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PATHOGENESIS OF CARDIAC HYPERTROPHY IN IRON DEFICIENCY ANAEMIA: THE ROLE OF NORADRENALINE

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Summary.—This study examined the effect of long-term administration of reserpine, an adrenergic blocking agent, on cardiac hypertrophy in animals with severe iron deficiency anaemia. This condition was induced by feeding rats on an iron-deficient diet for 30 days from the time of weaning. Anaemia was indicated by lowering of blood haemoglobin levels. Reserpine was administered i.p. (0·15 mg/kg body wt) every day during the experiment. Marked cardiac hypertrophy, as indicated by increased heart weight and increased size of cardiac muscle cells, was evidenced in iron-deficient rats, while the heart weights and myocardial cell size of drug-treated anaemic rats were in the normal range. The successful prevention of cardiac hypertrophy in anaemic iron-deficient rats by reserpine administration supports the hypothesis that noradrenaline plays a key role in the cardiac-hypertrophy process in iron deficiency anaemia.

FROM EARLIER STUDIES of isolated muscle (Spann *et al.*, 1967) and intact ventricle (Spann *et al.*, 1972) from pressure-overloaded hearts showing depressed systolic performance, it was concluded that cardiac hypertrophy, in the absence of failure, was associated with decreased myocardial contractility. However, more recently it has been shown that, depending on the nature of the stimulus, the degree and duration of ventricular-wall stress, and the age and health of the animal, hypertrophy results in a number of physiological and biochemical changes of the cardiac muscle reflecting augmented (Scheuer and Bhan, 1979; LeWinter, Engler and Karliner, 1980), normal (Sasayama *et al.*, 1976) or depressed (Carey *et al.*, 1978; Newman and Webb, 1980) mechanical function. Based on these facts, two types of hypertrophy of the cardiac muscle have been distinguished—physiological, with enhanced or normal contractility, and pathological, with decreased contractility (Wikman-Coffelt, Parmley and Mason, 1979; Grossman, 1980). Of clinical relevance is the possibility of preventing the transition from physiological to pathological hypertrophy. For this, it would be of the utmost importance to know the biochemical events that are involved in the pathogenesis of cardiac hypertrophy.

Cardiac hypertrophy has been produced by several methods, such as constriction of pulmonary artery and abdominal aorta, treatment with sympathomimetic amines and thyroxine, prolonged physical training, production of anaemia, and nutritional deficiency states (Fanburg, 1970). Recently, we have studied in our laboratory the effect of severe iron deficiency anaemia in the rat on noradrenaline levels and heart morphology (Rossi, Carillo and Oliveira, 1981). Significant decrease of myocardium noradrenaline concentration associated with cardiac hypertrophy, as revealed by increased wet heart weight and increased size of cardiac muscle cells, were observed in anaemic rats in comparison to controls. Taking into account that (a) an abnormally high urinary excretion of noradrenaline (Voorhess *et al.*, 1975) and a significant increase in plasma noradrenaline (Wagner *et al.*,

1979) have been reported in anaemic iron-deficient patients, (b) catecholamines overflow into the circulation only when the rate of utilization exceeds the capacity of the receptors to utilize it (Yamaguchi, DeChamplain and Nadeau, 1975) and (c) noradrenaline has been suggested (Laks and Morady, 1976) as the myocardial hypertrophy-inducing hormone (chronic infusion of subhypertensive doses of noradrenaline has been shown to result in left ventricular hypertrophy (Laks, Morady and Swan, 1973)), we have proposed that the low cardiac stores, the high plasma levels, and the increased urinary excretion of catecholamines in iron deficiency anaemia could be due to an increased release of neurotransmitter, associated or not with enhanced synthesis and a decreased reuptake mechanism, thus meaning a larger quantity of transmitter available to react with receptors, stimulating the myocardial muscle cells, and inducing protein synthesis and myocardial hypertrophy.

