Effect of Pyridoxine Deficiency on Blood Volume Measured by $RI^{131}SA$ and Organ Weights in Rats.* (27159)

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Our previous studies(1) have indicated that deficiency of pyridoxine in rats was associated with an elevation of serum Na and muscle K, accompanied by a reduction of muscle Na concentrations. This observation has been recently confirmed by Hartsook and Hershberger(2). These findings and the derangement of water metabolism in pyridoxine deficient rats reported by others(3), taken as a whole, have suggested the possibility of concomitant alteration in blood and plasma volumes. Differences in electrolyte retention by various organs associated with pyridoxine deficiency may also alter their moisture content.

The present work reports the effects of dietary deprivation of pyridoxine on blood and plasma volumes measured by radioiodinated serum albumin ($RI^{131}SA$) technic. The wet and dry weights of the heart, kidneys, liver, spleen, thymus, adrenals and testes from pyridoxine deficient rats were compared with respective organs from controls. The differences between the wet and dry weights from various organs were used as indices of moisture content.

Materials and methods. (1) *Blood volume measurements*: Pyridoxine deficiency was produced in male and female rats of the McCollum strain by standard procedures previously reported(1). The rats, 5-7 weeks old, were placed on a basal diet deficient in pyridoxine. Control animals received a supplement of pyridoxine HCl (20 mg/kg) added to the basal diet deficient in pyridoxine. All animals were housed in individual cages provided with wide screen bottoms. They were weighed weekly and were given water *ad libitum*. Upon onset of deficiency evidenced by acrodynia and reduction of serum glutamic-oxalacetic transaminase activity, pyridoxine deficient and non-depleted control animals were lightly anesthetized with nembutal and weighed. Each rat then received a single intravenous injection of 0.25 ml of fresh $RI^{131}SA$ solution in isotonic saline by way of the external great saphenous vein. The dose of injected radioactivity ranged from 0.5 to 0.8 μc. Cardiac blood samples were obtained from the animals at 12 ± 1 minutes following the injections. The blood samples were centrifuged and their hematocrits measured. The I^{131} radioactivity in aliquots of both whole blood and plasma were measured by means of scintillation spectrometry.

(2) *Organ weights*: The experimental design used to study the effect of pyridoxine deficiency on organ weight was similar to that used in previous work(1). After 6 weeks the animals were killed by severance of the neck vessels. The organs were removed, washed with isotonic saline, and gently dried with filter paper. The wet organs were weighed on an analytical balance, dried in beakers at 58-60°C for 24 hours, and their dry weights measured.

Results and discussion. The effects of pyridoxine deficiency on blood and plasma volumes of male and female rats are shown in Table I. Supplementation of the basal diet with pyridoxine increased growth. Changes in total blood volumes were proportional to the size of animals, but pyridoxine deficiency did not influence blood and plasma volumes expressed in terms of ml/100 g body weight. No differences between blood volumes of male and female rats were noted. Brown and Pike(4) studied the changes in blood volume in Vit. B_6 deficient and control pregnant and non-pregnant rats by means of an intrave-

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TABLE I. Blood Volumes of Normal and Pyridoxine-Deficient Rats.

Group	Pyridoxine supplement		Pyridoxine-deficient		Stock animals	
No. rats	5	5	5	5	6	2
Avg wt	278	198	108	133	311	205
	♂	♀	♂	♀	♂	♀
Plasma vol:						
Total (ml)	6.35 ± .68*	5.82 ±.09	3.96 ±.27	4.26 ±.11	12.10 ±1.34	8.61 ± .97
ml/100 g BW	2.69 ± .29	2.80 ±.04	3.67 ±.25	3.24 ±.08	3.96 ± .17	4.19 ± .09
Blood vol:						
Total	15.62 ±1.12	14.37 ±.59	7.65 ±.61	8.75 ±.45	21.91 ±3.08	14.53 ±1.49
ml/100 g BW	6.62 ± .48	6.89 ±.16	7.07 ±.15	6.60 ±.06	7.00 ± .24	7.08 ± .07

* Mean value ± stand. dev. of mean.

nous Evans blue dye method. These authors found that the pregnant and non-pregnant animals treated with desoxypyridoxine had the lowest total blood volumes. In contrast, in the present study where pyridoxine deficiency was produced by a pyridoxine deficient diet alone, deficient animals of both sexes had almost identical total blood volumes compared to their corresponding controls. Although the plasma volumes tended to be higher in deficient groups, the differences were not statistically significant. The average total blood volume of 6.82 ± .72 ml determined by the $RI^{131}SA$ procedure in 28 rats is considerably higher than the range of 4.69 to 5.67 ml/100 g body weight estimated by the Evans blue dye technic(4).

