Enhancement of Growth of Vit. B_6-Deficient Rats by Chlorpromazine Hydrochloride (SKF No. 2601-A) (25329)

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The beneficial effects of chloro-(3-dimethylamino-3′-propyl)-10-phenothiazine hydrochloride (chlorpromazine hydrochloride) on stressed, intact animals, as manifested by increased survival times under conditions of hemorrhagic shock(1,2,3,4,5), X-irradiation (6) and other types of stress(7) have been reported. The anti-stress results with this drug were not always consistent; however, differences in effects often appeared to be related to level of drug used. Chlorpromazine, at a level of 10 mg/kg/day, has been reported to retard growth of rats(8), and at a level of 3.4 mg/rat/day to increase urinary nitrogen excretion and also to reduce weight gain(9). The present study demonstrated that the drug may either enhance or depress growth, depending upon level administered and nutritional status of animal.

Methods. Weanling, Long-Evans strain rats were divided into 8 groups of 12 rats each (6 males and 6 females). Four groups were fed different levels of chlorpromazine in the absence of pyridoxine hydrochloride, and 4 groups served as controls at the same levels of drug in a complete diet (Table I). Composition of basal diet was as follows: casein (vit. free), 18%; sucrose, 65%; dextrose, 5%; salt mixture (USP XIV), 4%; alphacel, 4%; and cottonseed oil, 4%. Each kg of diet contained the following micronutrients (mg): thiamin hydrochloride, 10; riboflavin, 16; calcium pantothenate, 40; nicotinic acid, 100; 2-methyl-1,4-naphthoquinone, 1; biotin, 0.6; folic acid, 4; Vit. B_{12}, 0.05; and choline chloride, 1200. Each kg of diet also contained 10,000 USP units of Vit. A acetate, 1000 USP units of Vit. D and 125 mg of alpha-tocopherol acetate. All vitamins and chlorpromazine were added at the expense of sucrose except choline chloride, which was added at the expense of dextrose. Diets were fed *ad lib.* for 16 weeks. Animals were housed individually in metal cages with raised screen bottoms. Weight gains were recorded weekly and food consumption data were collected during second, sixth and fourteenth weeks. Studies on blood from tails of rats were made during fifth and sixth week of experiment. Standard analytical procedures were used on all blood tests (10). Studies were made on 24-hour urine samples collected from individual rats kept in metabolism cages during 2 periods of experiment: 1) 12-14 weeks and 2) 14-16 weeks after start of experiment. Urinary creatine and creatinine levels were measured by the method of Folin(11), while urinary phosphates were determined by the method of Fiske and Subbarow(12). Animals were sacrificed for autopsy by exsanguination while under light ether anesthesia at end of 16 weeks. Liver, kidneys, spleen, heart, adrenals, thymus, and testes were removed, trimmed, and weighed wet at time of autopsy. Livers were analyzed for Vit. B_6 by microbiological assay(13).

TABLE I. Additives to Basal Diet.

Group No.	Pyridoxine hydrochloride, mg/kg diet	Chlorpromazine HCl (SKF No. 2601-A), mg/kg diet
I	None	100
II	"	200
III	"	400
IV	10	100
V	"	200
VI	"	400
VII	"	None
VIII	None	"

Results. Growth and food consumptions. A significant improvement in growth of B_6-deficient rats occurred when chlorpromazine was included at 100 mg/kg of diet (Fig. 1, 2). This growth-promoting effect of the drug was less evident at the 200 mg/kg level and was absent at the 400 mg/kg level.

Weight gains of both male and female B_6-supplemented rats were less at all 3 levels of dietary chlorpromazine tested than in control rats (Group VII) (Fig. 3, 4). Food consump-

TABLE II. Average Amount of Food (g/Rat/Day) and SKF No. 2601-A (mg/kg Rat/Day) Consumed by Rats Receiving 100 mg Drug/kg Diet during 2nd, 6th and 14th Weeks.*

	Food consumption (g/rat/day)† and mg SKF No. 2601-A consumed/kg rat/day‡							
	B_6 deficient				B_6 supplemented			
	Controls		100 mg SKF No. 2601-A/kg diet		Controls		100 mg SKF No. 2601-A/kg diet	
Week	♂	♀	♂	♀	♂	♀	♂	♀
2	7.2 ± .6	7.2 ± .7	6.3 ± .8 (9.1)	6.0 ± .5 (9.9)	8.2 ± .7	10.2 ± .7	7.6 ± .9 (8.7)	8.6 ± .3 (10.4)
6	7.6 ±1.5	6.4 ± .8	6.7 ± .4 (6.8)	5.9 ± .3 (7.5)	12.4 ±1.0	12.1 ± .5	12.8 ±1.0 (6.6)	12.0 ± .4 (7.6)
14	6.2 ± .4	7.0 ±1.0	7.6 ± .7 (5.1)	6.8 ± .8 (5.9)	15.2 ± .8	11.7 ± .7	15.7 ±1.1 (5.6)	12.8 ±1.3 (7.0)

* Samples from 16 wk feeding period. † Includes stand. error of mean. ‡ Values given in parentheses.

tions and amounts of drug consumed by male and female rats receiving the most active level of drug (100 mg chlorpromazine/kg of diet) during second, sixth and fourteenth weeks of experiment are presented in Table II. No significant differences in food consumption were observed between drug-supplemented and control groups at any periods used for food consumption measurements.