For the reasons given above it seemed logical to determine whether the development of cardiac hypertrophy in anaemic iron-deficient rats could be influenced by administering adrenergic blocking agents. Successful prevention of myocardial hypertrophy by one of these drugs would provide strong support for the view that noradrenaline plays a key role in the cardiac hypertrophy process in iron deficiency anaemia. We report here our results on the administration of reserpine, a drug that depletes the tissue stores of catecholamines (Wiener, 1980), to anaemic iron-deficient rats.

MATERIALS AND METHODS

Weaned male Wistar rats from our outbred colony with an average weight of 38 g were used. They were housed (4–5 per cage) in polypropylene cages and assigned randomly into 6 groups maintained for 30 days on the following diets: Group C (control), consisting of 12 animals, was fed on a nutritionally adequate semipurified diet; Group CR (control plus reserpine), consisting of 15 animals, was fed on a semipurified diet and treated with reserpine; Group D (iron-deficient), consisting of 20 animals, was fed on a iron-deficient diet; Group DR (iron-deficient plus reserpine), consisting of 17 animals, was fed on an iron-deficient diet and treated with reserpine; Group DS (iron-deficient supplemented with iron), consisting of 15 animals, was fed on an iron-deficient diet supplemented with iron; Group DSR (iron-deficient supplemented with iron plus reserpine), consisting of 11 animals, was fed on an iron-deficient diet supplemented with iron and treated with reserpine. The diet was contained in glass dishes, and deionized double-distilled water was offered in glass drinking tubes. Food and water were freely available to all animals. The animals were weighed twice weekly. Reserpine was administered i.p. (0·15 mg/kg body wt) every day.

The semipurified diet contained (g/100 g): vitamin-free casein—16·0; soybean oil—15·0; sucrose—40·0; dextrose—20·0; vitamin mixture —1·0; salt mixture—4·0; agar—3·8; and choline —0·2. The vitamin mixture was composed of (mg/100 g); retinol (100,000 u/g)—900; cholecalciferol (200,000 u/g)—50; α-tocopherol—500; ascorbic acid—4,500; inositol—500; menadione —225; *p*-aminobenzoic acid—500; niacin—450; thiamin—100; riboflavin—100; pyridoxine HCl—100; Ca pantothenate—300; biotin—2; folic acid—9; cyanocobalamin—0·135; dextrose to 100 g. The salt mixture (Manna and Hauge, 1953) contained the following amounts of salts (g/100 g): NaCl—13·945; KI—0·079; KH_2PO_4 —38·9; $MgSO_4.7H_2O$—5·73; $CaCO_3$—38·14; $FeSO_4.7H_2O$—2·70; $MnSO_4.H_2O$—0.401; $ZnSO_4.7H_2O$—0.048; $CoCl_2.6H_2O$—0.002. This diet provided 17·4 kJ/g (4·15 kcal/g).

The iron-deficient diet was prepared by mixing 681 g of powdered skimmed milk, 227 g of sucrose, 50 g of soybean oil, 30 g of salt mixture, 10 g of vitamin mixture, and 2 g of choline (modified from McCall *et al.*, 1962). The salt mixture contained the following amounts of salts (g/100 g): NaCl—2·9; KI—0·0007; $MnSO_4.H_2O$—0·554; $CuSO_4.5H_2O$—0·073; dextrose to 100 g. The vitamin mixture was composed of (mg/100 g): retinol (100,000 u/g)—200; cholecalciferol (200,000 u/g)—50; α-tocopherol—700; thiamin—20; riboflavin—25; pyridoxine HCl—12; Ca pantothenate—80; niacin—150; menadione—10; folic acid—10; *p*-aminobenzoic acid—150; biotin—2; inositol—20; dextrose to 100 g. The general composition of this diet was 24·5% protein, 58·1% carbohydrate and 5·7% fat, and the calculated caloric value was 16·01 kJ/g (3·82 kcal/g). Values for the constituents in the powdered skimmed milk were provided by the supplier (Companhia Industrial e Comercial de Produtos Alimentares, São Paulo, Brazil): fat 1%, protein 36%, lactose 52%, salts 8%, and water 3%.

