Cardiac Hypertrophy in Rats with Phenylhydrazine Anemia

By TOM D. NORMAN, M.D., AND ROBERT D. McBROOM

The effect of phenylhydrazine-produced anemia upon the hearts of rats was studied with observations upon the accompanying hypertrophy. Results indicated that the mechanism of hypertrophy under these conditions may be one of myocardial injury rather than mechanical.

This investigation was undertaken for the purpose of studying the effect of anemia upon the hearts of rats, of determining whether cardiac hypertrophy occurs, and, if so, of studying the mechanism of hypertrophy. In spite of the widespread belief that cardiac hypertrophy does occur with anemia, Stembridge and Rigdon have indicated the lack of evidence to support many of the reported cases. Their report of only 1 acceptable heart hypertrophy in autopsies of 7 persons with sickle cell anemia emphasizes the paucity of authentic examples of cardiac hypertrophy due to anemia in man.

Widdowson and McCance found in piglets with a suckling anemia an increase in heart weight which they thought actually due to an increase in the number of normal muscle cells. A comparable study was made by Forman and Daniels in which cardiac hypertrophy apparently occurred in rats with a milk anemia; 2 rats given supplementary vitamin B did not have an increase in heart weight. Results in both of these studies may be questioned, since in any nutritional anemia the hypertrophy produced may be related to thiamine deficiency.

METHODS

Eighty female Holtzman rats, weighing from 139 to 254 Gm., were divided into groups as follows:

Group 1. Ten rats received intraperitoneal injections of phenylhydrazine hydrochloride (40 to 50 mg./Kg. body weight) every other day for 15 days.

Group 2. Ten rats received phenylhydrazine hydrochloride every other day for 24 to 27 days.

Group 3. Ten rats received phenylhydrazine hydrochloride every fourth day for 27 days.

Group 4. Ten rats received phenylhydrazine hydrochloride every other day for 28 days; additional daily intramuscular injections of 10 mg. of thiamine hydrochloride were given each animal beginning on the fourteenth day.

Group 5. Ten rats received phenylhydrazine hydrochloride every other day for 28 days.

Group 6. Thirty rats received no injections.

The animals in groups 1, 2, 3, and 4 were killed after the stated period of drug injection; those in group 5 were killed 56 days after the initial injection, receiving no phenylhydrazine during the last 28 days of the experiment; the rats in group 6 were killed after a single hemoglobin determination at initiation of the experiments.

The phenylhydrazine hydrochloride for the injections was recrystallized 3 times before making a 1.55 per cent solution in normal saline. All of the rats were kept in wire cages, 2 per cage, and were given Purina Laboratory Chow and water as they desired.

Hemoglobin determinations were made by the cyanmethemoglobin method upon 2 occasions in the rats of group 1, and from 3 to 7 times in the other animals except those in group 6 in which a single observation was made. These studies were done on days between the phenylhydrazine injections.

When the animals were killed each heart was excised by cutting across the blood vessels at their junction with the heart, opened, washed and blotted dry before being weighed on a Voland balance. The atria were then excised from the ventricles and the right ventricle from the septum; the left ventricle with attached septum and the right ven-

*This method is furnished by the Department of Hematology, Walter Reed Army Institute of Research, and is derived from Sunderman's modification of Stadie's method (Am. J. Clin. Path. 23: 519-568, 1953).
tricle were then weighed separately. A portion of the anterior wall of the left ventricle was placed in a drying oven at a temperature of 90 to 100°C for 3 or more days, until a constant weight could be recorded. A similar part of the left ventricle was placed in 10 per cent formalin and the remainder of the heart was preserved by freezing. The spleen, liver, and kidneys from each rat were also weighed and, with the lungs and heart, were prepared for histologic studies.

The weights of the hearts were expressed in relation to body weight as heart weight-body weight ratios ($X10^{-4}$) and as regression lines plotted on log-log graphs. These lines were determined by the equation $\log Y = \log a + b \log X$ where $X$ = body weight and $Y$ = heart weight and $a$ and $b$ are derived parameters. The use of the linear expression of the heart weights was suggested by the work of Walter and Addis in demonstrating the exponential growth of such organs as the heart of the rat.

For a further study of the significance of the differences between the heart weights of the rats in the control and the various experimental groups, analyses of covariance were made. A probability ($p$) value of 0.01 or less was regarded as significant.

Analysis of variance was used to determine the significance of the difference between the per cent water in heart tissue and left-right ventricle ratios in the various groups. A probability value of 0.02 or less was regarded as significant.

**RESULTS**

**Blood Hemoglobin.** Figure 1 illustrates the anemia of the various groups at intervals after the beginning of treatment. The anemia produced by the less frequent injections of phenylhydrazine (group 3) was unexpectedly similar to that of the animals injected every 2 days, being significantly less only in the period of the first 10 days.

