THE DISTRIBUTION OF THE DIFFERENT PROTEIN FRACTIONS IN NORMAL AND HYPERTROPHIZED CATS' HEARTS.

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(With 6 Tables in the text.)

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While trying to work out the mechanism of heart hypertrophies (HAJDU and BEZNÁK, 1944) the question arose of how the composition of the heart muscle itself changes during hypertrophy. Several authors have proved that there is a certain connection between the activity of an organ and its bio-chemical composition. WEBER and MEYER (1933) found that the distribution of the N, resp. protein fractions is different in white and red muscles. HITCHINGS and WEARN (1944), working on the distribution of intra- and extra-cellular electrolytes, found in rabbits that the extra-cellular electrolytes increased during the first few days, after the lesion of the aortic valves, but on the 6th day — that is, when hypertrophy was already present — the distribution became normal. Several authors proved the connection between activity and phospholipid content. Thus MAYER and SCHAEFFER (1914) in the case of liver and lungs, BLOOR, OKEY and CORNER (1930), KAUFMAN (1927) in the case of the active corpus luteum, BLOOR (1934) in the case of the mammary gland, MASUDA and BLOOR (1932), BIERICH DETZEL and LANG (1931) in the case of fast-growing tumors and BLOOR (1927) in the case of muscle. According to BLOOR and SNIDER (1934) the cholesterol content of working muscle also increases.

LUDEWIG and CHANUTIN (1936) found, contrary to this, no increase in the phospholipid content of hypertrophized rats' hearts counted per unit surface, nor did they find a difference in the creatin concentration

of these hearts (LUDEWIG and CHANUTIN, 1936). We were particularly interested in the protein content of hypertrophized heart muscle, resp. the distribution of protein in different fractions. Protein being the contracting substance of muscle, we wanted to find out whether the protein content or its distribution in different fractions changes during hypertrophy or whether there is in the course of the hypertrophy a phase in which a difference can be found.

METHODS.

Our experiments were carried out on cats of both sexes weighing 3.2 ± 0.6 kg. The cats lived on a mixed diet. In the first part of the experiments we carried out determinations on normal cats' hearts. The chest and pericardium of the animals were opened in Pernocton anaesthesia (0.8 ml/kg.). The heart was cut from the big vessels and perfused with ice-cold Ringer solution from the coronary arteries. The heart was then quickly freed from its pericardium and fatty tissue and the two atria were cut off. The right ventricle was cut along the septum and both parts (right ventricle, left ventricle+septum) frozen with CO_2 snow and weighed separately.

The lesion of the valves was carried out in ether anaesthesia by pushing down a long, round-headed rod through the left carotid artery into the ascending aorta and tearing at least one, possibly two, valves to pieces. The animals soon recovered after the operation and during the first week did not differ from normal ones. In the course of the second and third weeks some of the animals (in which as was later proved the valvular lesion was greatest) showed some slight signs of dyspnoe and avoided walking about much. In the course of our observation (30 days) we never saw serious decompensation (edema, cyanosis). We controlled the heart by taking electrocardiograph records; one from the heart before, one immediately after the valvular lesion, and one at the time when the heart was used for determinations. At different intervals the hearts of the animals were treated in the above described manner in Pernocton anaesthesia.

Determinations were always carried out on the part of the heart containing the left ventricle+septum. This was finely ground in a small Latapie mill and its total N-content estimated by simple micro-Kjeldahl method. From the remaining ground tissue the protein was then extracted. This was carried out by a slight modification of the WEBER and MEYER method (l. c.). The difference consisted mainly in employing

smaller amounts — since working with heart muscle we could not use as much tissue as WEBER and MEYER did. The extraction was carried out on 3.7 ± 0.8 g. ground heart muscle. The course of the extraction was the following: 5 extractions with 50—50 ml. of WEBER I solution, 6 hours each. The extraction was carried out according to WEBER at 0 C° temperature. The total time of extraction was 40 hours. In this way the N-fractions of the heart muscle were divided into two parts: one soluble which was in the 450 ml. of united WEBER I and WEBER II solutions, and one insoluble remaining in the muscular residue after the extraction.