The composition of the iron-deficient diet supplemented with iron was the same as the last except for the supplementation of 108 mg of $FeSO_4.7H_20$/100 g of diet (National Research Council).

Caloric values were calculated from weight percentages based on standard physiological fuel values, *i.e.*, 16·8 kJ/g (4 kcal/g) protein and carbohydrate, and 37·6 kJ/g (9 kcal/g) fat.

At the end of the experimental period the surviving rats were killed under light ether anaesthesia by exsanguination from the abdominal aorta between 2 and 5 p.m. The thoracic cavity was opened, exposing the still beating heart. The hearts were rapidly removed, rinsed in cold 0·9% NaCl solution, blotted, and weighed. The hearts were taken from 3–6 animals in each group and fixed as a whole in neutral 10% formalin for histological study. Both ventricles of each heart were isolated and cut into 3 fragments by 2 coronal sections at equal intervals. Each block was serially cut at 6 μm in the same direction, and sections were stained with haematoxylin and eosin. The relative diameter of representative muscle fibres was measured with the help of camera-lucida drawings projected from preparations. Areas containing properly orientated cross-sections of myocardial fibres were selected from each section (excluding papillary muscles and subendocardium), and the minor diameter was measured in each 500–1,000 fibres of each heart, in such way that at least 3,000 measurements were performed for each group. Tissue catecholamines of the hearts from the remaining rats were separated and subjected to fluorimetric assays according to the procedure of Anton and Sayre (1962, 1968). This method involves extraction of catecholamines from tissue homogenates with butanol, return of the amine to an aqueous phase, and the oxidation of the subsequent eluate to a fluorescent trihydroxyindole derivative in the presence of potassium ferricyanide and alkaline ascorbate. The fluorescence was read in an Aminco-Bowman spectrofluorometer. Readings were made at activation wavelengths of 409 and 422 nm and at emission wavelengths of 519 and 529 nm for the assessment of noradrenaline and adrenaline respectively. The catecholamine values are given in μg free base/g of wet tissue weight.

Blood haemoglobin concentration was monitored at the time the animals were killed by taking 20 μl of blood from the inferior vena cava before they were killed. Haemoglobin was assayed by the cyanomethaemoglobin spectrophotometric method (Drabkin, 1948).

Experimental data were analysed by Student's *t* test (Snedecor and Cochran, 1967). Unless specified, data are presented as mean ± s.e.

RESULTS

The body weights and growth rats of different groups are given in Table I. Since the body weight of animals is an important source of variability of organ weight (Setnikar and Magistretti, 1965), it seems obvious that the organ weight should be corrected for differences in body weights. Body- and heart-weight data on a large group of normal male Wistar rats in the body-weight range of 50 to 450 g were

TABLE I.—*Body weights (initial and final) and growth rates of rats from different groups*

Group	Body weight		Growth rate (g/day per rat)
	Initial	Final	
C	38·17 ± 1·10 (n = 12)	124·17 ± 3·81 (n = 12)	2·87
CR	37·00 ± 1·30 (n = 15)	96·15 ± 3·83 (n = 13)	1·97
D	39·05 ± 0·87 (n = 20)	103·22 ± 4·82 (n = 18)	2·14
DR	39·13 ± 1·35 (n = 17)	77·07 ± 2·72 (n = 15)	1·26
DS	39·13 ± 1·07 (n = 15)	117·38 ± 7·07 (n = 13)	2·61
DSR	37·82 ± 0·96 (n = 11)	98·22 ± 5·22 (n = 9)	2·01

Values are mean ± s.e. Group C, semipurified diet; Group CR, semipurified diet plus reserpine; Group D, iron-deficient diet; Group DR, iron deficient diet plus reserpine; Group DS, iron-deficient diet supplemented with iron; Group DSR, iron-deficient diet supplemented with iron plus reserpine.