**Heart Weights.** The mean of the heart to body weight ratio ($X10^{-4}$) for each experimental group is shown in table 1. The ratio for the control group of rats was 34.7. This figure is lower than the mean ratio for any of the treated rats, but only slightly lower than that of the rats treated for 15 days (35.9). The largest mean ratio (41.9) was present in the rats made anemic for 28 days and killed 28 days later. The initial mean body weights (table 1) varied to some extent, but the mean final body weights varied even more. In view of these variations in the final body weights and the recognized variation in the heart-body weight ratio with changes in body weight, it was decided that statistical analyses of the differences in heart-body weight ratios would not be valid.

The control regression line has been used as a basis of comparison for each of the other experimental groups (figs. 2 to 6), with 1 standard error of the estimate plotted for the control and each treated group’s regression.
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line. The regression line constructed from the heart and body weights of the control animals (group 6) illustrates the increase in heart weight as body weight increases. From this, a 220 Gm. rat would be expected to have a heart weight in the range of 0.745 ± 0.039 Gm. In figure 2 the data are interpreted to mean that there has been no significant change of heart weights in either direction in the group 1 rats (killed after phenylhydrazine treatment every other day for 15 days). The data for the rats which were killed after phenylhydrazine treatment on alternate days for 24 to 27 days (group 2) formed the regression line shown in figure 3; this group appears to have heart weights significantly greater than

Fig. 2 Top, left. Regression lines from data of rats killed after 15 days of phenylhydrazine injections every other day (group 1) and of control rats. Lighter lines indicate 1 standard error of the estimate.

Fig. 3 Bottom, left. Regression lines from data of rats killed after 24 to 27 days of phenylhydrazine injections on alternate days (group 2) and of control rats. Lighter lines indicate 1 standard error of the estimate.

Fig. 4 Top, right. Regression lines from data of rats killed after 27 days of phenylhydrazine injections every fourth day (group 3) and of control rats. Lighter lines indicate 1 standard error of the estimate.

Fig. 5 Bottom, right. Regression lines from data of rats killed after 28 days of phenylhydrazine injections every other day (group 4) and of control rats. The treated animals also received injections of thiamine hydrochloride after the fourteenth day. Lighter lines indicate 1 standard error of the estimate.
those of the control rats. The heart weights of animals killed after receiving injections every fourth day for 27 days (group 3) formed the regression line in figure 4, indicating a significant increase in the heart weights of these animals in comparison with the control group. The regression line for the group 4 rats (killed after receiving phenylhydrazine every other day for 28 days as well as injections of thiamine hydrochloride) demonstrates a significant increase in these heart weights over those of the control group (fig. 5). Figure 6 illustrates the regression line developed by the data obtained from the rats which received phenylhydrazine every other day for 28 days and were allowed to live for 28 more days (group 5); the difference between the line for this group of animals and that for the controls is of questionable significance because of the large standard error of the estimate (± 0.298).

The significance of the difference between heart weights of the control and various experimental groups, as determined by analyses of covariance using body and heart weights, demonstrated in groups 2 through 5 that the probability value was less than 0.01, and that therefore the differences were significant; in group 1 (phenylhydrazine-treated for only 2 weeks) the p value was greater than 0.05 and therefore the difference was not significant.

Myocardial Water Content. The mean water content in the myocardium of the rats is listed in table 1. If one animal with an unusually high value is deleted from group 3, the highest mean value was 79.5 per cent. Although the mean water content was slightly greater in the hearts of the animals with evidence of increased heart weight, the difference between the means of the various groups is not regarded as significant, since a p value of greater than 0.1 was obtained by analysis of variance.

Left to Right Ventricle Ratio. The left to right ventricle ratios for the various groups are given in table 1. The difference between the mean ratios of the various groups is not considered significant, since a p value of greater than 0.1 was obtained by analysis of variance. Therefore, even though the mean ratio was slightly less in each of the treated groups than in the control group, the increased weight of the hearts was not considered to be unequally distributed between the ventricles.

Histologic Observations. A large amount of brown pigment and foci of hematopoiesis were present in the livers and spleens, but not in the hearts, of the treated rats. A mild chronic interstitial pneumonitis was in general more severe in the treated than the control animals. There was no obvious difference in the size of the myocardial fibers, but actual measurements of these cells were not made. No inflammatory changes were present in the myocardium.

Discussion

The degree of anemia found in these rats was less than that expected on the basis of the known effect of phenylhydrazine and the general appearance of the animals. It is thought that the actual hemoglobin values of the treated animals were somewhat lower than those recorded, because of the presence of Heinz bodies resulting from the phenylhydrazine. Subsequent studies of animals made anemic with this drug have shown that blood hemoglobin values are approximately 1 Gm.
lower after centrifugation of the cyanomet-hemoglobin solutions.

