Proteins of Heart in Experimental Cardiac Hypertrophy in the Rat.* (24452)

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Rapid cardiac hypertrophy follows exposure of rats to a reduced atmospheric pressure with increased environmental temperature(1). For example, after exposure to these conditions for 4 hours a day, a 50% increase in heart weight occurs after 12 days. A procedure has been developed for quantitative determination of cardiac protein fractions(2). The findings on growing rats have been reported(3). It was desirable to investigate the protein fractions of the hypertrophic hearts.

Methods. Hypertrophy was produced as previously described(1). Female rats of the Addis-Slonaker strain weighing 150 g were exposed to a simulated altitude of 25,000 ft. at 100°F for 4 hours a day. After average accumulated exposure time of approximately 48 hours, the mortality was 40%, deaths occurring mainly during the first 8 hours. The morning following last exposure, the animals were sacrificed and the hearts prepared as previously described(2), without the auricular appendages. Protein fractionation was carried out using procedure A. Briefly, the steps include the following: (1) grinding with sand; (2) extraction with 0.28 M Sucrose, .01 M Versene at pH 5 and centrifugation at 19,000 g (supernatant Fraction I); (3) extraction of residue with 0.28 M Sucrose, .01 M Versene at pH 7.4 and centrifugation at 650 g followed by 19,000 g (supernatant Fraction IA and residue mitochondria Fraction III); (4) extraction with 0.6 M KCl phosphate-pyrophosphate-versene buffer (supernatant Fraction II) and centrifugation to obtain residue (R). Fractions I, IA and II were subfractionated electrophoretically with Kern microelectrophoresis apparatus (type LK 30) which utilizes the interference principle. No unusual difficulties were encountered in analysis of the hypertrophic hearts. It was observed, however, that Fraction I, which is normally only slightly tinged with pink, was now distinctly red. Fraction II was considerably more turbid than usual but did not appear to be more viscous.

Results. In general, weight did not change during exposure to experimental conditions. Animals with weight loss exceeding 5 g were discarded. Fourteen hypertrophic hearts from 150 g rats with average weight of 794 mg were analyzed. The expected heart weight for unexposed animals was 541 mg. The findings were compared with the predicted values for 150 g rats obtained from the regression equations previously derived(3). The results are shown in Table I.

The total protein increased 42%. Individual protein fractions grew in a disproportionate manner. Several protein fractions exceeded general growth of the organ. Fractions I_5, II and mitochondria (III) exhibited the largest increases.

TABLE I. Quantitative Analysis of Protein Fractions from Hypertrophic Hearts.

	Pobs. (mg)	P 150 (mg)	Pobs./P 150	$R\%_{150}$
P_t	114.7 ±3.1*	80.7	1.42	.52
I_t	24.5 ± .9	16.9	1.45	.62
I_1	3.0 ± .1	2.8	1.07	.53
I_2	3.5 ± .2	2.4	1.46	.65
I_3	3.9 ± .2	2.9	1.34	.72
I_4	4.9 ± .3	3.4	1.44	.51
I_5	9.2 ± .4	4.6	2.00	.67
IA_t	9.0 ± .4	6.9	1.30	.41
IA_1	2.1 ± .1	1.8	1.17	.43
IA_2	4.5 ± .2	3.3	1.36	.52
IA_3	2.4 ± .2	1.7	1.41	.27
II_t	30.2 ±1.3	20.0	1.51	.84
$II\alpha$	5.2 ± .3	4.5	1.16	.90
$II\beta$	19.7 ± .8	11.0	1.79	1.00
$II\gamma$	5.3 ± .5	3.9	1.36	.63
III	9.6 ±1.1	5.6	1.71	.62
R	41.0 ± .9	30.6	1.34	.30

* Stand. error.

P_t = total protein; I_t, IA_t, II_t, III and R refer to total protein of major fractions; other subscripts refer to specific subfractions. Pobs. = experimental values; P 150 = calculated from regression equations[3] for 150 g normal rats; Pobs./P 150 = ratio of value for hypertrophic heart to normal heart; $R\%_{150}$ = increase in protein (mg)/g gain in body wt calculated from equations[3].

* This project was supported by grants from U.S.P.H.S. and Am. Heart. Assn.

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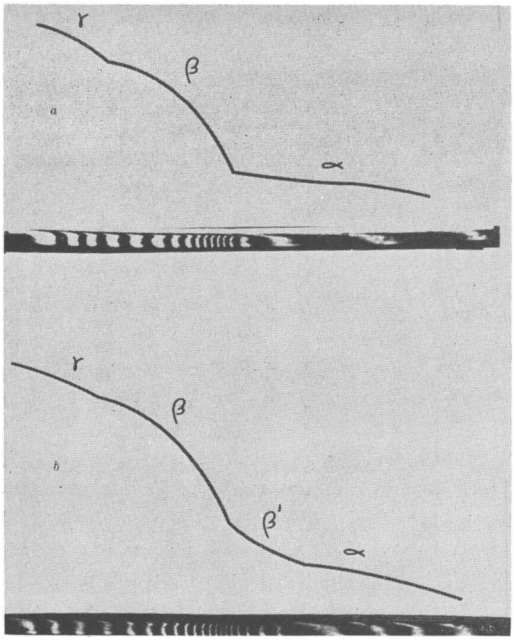

FIG. 1. Fraction II for Normal Heart (a) and Hypertrophic Heart (b). Extrapolation of electrophoretic pattern obtained with a Kern microelectrophoresis apparatus.