collected, and expressed as a weight curve of heart weight relative to body weight. By using this method it was possible to compare the wet heart weight of rats from Groups C, CR, D, DR, DS and DSR to wet heart weights of equal body-weight controls (predicted), as done by Grove, Nair and Zak (1969). The wet heart weight of D rats—560·88 ± 16·09 mg—was significantly higher than the predicted heart weight of equal body weight controls (418·44 mg) (Table II). However, the heart weights of C—464·50 ± 21·67 mg, CR—385·45 ± 19·12 mg, DR—365·38 ± 16·19 mg, DS—474·23 ± 21·42 mg, and DSR—410·00 ± 23·14 mg were similar to the predicted wet heart weight of equal-body-weight controls (489·36 mg, 407·80 mg, 347·52 mg, 469·50 mg and 409·22 mg respectively).

Results of the morphometric study are tabulated in Table II. The means and standard deviation of the minor diameters of cardiac muscle cells for each group are given. The size of the myocardial cells of anaemic iron-deficient rats from Group D —11·73 ± 2·83 μm—was markedly greater than those of rats from Groups C—7·61 ± 1·43 μm, CR—7·52 ± 1·54 μm, DR—7·87 ± 1·73 μm, DS—7·80 ± 1·41 μm, and DSR—7·83 ± 1·21 μm. On the other hand, the average cell diameter of Groups C, CR, DR, DS, and DSR were very similar among themselves. This can be clearly seen when the percentile frequency distributions of fibre diameters in each group were then plotted, including fibres from all ventricles (Fig. 1). In order to assess the real effect of anaemia and reserpine administration on myocardial cell size, hearts were taken from a group of normal rats in the body-weight range of 70–150 g, and a cardiac muscle cell diameter curve relative to body weight was obtained, making it possible to predict the myocardial cell diameter of equal-body-weight control animals (predicted). The size of the myocardial cells of anaemic rats of Group D was markedly greater than that of the respective equal-body-weight control, while the average cell diameters of rats in Groups C, CR, DR, DS, and DSR were very close to the respective predicted mean myocardial cell diameter (Table II).

The blood haemoglobin levels of rats from different experimental groups are

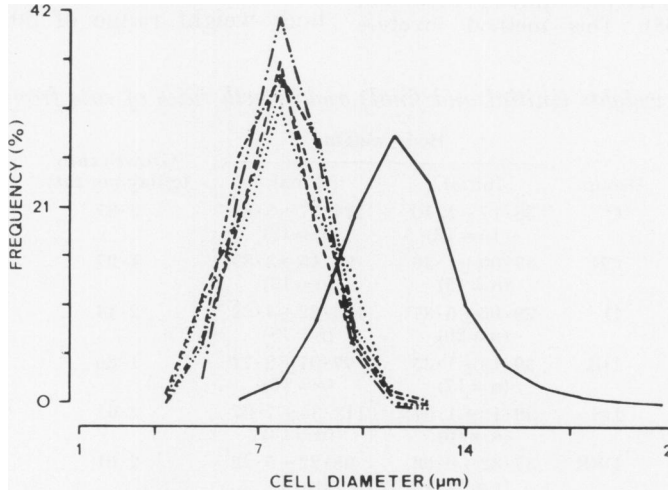

FIG. 1.—Percentile frequency distributions of myofibre diameters in different groups: Group C, semipurified diet (–·–·–·–); Group CR, semipurified diet plus reserpine (– – – – – –); Group D, iron-deficient diet (——); Group DR, iron-deficient diet plus reserpine (·····); Group DS, iron-deficient diet supplemented with iron (–···–···–); and Group DSR, iron-deficient diet supplemented with iron plus reserpine (—··—··—).