The term myocardial hypertrophy is used in this presentation for any increase in the weight of the heart, although myocardial fiber size has not actually been measured. The lack of significant difference in the myocardial water content of the control and various experimental groups of rats is evidence that the increase in heart weight in these animals was not due to a local change in tissue fluids.

Covariance analysis demonstrated conclusively that the enlargement of the hearts in animals treated with phenylhydrazine for periods greater than 15 days was of statistical significance, in general agreement with deductions made from the presentation of the data as regression lines. Several features of this hypertrophy are opposed to mechanical factors as a cause for the heart enlargement. The time required for the development of the hypertrophy was considerably longer than the 2 days demonstrated by Rather as necessary for the development of hypertrophy in hypertensive rats. In addition, cardiac hypertrophy existed 28 days after the end of a 28 day phenylhydrazine treatment period; the hemoglobin was at or above the pretreatment levels during most of these 28 days following treatment with the chemical. Although the large standard error of the estimate indicates that this may be near the critical point for regression of the hypertrophy, the fact that regression was relatively slow in these animals is of importance, since it has been shown by Hall and his associates that there was a complete regression of assumed heart hypertrophy after 15 days, when rats with hypertension due to ligation of the renal artery had the altered kidney removed. The fact that the left to right ventricle ratios in each experimental group of rats were approximately the same may contribute something to an understanding of the pathogenesis of the hypertrophy, since mechanical factors as a rule produce hypertrophy primarily of one ventricle or the other. Other studies have shown that cardiac output is not altered in chronically anemic animals until the hemoglobin is lower than 7.0 Gm./100 ml. of blood; it is doubtful whether the level of anemia produced had any effect upon cardiac output in the present experiments. The length of time necessary for myocardial hypertrophy to occur, its slowness in regressing, and a relatively equal increase in weight of both ventricles provide evidence that the mechanism of hypertrophy was non-mechanical. An increase in heart weight in animals anemic to a degree that is probably not adequate to alter cardiac output supports this concept. Myocardial injury of an undetermined type offers the best explanation for the increase in heart weight which was produced.

The mean weight gain (table 1) in the various experimental groups was as expected, with the exception of group 5. These animals were killed after a period of observation longer than any of the other groups, but had a weight gain only slightly more than that of the group observed for the shortest period of time. The reasons for their failure to gain are not clear. With the possible exception of rats of group 5, it seems extremely unlikely that a nutritional factor entered into these experiments; the fact that hypertrophy was present in the group of rats receiving thiamine is additional evidence that the increases noted in the heart weights were on a non-nutritional basis.

Can the presence of a mild chronic pneumonitis in these animals, more severe in the treated groups, possibly be related to the development of the heart hypertrophy? Its presence in both control and treated animals, although milder in the control rats, together with an absence of inflammatory changes in the myocardium and the lack of a change in the left to right ventricle ratio, appears to be evidence enough to rule it out as a cause of the hypertrophy.

SUMMARY

Fifty rats, made anemic by repeated injections of phenylhydrazine hydrochloride, and varying in groups of 10 by the frequency of injections, duration of treatment, the additional daily injection of thiamine hydrochloride, and the time of killing after cessation of
the treatment, were compared with 30 rats who were not anemic. A statistically significant increase in heart weight occurred in rats treated for approximately 4 weeks; this increase in weight was not prevented by the administration of thiamine hydrochloride. The increase in heart weight was present 28 days after the end of treatment of animals for a period of time considered adequate for hypertrophy to occur, but the range of heart weights in these rats was greater.

Acknowledgments
The technical assistance of Judith Forbes, Betty Horn, and Marguerita K. Krestensen, B.A., M.T. (ASCP), is gratefully acknowledged. Credit is also due Helen W. Reilly, B.S., and James G. Hiltun, Ph.D., for aid with the statistical analyses.

SUMMARIO IN INTERLINGUA
Cinquanta rattos, facite anemic per repetite injectiones de hydrochloruro de phenylhydrazina e classate in gruppos de 10 individuos secundo le frequentia del injectiones, le duration del tractamento, le diurne injectiones supplementari de hydrochloruro de thiamina, e le tempore de lor sacrificio post le cessation del tractamento, eseva comparete con 30 rattos que non esseva anemic. Un augmento del peso del corde de grados de significacion statistic occurreva in rattos tractate durante approximativamente 4 septimanas. Iste augmento del peso cardiaque non esseva prevenite per le administration de hydrochloruro de thiamina. Le augmento del peso cardiaque eseva presente 28 dies post le fin del tractamentino in omne animales que habeva recipite un tractamento considerate como sufficienente pro causar le hypertrophia, sed post iste intervallo le variabilitate del pesos cardiac eseva plus pronunciate.

REFERENCES
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Circ Res. 1958;6:765-770
doi: 10.1161/01.RES.6.6.765

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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