Blood studies. Chlorpromazine at the 3 die-

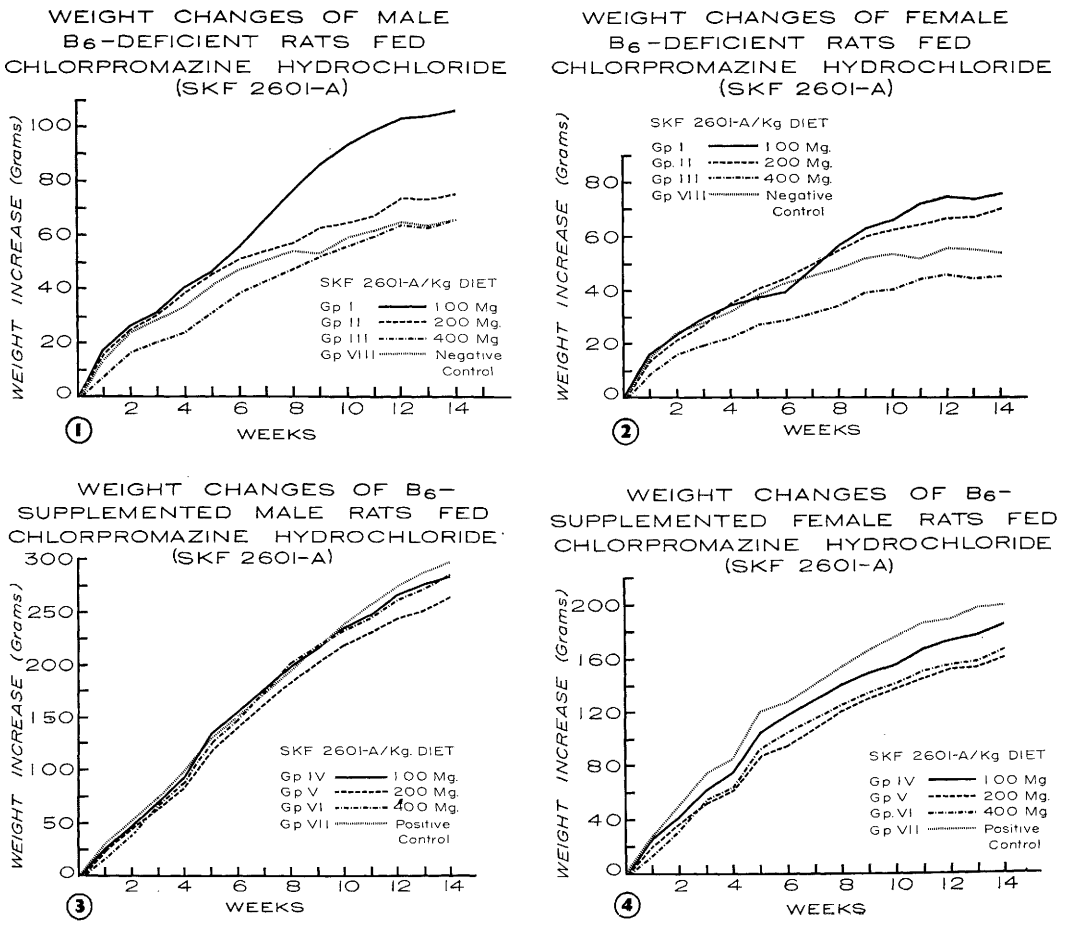

FIG. 1-4.

TABLE III. Summary of Blood Studies on B_6-Deficient and Control ($+B_6$) Rats With and Without Chlorpromazine HCl (SKF No. 2601-A).

Group No.	B_6	Supplements SKF #2601-A, mg/kg diet	Hematocrit,* ml/100 ml	R.B.C.* $\times 10^6$/mm^3	Hemoglobin,* g/100 ml	Total† W.B.C./mm^3	Differential W.B.C.† Lympho-cytes	Neutro-phils	Eosino-phils	Baso-phils	Mono-cytes
							%				
					Males						
I	—	100	41	6.73	13.4	11,250	52	43	1	2	2
II	—	200	42	7.26	14.1	10,130	67	30	.3	2	.3
III	—	400	46	7.28	14.9	10,330	50	46	2	0	2
IV	+	100	39	5.97	13.5	10,780	65	32	1	0	1
V	+	200	38	6.39	13.4	10,930	72	25	1	.3	1
VI	+	400	34	5.77	13.0	10,970	62	34	4	0	.3
VII	+		35	5.63	12.7	12,730	62	34	2	0	2
VIII	—		38	5.60	13.2	13,330	51	47	0	0	2
					Females						
I	—	100	42	8.48	14.2	13,800	67	26	3	0	5
II	—	200	41	8.04	13.8	11,400	54	37	3	0	7
III	—	400	45	8.07	14.3	10,600	70	25	3	0	3
IV	+	100	39	6.90	12.9	13,700	70	25	1	0	4
V	+	200	40	6.51	13.7	11,500	69	25	3	0	3
VI	+	400	41	6.38	13.6	13,200	77	16	3	0	4
VII	+		42	6.68	14.5	10,600	77	14	3	0	6
VIII	—		44	6.99	13.4	7,300	68	27	1	0	3

* Avg of 6 rats/group.