TABLE II.—*Hearts weights (observed and predicted), diameter of muscle fibres (observed and predicted), blood haemoglobin levels and heart noradrenaline of rats from different groups*

Group	Heart weight (mg)				Diameter of muscle fibre (μm)**		Haemoglobin (g/100 ml)	Noradrenaline (μg/g)
	Observed	Predicted	% of predicted	P*	Observed	Predicted		
C	464·50 ± 21·67 (n = 10)	489·36	−5·08	NS	7·61 ± 1·43	7·80	13·75 ± 0·62 (n = 11)	0·749 ± 0·046 (n = 9)
CR	385·45 ± 19·12 (n = 11)	407·80	−5·48	NS	7·52 ± 1·54	7·62	14·36 ± 0·47 (n = 12)	0·035 ± 0·006 (n = 11)
D	560·88 ± 16·09 (n = 17)	418·44	+34·04	$P < 0·00001$	11·73 ± 2·83	7·62	5·81 ± 0·54 (n = 18)	0·436 ± 0·005 (n = 13)
DR	365·38 ± 16·19 (n = 13)	347·52	+5·14	NS	7·87 ± 1·73	7·48	6·84 ± 0·50 (n = 14)	0·023 ± 0·005 (n = 8)
DS	474·23 ± 21·42 (n = 13)	469·50	+1·00	NS	7·80 ± 1·41	7·75	13·81 ± 0·27 (n = 13)	0·727 ± 0·031 (n = 11)
DSR	410·00 ± 23·14 (n = 9)	409·22	+0·19	NS	7·83 ± 1·21	7·58	13·50 ± 0·67 (n = 9)	0·052 ± 0·67 (n = 7)
C × CR	$P < 0·001$						NS	$P < 0·00001$
D × DR	$P < 0·00001$						NS	$P < 0·00001$
DS × DSR	$P < 0·05$						NS	$P < 0·00001$
C × D	$P < 0·001$						$P < 0·00001$	$P < 0·00025$
C × DS	NS						NS	NS
D × DS	$P < 0·0025$						$P < 0·00001$	$P < 0·0001$
CR × DR	NS						$P < 0·00001$	NS
CR × DSR	NS						NS	NS
DR × DSR	NS						$P < 0·00001$	$P < 0·01$

Values are mean ± s.e. *P = Measured *vs* predicted. **Mean ± standard deviation. NS = not significant. Group C, semipurified diet; Group CR, semipurified diet plus reserpine; Group D, iron-deficient diet; Group DR, iron-deficient diet plus reserpine; Group DS, iron-deficient diet supplemented with iron; Group DSR, iron-deficient diet supplemented with iron plus reserpine.

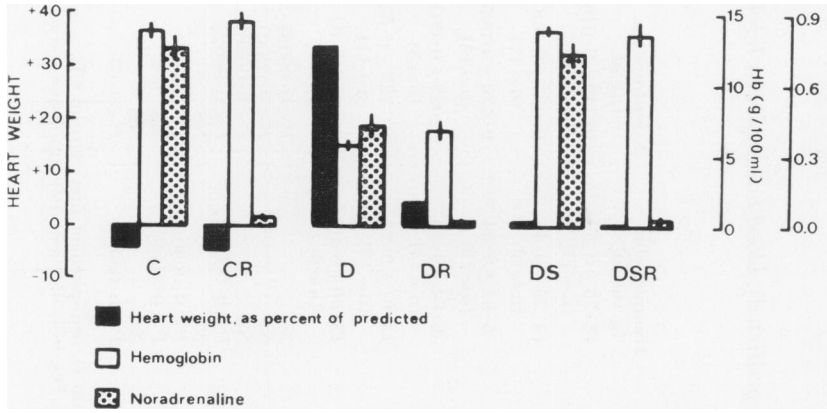

FIG. 2.—Bar graph showing the mean values of percentual change of wet heart weight compared to heart weight of equal-body-weight controls (predicted), cardiac noradrenaline and blood haemoglobin levels in different groups: Group C, semipurified diet; Group CR, semipurified diet plus reserpine; Group D, iron-deficient diet; Group DR, iron-deficient diet plus reserpine; Group DS, iron-deficient diet supplemented with iron; and Group DSR, iron-deficient diet supplemented with iron plus reserpine. The vertical bars represent the standard errors of the mean.