Organ weights of the experimental animals are listed in Tables II and III. Table II shows statistically increases in wet and dry weights of liver, kidneys, adrenals and testes of pyridoxine deficient male rats compared to those that received pyridoxine. However decreased wet and dry weights of thymus were observed in the pyridoxine deficient group. These differenecs were accentuated in the pyridoxine-deficient group by administration of the antagonist, desoxypyridoxine. Weights of heart and spleen in pyridoxine deficient male rats were close to those of the control group. Table III shows a large increase in liver weight in pyridoxine deficient female animals accompanied by a thymus weight decrease similar to that found in the deficient male rats. In the deficient females differences in kidney and adrenal weights corresponding to those in males were not observed, but spleen weight increased. Administration of desoxypyridoxine to female rats was associated with significant weight increases of liver, kidney, and spleen weight accompanied by a concomitant decrease of thymus weight. Heart weight was not affected by pyridoxine deficiency.

For comparison with these data organ weights of stock male and female rats were determined. These are shown in the last columns of Tables II and III. Organ weights of the stock animals and pyridoxine treated rats differed only slightly. Thus it would appear that the weight differences between tissues obtained from pyridoxine deficient rats and pyridoxine treated animals may be closely related to a dietary lack of pyridoxine supplement.

Comparison of the alterations in the wet and dry weights of liver, kidneys, spleen, adrenal, and testes of pyridoxine deficient rats with their controls suggests that these changes represent true hypertrophy rather than only increase in tissue fluid content. Conversely these data suggest that atrophy of thymus may also be associated with pyridoxine deficiency.

Agnew(5) and Olsen and Martindale(6) had previously reported cardiac, renal and hepatic hypertrophy in pyridoxine deficient rats. The present work confirms their observations of liver and kidney increases but does not indicate a similar effect in heart tissue. In the present study the heart weights of pyridoxine depleted or desoxypyridoxine treated rats did not differ significantly from those of pyridoxine treated controls, although the data did demonstrate a close relationship of

hypertrophy of spleen, adrenal, and testes to pyridoxine deficiency. The decreased thymus weights in pyridoxine deficient rats, first observed by Butler and Morgan(7), indicate a specific involution of this organ attributable to pyridoxine deficiency. Although the specific reasons for the large weight changes observed in these vital organs are not known, it is possible that these changes may be an initial step toward additional studies of func-

TABLE II. Organ Weights of Pyridoxine and Desoxypyridoxine Fed Male Rats.

Group	Pyridoxine		No supplement		Desoxypyridoxine		Stock	
No. rats	7		6		5		3	
Avg wt	186		133		106		156	
Organ	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	Wt (mg)							
Heart	320 ± 13*	74 ± 3	317 ± 11	74 ± 2	332 ± 20	84 ± 3	347 ± 11	81 ± 2
Liver	2920 ± 80	861 ± 18	3160 ± 50†	950 ± 25	3730 ± 110†	1140 ± 36	3120 ± 110	947 ± 27
Kidney	710 ± 18	179 ± 4	847 ± 34†	213 ± 8	1163 ± 56†	296 ± 12	735 ± 19	181 ± 6
Spleen	222 ± 11	52 ± 10	218 ± 16	52 4	246 15	61 4	225 39	54 2
Thymus	245 ± 12	57 ± 4	153 ± 16†	35 2	84 ± 24†	18 ± .7	240 39	44 2
Adrenal	19 ± 2	7 ± .5	26 ± 1†	9 ± .6	33 ± 7†	11 ± .9	23 5	7 .9
Testes	1330 ± 50	180 ± 10	1710 ± 90†	230 ± 10	2080 ± 160†	290 ± 20	1580 ± 20	210 ± 10

* Mean organ wt/100 g body wt ± stand. dev. of mean.
† Statistically significant difference from pyridoxine-treated group, $P < .05$.
†† $P < .01$.