Close inspection of the interference patterns suggested that I_5 contained a second component. The possibility that this was due to myoglobin was offered by the following: It was pointed out above that Fraction I of the hypertrophic hearts was distinctly red. This suggested that an increase in hemoglobin and/or myoglobin had taken place. To test this point, the concentrated Fraction I was subjected to electrophoresis on paper at pH 8.9 with buffer using Spinco Electrophoretic apparatus. Unstained strips were compared to strips stained in the usual manner with brom thymol blue. Examination of these strips indicated that the pigment moved with the slowest moving Fraction I_5. The pigment had 2 components. By comparison with rat hemoglobin the faster pigment component was ascribed to this substance, the slower was assumed to be myoglobin. If this is the case it must be concluded that some increase in hemoglobin and a considerable increase in myoglobin content had taken place. Nearly undetectable amounts of pigment were present in normal hearts under similar conditions.

Fraction II β exhibited a second component with hypertrophy (Fig. 1). The fractions of II designated α, β and γ in this laboratory follows Dubuisson's designation α, β and contractin. II β corresponds to actomyosin of other investigators.

Discussion. The data describe changes in protein fractions of the heart in which hypertrophy was induced due to an increase in work load. It was not expected that the information would be useful in interpreting the events which had taken place since specific functions have not yet been assigned to each fraction. Nevertheless the apparent increase in myoglobin and the factual increase in mitochondria indicate an expansion of the oxygen transporting and oxygen utilizing systems. The changes in II β indicate presence of increased quantities of contractile protein. These findings are consistent with suggestions made previously that during metabolic disequilibrium changes in protein content are governed by the functional demand for services of the individual proteins which take place during the change(3).

It was of interest to compare changes observed with hypertrophy to that seen in growth. From data previously reported(3) the net deposition of protein for each fraction/g gain in body weight for 150 g rats was calculated (column 5, Table I). Comparison of this data with that derived from hypertrophic hearts suggested that hypertrophy is not simply an exaggerated growth. Certain differences were quite striking. For example Fraction II α was increased rapidly during normal growth but not with hypertrophy. Evidently the increased demand for the services of each fraction during an induced hypertrophy under an increased work load differs in certain respects from the demands made during normal growth.

Summary. Protein fractions of hearts from rats in which cardiac hypertrophy was produced by exposure to elevated environmental temperature and reduced atmospheric pressure were studied. Increases occurred in all fractions but in a disproportionate manner. Largest increases were observed in the following fractions: (1) I_5, due in part to an apparent increase in myoglobin, (2) in the contractile Fraction II β, due in part to appear-

ance of a component not normally demonstrated, and (3) in the mitochondrial fraction. It is suggested that these changes represent an adaptation to work demands placed upon the heart under experimental conditions.

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Received September 15, 1958. P.S.E.B.M., 1958, v99.

Brain Excitability in Relation to Age and Hypophysectomy. (24453)

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A type of brain excitability may be determined by measuring minimal electroshock threshold (EST) of rats. A number of variables have been reported to change this response: body weight, nutritional factors, endocrine imbalances, etc.(1-5). The undefined significance of body weight and of other variables occurring in chronic experiments makes it difficult to obtain, by EST measurement, an exact perspective of the role of the endocrine system on sensitivity of the central nervous system, and this may account for disparities in conclusions(2-5). In an attempt to explain the interrelationship of EST with body, brain, and adrenal weights, and age in intact growing rats, and in early and late hypophysectomized rats the following experiments were performed.

Materials and methods. Two hundred and eight, male Sprague-Dawley rats were used. Body weight and EST measurements were made on each of 10 groups of 10 intact rats, on each of 8 groups of 10 early hypophysectomized rats, and on each of 2 groups of 9 and 2 groups of 10 late hypophysectomized rats. Intact animals were exposed to electrical stimulation only once at 10 different time intervals between 23 and 95 days of age; the early hypophysectomized rats at 8 intervals between 25 and 137 days of age; and the late hypophysectomized rats at 4 intervals between age 70 and 137 days. Electroshock was produced by a 60-cycle stimulator with a 0.16 second duration through corneal electrodes. Tissues were removed and weighed 30 minutes after electroshocking. Coefficients of correlation and regression between EST and the other factors examined (organ and body weights and age) were statistically evaluated.

The results are summarized in Table I. Intact animals showed an increase in body, brain, liver, and adrenal weights; also EST increased with age. Early hypophysectomized rats (operated at 23 days) exhibited an initial drop in body weight followed by an increase. Brain and adrenal weights decreased; whereas liver weights and EST increased, although less rapidly than in intact animals. Late hypophysectomy (operation at 67 days) suppressed growth; body and brain weights remained steady, but adrenal and liver weights decreased. The adrenals appeared to atrophy whereas EST continued to increase parallel to rate of increase observed in the intact animal.

Discussion. In intact, growing animals, absolute value of EST increases with age and consequently with body, and other organ weights. In the hypophysectomized animals, *in which body and brain weight increase is inhibited,* EST still increases with time. When EST values were plotted against age, straight lines resulted; their coefficients of regression have been calculated by standard statistical methods. At 95% confidence intervals, there was no significant difference between the coefficients for intact (0.362) and late hypophysectomized rats (0.324). On the other hand, this coefficient was significantly different (0.220) for early hypophysectomized animals. Age appears to be most directly related to EST in both hypophysectomized and normal animals. Instead, if EST