In the soluble part we determined the non protein-N content by precipitation with sulphosalicylic acid. The separation of the proteins took place according to WEBER into myosin, globulin and myogen fractions. The myosin fraction was precipitated by dyalising through a cellophane membrane at 0 C° for 24 hours with constant shaking against a m/130, pH 6.3 phosphate buffer solution. The precipitate was centrifuged and its N-content determined. To 50 ml. of the myosin-free solution 4 times 25 ml. of a m/90 and pH 5.0 acetate buffer solution was added with constant shaking, then the whole was put into an ice-chest and shaken at 0 C° for an hour. The precipitate — corresponding to the globulin fraction — was then centrifuged and its N-content determined. The remaining solution containing the myogen fraction — soluble in these circumstances — was evaporated and its N-content determined. All N-determinations were carried out by simple micro-Kjeldahl analysis. In this way the soluble N-content of the heart was divided into 4 fractions: Non protein-N, myosin-, globulin-, and myogen-N.

The other part of the heart, that is the part remaining after the extraction, was treated in a way described by SPENCER, MORGULIS and WILDER (1937). The extracted muscular residue was dried with acetone and autoclaved for 3 hours at a pressure of 7 atmospheres. After centrifugation we determined the N-content of the remains, that is the fraction insoluble even under these circumstances, and called it Remaining-N. In the solution after centrifugation we precipitated the collagen and determined the N-content of the precipitate (collagen-N). The N-content of the solution after precipitation was also determined and called fluid-N. All N-determinations were carried out by simple micro-Kjeldahl procedure.

EXPERIMENTAL RESULTS.

Before describing our experimental results, we must say a few words about the experimental error of the methods used in the course of this work. We made 3 parallels to determine the N-content of the hearts. After the WEBER extraction the N-content of the extract was determined on 3 samples. The non protein-N was also determined on 3 samples. The determination of the myosin, globulin-, and myogen-N was made on 2 parallels in such a way that 100—100 ml. of the myosin extract was placed in two exactly similar dyalisors. After the myosin precipitate was centrifuged off 25—25 ml. of each was used for the precipitation of the globulin fraction; and the N-content of the two remaining fluids was also determined (Myogen-N). Of the non-extractable, acetone-dried muscle residue 3 samples were autoclaved; thus we had 3 parallel determinations of the remaining-, fluid-N, and collagen-N. The experimental error of these determinations is shown in Table I.

TABLE I.

Experimental error of the methods employed.								
Heart-N.	Extractable-N.	Non protein N.	Myosin-N.	Globulin-N.	Myogen-N.	Collagen-N.	Remaining-N.	Fluid-N.
± 1·8 %	± 1·5 %	± 2·3 %	± 4·1 %	± 8·8 %	± 6·8 %	± 5·5 %	± 12·1 %	± 6·9 %

Table II contains heart weights of cats whose aortic valves had been destroyed 9—29 days previously. Table II. shows that the hearts

TABLE II.

		Heart weight in g.			Heart weight in g. per kg. body-weight.			Left ventricle + septum weight in g.			Left ventricle + septum weight in g per kg body weight.			Left ventricle + septum per right ventricle		
	No	M	± μ	k	M	± μ	k	M	± μ	k	M	± μ	k	M	± μ	k
Normal	12	9·2	1·75	2·8	3·15	0·44	2·1	7·1	1·5	3·4	2·4	0·44	3·8	3·6	0·6	3·4
Hypertrophized	13	11·5	2·5		3·85	1·05		9·3	1·9		3·5	0·94		4·6	0·9	

of cats hypertrophize during that time and that this hypertrophy relates chiefly to the left ventricle. Table III. shows that the dry matter content of the heart does not change during hypertrophy. The content in dry matter is the same in the right and left ventricle whereas —

TABLE III.

Dry matter content of the hearts in %.								
Normal left ventricle + septum.			Hypertrophized hearts. left ventricle + septum.			Right ventricle.		
No	M	± μ	No	M	± μ	No	M	± μ
9	21·3	1·1	8	22·1	0·9	8	22·1	0·9
	→ k = 1·8 ←			→ k = 0·0 ←				

as seen in Table II. — only the left ventricle hypertrophized. Table IV. shows the N-content of the left ventricle in %. The hypertrophized

TABLE IV.