TABLE III. Organ Weights of Pyridoxine and Desoxypyridoxine Fed Female Rats.

Group	Pyridoxine		No supplement		Desoxypyridoxine		Stock	
No. rats	4		6		5		5	
Avg wt	148		110		91		128	
Organ	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	Wt (mg)							
Heart	343 ± 12*	82 ± 3	367 ± 22	85 ± 5	364 ± 12	85 ± 4	366 ± 8	83 ± 1
Liver	2980 ± 120	878 ± 34	3390 ± 90†	979 ± 24	3640 ± 180†	1096 ± 65	3000 ± 60	920 ± 12
Kidney	812 ± 30	199 ± 4	804 ± 18	209 ± 3	1121 ± 23†	275 ± 8	738 ± 23	188 ± 5
Spleen	199 ± 13	50 ± 3	253 ± 17†	61 ± 3	283 ± 25†	67 ± 7	188 ± 5	57 ± 5
Thymus	257 ± 8	60 ± .2	163 ± 18†	37 ± 3	80 ± 7†	18 ± 5	256 ± 27	57 ± 6
Adrenal	30 ± 1	11 ± .4	33 ± .2	11 ± .4	37 ± 2	12 ± 1	34 ± 2	11 ± .5

* Mean organ wt/100 g body wt ± stand. dev. of mean.
† Statistically significant difference from pyridoxine-treated group, $P < .01$.

tional disturbances in pyridoxine deficient organs.

Summary. Total blood and plasma volumes measured by use of radioactive iodinated serum albumin ($RI^{131}SA$) were not altered from normal by chronic pyridoxine deficiency in male and female rats. The average total blood volume as determined by this radioisotopic technic is somewhat higher than values obtained elsewhere by the Evans blue dye method.

Organ weight changes have been measured and compared in pyridoxine deficient, desoxypyridoxine treated, and pyridoxine treated rats. Wet and dry weights of the liver, kidney, adrenal, spleen and testes of the deficient animals were greater compared to the pyridoxine treated rats. Administration of the antivitamin, desoxypyridoxine, to pyridoxine deficient rats not only increased the apparent hypertrophy of these organs, but also accentuated the marked atrophy of thymus associated with pyridoxine deficiency.

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Monoamine Oxidase Activity in Human Tissues and Intestinal Biopsy Specimens. (27160)

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Knowledge of the tissue distribution of monoamine oxidase (MAO) has been of value in determining the sites of inactivation for amines such as 5-hydroxytryptamine and norepinephrine. Such information is also helpful in attempting to explain the pharmacologic actions of MAO-inhibiting drugs. It is more desirable to consider human amine metabolism in terms of MAO levels in human rather than animal tissues because wide variation between species in the distribution of this enzyme are known to occur(1). Since previous studies(1) of this enzyme in humans have been limited to only 6 tissues, a more complete survey of MAO in human tissue was undertaken and is reported here.

Heretofore, estimation of MAO activity in living humans was dependent on indirect methods of evaluation such as the assay of one of its substrates, *e.g.,* tryptamine, in the urine(2). The recent development of a highly sensitive method for assay of MAO in tissues(3) permits direct measurements of enzyme activity in small tissue specimens. The results of applying this technic to biopsies of intestinal mucosa from normal subjects are presented.

Materials and methods. Specimens of tissue were obtained within 8 hours after death from each of 7 patients. Of these, 5, ages 22 to 60, died following unsuccessful cardiac surgery; one, age 31 died of a convulsive disorder; and one, age 2, died of congenital hydrocephalus. None had received hormones or drugs known to alter MAO activity. Tissue specimens were frozen promptly until time of assay, within 48 hours. Tissues with gross abnormalities in appearance were not studied. Utilization of postmortem material for determining distribution of MAO was considered valid because the activity of this enzyme is quite stable even at room temperature for 24 hours(1). Furthermore, sympathetic ganglia obtained at surgery have MAO activity in the same range as those obtained at autopsy (3).