connective tissue conceivably takes place in cases of advanced muscular atrophy. From the fact that the stroma content of

muscular tissue underwent no change in the present experiments, I conclude that the observed muscles had not atrophied. Aging itself very rarely seems to produce so-called 'senile muscular atrophy' (Adams, Denny-Brown & Pearson, 1953), nor was that condition observed in these experiments.

The two series of experiments clearly indicate that the composition of the muscle cell may normally vary within wide limits. To some extent it seems capable of adapting itself to the degree of activity, as reflected by a rising myofilamental density during heavier work and a diminishing myofilamental density when the activity is restricted. Thus the composition of normal muscle cells is not fixed. I have previously proposed that the term 'allomorphism' should be applied to such fluctuations in the normal cell composition (Helander, 1958). The same term may be applied to other cells, should they be capable of undergoing similar adaptive changes.

Clearly, therefore, the skeletal muscular tissue responds in two ways to changes in the degree of functional activity. In the first place exercise is accompanied by hypertrophy and inactivity by hypotrophy; in the second place the amount of contractile substances in the muscle cell changes, rising with exercise and diminishing with restricted activity. Both these processes exert a modifying influence on the contractile strength of the muscle.

In general the contractile strength of muscular tissue is described as directly proportional to the effective cross-sectional area of the muscle. But since the amount of contractile substances may vary widely from one muscle cell to another, it would perhaps be more correct to use the effective cross-sectional myofilamental area as a basis for calculating the muscle's contractile strength.

SUMMARY

1. A modified method for calculating the protein composition of muscular tissue is described. An extraction procedure enables determination of the amounts of myofilamental proteins, sarcoplasmic proteins, stroma proteins, non-protein nitrogen, total nitrogen and water.

2. A first series of experiments were performed on forty-eight guinea pigs divided into three groups of sixteen. The animals in one of these groups were kept in very small cages so as to restrict their activity, those in another group were exercised and those in the last group served as controls. The myofilamental protein content was higher in the calf-muscles of the exercised animals than in either of the other two groups.

3. In a second series of experiments rabbits were kept up to 3 years in cages designed to severely restrict their activity. This gave rise to a modification of the composition of the thigh muscles manifested by reduced myofilamental content and enhanced sarcoplasmic content.

4. It is concluded that skeletal muscles adapt themselves to the degree of functional activity, not only by showing hypertrophy after exercise and hypotrophy after restricted activity but also by alterations in the composition of the muscle cells. Exercise thus enhances the myofilamental density in the muscle cell, whereas restricted activity reduces its myofilamental density and instead increases the sarcoplasmic content. Both these processes are liable to influence the contractile strength of the muscular tissue.

REFERENCES

Adams, R. D., Denny-Brown, D. & Pearson, C. M. (1953). *Diseases of Muscle.* New York: Harper Brothers.

Fischer, E. & Ramsey, V. W. (1946). *Amer. J. Physiol.* **145**, 571.

Helander, E. (1957). *Acta physiol. scand.* **41**, suppl. 141.

Helander, E. (1958). *Nature, Lond.*, **182**, 1035.

Mölbert, E. & Jijima, S. (1959). *Verh. dtsch. path. Ges.* **42**, 349.

Perry, S. V. (1956). *Phys. Rev.* **36**, 1.

Pratt, F. H. & Eisenberger, J. P. (1919). *Amer. J. Physiol.* **49**, 1.

Sassa, K. & Sherrington, C. S. (1921). *Proc. Roy. Soc.* B, **92**, 108.

Snedecor, G. W. (1956). *Statistical Methods*, 5th ed. Ames, Iowa: State College Press.

Spector, W. S. (1956). *Handbook of Biological Data.* Philadelphia: W. B. Saunders Co. Ltd.

Ufland, J. M. (1932). *Arbeitsphysiologie*, **6**, 653.