N-content of left ventricle + septum in %						Extractable N as % of the total N content					
Normal			Hypertrophized			Normal			Hypertrophized		
No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ
11	2·76	0·13	12	2·96	0·11	8	68·2	5·6	9	67·6	3·0
	→ k = 4·0 ←						→ k = 0·3 ←				

left ventricle contains somewhat more N than the normal one (significant difference 4.0). This surplus N seems to be divided very evenly between the different N-fractions for — as seen in following Tables — we found no difference between the N-fractions in normal and hypertrophized heart muscle. It is also to be seen from Table IV. what percent of the total N-content of the heart muscle can be extracted by the described method. In this respect normal and hypertrophized muscles do not differ. Table V. and Table VI. show the distribution

TABLE V.

The distribution of the extractable N into different fractions in %.																				
Myosin- N.				Globulin- N.				Myogen- N.				Non-protein- N.								
Normal			Hypertrophized			Normal			Hypertrophized			Normal			Hypertrophized					
No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ	No	M	± μ
8	22.5	3.4	9	26.5	7.8	8	12.2	4.3	9	11.5	5·3	8	13.8	3.4	9	18.4	8.0	8	10.7	2.8	9	17.4	4 5
	→ k = 1.4 ←					→ k = 0.3 ←						→ k = 1.6 ←						→ k = 0.4 ←					

TABLE VI.

The distribution of the non-extractable N into different fractions in %.

Collagen- N.						Fluid- N.						Remaining- N.					
Normal			Hyper-trophized			Normal			Hyper-trophized			Normal			Hyper-trophized		
No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$	No	M	$\pm \mu$
8	15·7	2·7	9	13·4	1·5	8	9·4	2·7	9	11·1	2·9	8	7·5	2·2	9	5·0	0·9
	→ k = 2·1 ←						→ k = 1·2 ←						→ k = 2·8 ←				

of the different N-fractions. There is no difference between normal and hypertrophized muscle. Perhaps the remaining-N — that is, the part which is insoluble in WEBER solutions and ever after autoclave treatment — decreases somewhat in hypertrophized heart-muscle (significant difference 2.8).

DISCUSSION.

In the first part of our experiments we determined the protein fraction of normal cats' hearts. In 3 cases we also estimated the different protein content of the psoas muscle of the cat. The N-content of the psoas muscle was always more ($3.46 \pm 0.13\%$) than that of the heart ($2.76 \pm 0.13\%$).A larger portion of this greater amount of N could be extracted according to WEBER ($81.0 \pm 4.9\%$), whereas from the heart muscle only $68.2 \pm 3.6\%$ of its N-content could be extracted. Similar results were obtained in the case of skeletal muscles of rabbits by WEBER and MEYER (l. c.) who found that in the case of white muscles 85%, in the case of red muscles 76% of their respective N-content could be extracted. There is accordingly a certain difference in the extractability of the N-content of different muscles (white muscle, red muscle, heart muscle). DEUTICKE (1926, 1930, 1932) found that more or less protein could be extracted from frog muscles according to the state of the muscle in question. If we assume that the different N-fractions of the cats' skeletal muscles are about the same as the values given by WEBER and MEYER in the case of rabbits, we find the following differences between the N-fractions of heart and skeletal muscles: the heart muscle contains less extractable N, accordingly more stroma-N. The non protein-N fraction is greater in the heart muscle than in the skeletal muscle, and the myosin-, globulin-, and myogen-N (especially the myosin fraction) is less. As SZENT-GYÖRGYI and his collaborators have proved (1941)

that the myosin is only extractable in the presence of adenosintriphosphate, we first thought of the possibility that the heart muscle contained less adenosintriphosphate or one that was destroyed faster and in consequence less myosin could be extracted. Therefore we performed the following experiment: in 3 cases we divided the heart muscle: one part was extracted in the usual way, to each of the third to ninth extractions of the other part we added 5—5 mg adenosintriphosphate. There was no difference in the amount of N extracted. That we find less myosin-N in the heart is therefore probably due to the fact that the heart muscle contains a smaller amount of myosin than skeletal muscle does, and not to an experimental error. Red muscle stands nearer to heart muscle inasmuch as it contains less extractable and therefore more stroma-N than white muscle.

We may mention that ALBURN and MYERS (1939) estimated the collagen content of different parts of dogs' hearts. They worked with the same method, of SPENCER, MORGULIS and WILDER (l. c.), which was used in our experiments. If we take the N-content of the dog's heart to be the same as that of cats' hearts (2.76%) we find, calculating with their data, that 15.5% of the total N-content is collagen-N. This value is in amazing accordance with our findings in cats' hearts according to which 15.7% of the total-N is Collagen-N.