shown in Table II. The blood haemoglobin of rats on the iron-deficient diet only ($5{\cdot}81 \pm 0{\cdot}54$ g/100 ml) and on the iron-deficient diet and treated with reserpine ($6{\cdot}84 \pm 0{\cdot}50$ g/100 ml) were markedly diminished in comparison to the haemoglobin levels of rats fed a semipurified control diet only ($13{\cdot}75 \pm 0{\cdot}62$ g/100 ml), of rats fed a semipurified control diet and treated with reserpine ($14{\cdot}36 \pm 0{\cdot}47$ g/100 ml), of rats fed on an iron-deficient diet supplemented with iron only ($13{\cdot}81 \pm 0{\cdot}27$ g/100 ml), and of rats fed an iron-deficient diet supplemented with iron and treated with reserpine ($13{\cdot}50 \pm 0{\cdot}67$ g/100 ml) (Table II).

Table II shows the average values of concentration of noradrenaline in the hearts of rats from Groups C, CR, D, DR, DS and DSR. The heart noradrenaline, expressed as µg/g wet tissue weight, of anaemic rats from Group D—$0{\cdot}436 \pm 0{\cdot}005$ µg/g—was significantly lower than that of controls from Group C—$0{\cdot}749 \pm 0{\cdot}046$ µg/g. However, no differences were found between the values of myocardial noradrenaline concentration in rats fed an iron-deficient diet supplemented with iron —$0{\cdot}727 \pm 0{\cdot}031$ µg/g—and that of C rats. Furthermore, a marked depletion of myocardium noradrenaline levels was observed in all groups chronically treated with reserpine (Group CR—$0{\cdot}035 \pm 0{\cdot}006$ µg/g, Group DR—$0{\cdot}023 \pm 0{\cdot}005$ µg/g, and Group DSR—$0{\cdot}042 \pm 0{\cdot}005$ µg/g).

Figure 2 illustrates the percentage change of wet heart weight compared to heart weight of equal-body-weight controls, cardiac noradrenaline and blood haemoglobin levels in Groups C, CR, D, DR, DS and DSR.

DISCUSSION

In the present investigation the experimental procedure used for inducing iron deficiency anaemia in rats was based on the model of McCall *et al.* (1962). The experimental design employed allowed the rats fed on an iron-deficient diet to develop a severe anaemia. The nutritional adequacy of the iron-deficient diet, other than iron, was evidenced by continued growth as well as normal haemoglobin levels of rats given the same diet supplemented with iron (Group DS) in comparison with controls fed a nutritionally adequate semipurified diet (Group C).

Rats of Group D fed an iron-deficient diet showed restriction of body-weight gain. This retardation in growth may be explained by inadequate availability of

calories from carbohydrates due to possible decreased activities of intestinal disaccharidases in iron deficiency (Sharma, Singh and Simlot, 1973). Besides, the reserpine-treated rats of Groups CR, DR and DSR showed an impairment of body weight gain of 31%, 41% and 23% in comparison to their respective control groups C, D and DS. Decreased weight gain has been reported in rats treated with reserpine (Iversen *et al.*, 1967). The mechanism of the growth-inhibiting effect of reserpine is unknown. However, it can be speculated that the mild changes in stool consistency observed in reserpine-treated animals might reflect a slight impairment of intestinal absorptive function. Another possibility is that reserpine could directly affect the matabolism of carbohydrates, lipids and proteins in the organism. Also the drug can produce a variety of endocrine changes in laboratory animals (Gaunt, Chart and Renzi, 1963), perhaps caused by depletion of dopamine in the median eminence that is involved in the regulation of the secretion of hormones of the adenohypophysis. The more pronounced growth retardation observed in rats of Group DR can be explained by an additive effect of iron deficiency and reserpine treatment.