In the second part of our experiments we wanted to find out whether the protein content or its distribution into different fractions shows any change during hypertrophy or whether there is in the course of the hypertrophy a phase in which a difference can be found. Therefore we carried out estimations at different times after the destruction of the aortic valves. The data here published are from hearts observed 8—10, that is 27—29 days after the valvular lesion. As there was not the slightest difference in the estimated values, the Tables of this paper contain the combined results. To decide the question finally, estimations should be carried out immediately (2—3 days) after the operation and several months later. The N-fractions of decompensated hearts should also be determined separately.

The conclusion to be drawn from these experiments is that — at least in the early and middle phases of the hypertrophy — the different N-fractions increase at the same rate, because their percentual proportions remained unchanged. We do not know what significance the fact of the N-content of hypertrophized heart-muscle being somewhat increased may have. This increase is about 7%. Since the N-content of the different fractions in normal and hypertrophized hearts

shows a great variability, this difference could hardly be valued even if the whole increase was due to the increase of a single N-fraction.

It was found in our earlier experiments on rats (l. c.) that the percentual dry matter content of the heart corresponds — even in the early phases of the hypertrophy — to the normal values. Table II. shows the same to be true in the case of cats' hearts.

Our experiments prove that as the quantitative and qualitative distribution of the heart's protein content remains unaltered, the heart increases its mass in such a way that the composition of the new matter corresponds exactly to that of the old.

SUMMARY.

1.) Cats' hearts contain somewhat less N than skeletal muscle does.

2.) A smaller part of the heart's N-content can be extracted according to Weber than of the skeletal muscle.

3.) Hypertrophized heart muscle contains somewhat more N than normal heart muscle.

4.) The distribution of the N-content into different fractions (myosin, globulin, myogen, Rest, collagen, remaining, fluid N) is the same in normal and hypertrophized heart muscle.

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REFERENCES.

Alburn, H. E., Myers, V. C. (1939). *J. Biol. Chem.* **131.** 713.
Beznák, M., Hajdu, I. (1944). *Orvostud. Közl.* No. 14.
„ „ (1946). *Schweiz. Med. Wschr.* **76.** 390.
„ „ (1944). *Orvostud. Közl.* **35.** 700.
„ „ (1945). *Schweiz. Med. Wschr.* **75.** 300.
Bloor, W. R (1927). *J. Biol. Chem.* **72.** 327.
Bloor, W. R., Okey, G. W. R., Corner, (1930). *J. Biol. Chem.* **86.** 291.
Bloor, W. R. Cit. Bloor, W. R. Snider, R. H. (1934). *J. Biol. Chem.* **107.** 459.
Bloor, W. R., Snider, R. H. (1934). *J. Biol. Chem.* **107.** 459.
Detzel & Lang (1931). *Z. Physiol. Chem.* **201.** 157.
Deuticke, H. J. (1926). *Verh. d. ges. deutsch. Naturforsch. u. Aerzte. Sept. 19-26.* Ber. ü. ges. Phys. 38. 372.

Deuticke, H. J. (1930). *Pflügers Arch.* 224. l.
„ (1932). *Hoppe-Seylers Z.* 210. 97.
Hajdu, I. Beznák M. (1943). *Orvostud. Közl.* No. 19.
„ „ (1945). *Schweiz. Med. Wschr.* **75.** 665.
Hitchings, G. H., Wearn, J. T. (1941). *J. Biol. Chem.* **140.** LXI.
Kaufman, C. (1927). *Z. Geburtsh. u. Gynäk.* **91.** 668.
Ludewig St., Chanutin, A. (1936). *J. Biol. Chem.* **115.** 327.
„ „ (1936). *Arch. Int. Med.* **57.** 887.
Masuda, T., Bloor, W. R. (1932), *J. Clin. Inv.* **11.** 667.
Mayer and Schaeffer (1914). *J. Physiol. et Path. Gén.* **16.** 325.
Spencer, H. C., Morgulis, S., Wilder, V. M. (1937). *J. Biol. Chem.* **120.** 257.
Szent-Györgyi, A. et al. (1941). *Studies from the Inst. of Med. Chem. Univ. Szeged Vol. I.*
Weber, H. H., Meyer, K. (1933). *Biochem. Ztschr.* **266.** 137.