The absolute weights of hearts and diameters of muscle fibres from D animals were significantly greater than the predicted heart weight and muscle-fibre size of equal-body-weight controls, while the heart weights and myocardial cell diameters from Groups C, CR, DR, DS, and DSR were not different from those of their respective body-weight controls. The concentration of noradrenaline in the hearts of D rats was significantly decreased compared with that in C and DS animals. However, the concentration of noradrenaline in the hearts of DS rats was unaltered when compared with that in controls (C). On the other hand, the cardiac noradrenaline of rats treated with reserpine from Groups CR, DR, and DSR was extremely depleted. In other words, the findings in the present investigation, besides confirming our previous results (Rossi *et al.*, 1981), clearly show that the administration of reserpine completely inhibited the cardiac hypertrophy, as indicated by increased heart weight and increased myocardial cell size, induced by iron deficiency anaemia.

Since it is generally agreed that an increased cardiac work load results in increased wall tension leading to the synthesis of protein and myocardial hypertrophy, one possible explanation for the inhibition by reserpine of anaemia-induced cardiac hypertrophy would be that the hearts of reserpine-treated anaemic rats of Group DR were subjected to a decreased work load in comparison with untreated anaemic animals of Group D. It is known that chronic anaemia increases the cardiac output probably caused by an increase in heart rate and stroke volume in both man and laboratory animals (Varat, Adolph and Fowler, 1972). However, not only were the reserpine-treated anaemic rats subjected to the same iron-deficient diet as the untreated anaemic group, they also displayed the same degree of anaemia.

It could also be argued that reserpine might cause hypotension leading to a decreased work load for the heart. It is well known that reserpine causes a fall in blood pressure frequently associated with bradycardia (Wiener, 1980); hence one would expect that the reserpine-treated control rats of Groups CR and DSR would have significantly smaller hearts than the respective body-weight control animals. However, the observed heart weights and cardiac muscle-cell diameters in these groups were found to be very close to the predicted heart weights and myocardial cell size of the respective body-weight controls.

Thus we are left with the possibility that noradrenaline may have a direct growth-promoting effect on cardiac muscle. It has been reported that catecholamines affect the cell growth and division of *Tetrahymena pyriformis* (Iwata, Kariya and Fujimoto, 1969). Also, catecholamines increase RNA synthesis in chick embryo

glands (Caldarera, Giorgi and Casti, 1970) and stimulate nucleic acid metabolism in cardiac muscle cells (Wood, Lindenmayer and Schwartz, 1971). Laks *et al.* (1973) have shown that chronic infusion of subhypertensive doses of noradrenaline results in left ventricular hypertrophy. Similarly, Mallov (1973) has demonstrated that chronic s.c. administration of suspensions of adrenaline, noradrenaline, isoproterenol (isoprenaline) and phenylephrine in oil promotes an increase in the rate of protein synthesis in rat heart. Noradrenaline given chronically *in vivo* has been shown to reduce the hormone-sensitive adenylate-cyclase system and to increase the activity of a specific 3′–5′–AMP phosphodiesterase, both changes involving the synthesis of new protein (Weiss, 1973). Recently Östman-Smith (1979) has reported that chemical sympathectomy with guanethidine treatment completely abolished the exercise-induced cardiac hypertrophy in rats. More recently, we have demonstrated an increase in myocardial noradrenaline levels and heart hypertrophy in both malnourished chronic alcoholic rats (Rossi, 1980a, b) and protein-energy malnourished rats (Rossi *et al.*, 1980). It is therefore quite a plausible hypothesis that noradrenaline is the cardiac hypertrophy-inducing hormone in iron deficiency anaemia—increased physiological and pathological demands for cardiac work induce augmentation of protein synthesis mediated by release of noradrenaline by the sympathetic nervous system into the myocardium. In other words, noradrenaline may be the hormone that puts in motion the biochemical machinery involved in protein synthesis. Moreover, it can be pointed out that the implications of the present results showing the involvement of noradrenaline in the intrinsic mechanism of the cardiac-hypertrophy process could be more than of mere academic interest.

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