† Avg of 3 rats/group.

TABLE IV. Urinary Creatine/Creatinine Ratio for Vit. B_6-Deficient or B_6-Supplemented Rats Administered Chlorpromazine HCl (SKF No. 2601-A).

Group No.	Vit. B_6	SKF #2601-A, mg/kg diet	Creatine/Creatinine ratio* ♂ 12-14 wk	♂ 14-16 wk	♀ 12-14 wk	♀ 14-16 wk
I	—	100	.38 ± .1	.48 ± .2	.97 ± .3	1.14 ± .5
II	—	200	1.29 ±1.0	.52 ± .2	4.67 ±1.5	5.48 ±2.5
III	—	400	1.49 ±1.2	1.73 ±1.0	8.79 ±8.3	4.04 ±2.5
IV	+	100	.10 ± .03	.22 ± .08	.26 ± .05	.43 ± .2
V	+	200	.12 ± .05	.14 ± .06	.33 ± .09	.26 ± .06
VI	+	400	.14 ± .04	.10 ± .05	.36 ± .03	.25 ± .05
VII	+		.14 ± .04	.06 ± .03	.24 ± .03	.25 ± .03
VIII	—		1.00 ± .3	.89 ± .3	3.93 ±1.7	2.24 ± .8

* Includes stand. error of mean.

tary levels (100, 200, and 400 mg/kg) increased red blood cell counts and hemoglobin values in male and female B_6-deficient rats, but did not affect normal rats (Table III). White blood cell counts were elevated in female B_6-deficient animals but were reduced in male B_6-deficient rats receiving chlorpromazine. This same trend toward alteration of WBC count was noticed in B_6-supplemented male and female rats receiving the drug. Calculations of mean corpuscular volume and mean corpuscular hemoglobin revealed that red cells of B_6-deficient rats receiving chlorpromazine were consistently smaller and contained less hemoglobin than did B_6-deficient controls.

Urine studies. Creatinine excretion was related to food intake in both B_6-deficient and normal (B_6-supplemented) rats. Creatine excretion was elevated above normal levels in control B_6-deficient male and female rats (Group VIII); however, the lower level of chlorpromazine (Group I) induced a significant reduction of creatine urinary excretion. In B_6-supplemented rats, receiving chlorpromazine, creatine levels were not different from those of normal control rats (Group VII). These results are reflected in the creatine/creatinine ratios reported in Table IV. A tendency for all 3 levels of chlorpromazine to lower phosphate excretion in both B_6-deficient and control male rats was also noted; however, this trend was not observed for female rats.

Autopsy data. Hearts and adrenal glands of male and female B_6-deficient rats were more nearly normal in weight (smaller) in animals receiving chlorpromazine (Groups I, II and III) than they were in negative controls (Group VIII, Table V). Gonads of both male and female B_6-deficient rats receiving chlorpromazine (especially on lower level of drug [Group I]), and uteri of female rats were increased in weight over those of B_6-deficient controls.

Liver B_6 levels. Chlorpromazine did not significantly alter storage level of Vit. B_6 in livers of male or female B_6-deficient rats or in normal rats.

Discussion. In B_6-deficient rats receiving low levels of chlorpromazine, an increase in body weight occurs with only a slight increase in food consumption. The more normal relative organ weights of rats on pyridoxine-deficient diets containing low levels of chlorpromazine indicate an increase in available Vit. B_6 for utilization by the animals. In deficient animals the drug also reversed to a large extent the loss of metabolically important urinary creatine and phosphates. These activities are in all respects similar to those of adding vitamin to the diet of animals.

Similar levels of drug in Vit. B_6-supplemented rats had no effect in our tests other than to cause some depression in weight gain. Whether much lower doses of chlorpromazine would increase the weight of Vit. B_6-supplemented rats is not known. Observations have been made in our Microbiology Section (unpublished) that very low levels of chlorpromazine stimulate growth of *L. acidophilus,* whereas higher levels of drug inhibited growth.

Although there was no increase of pyridoxine in livers of animals which showed enhancement of body weight when fed a pyridoxine-deficient diet with chlorpromazine HCl, utili-

TABLE V. Autopsy Data on Rats Sacrificed at End of Experiment.

Group No.	Supplement SKF 2601-A, mg/kg diet	Supplement Vit. B_6	Avg relative organ weights (per 100 g of body wt) Liver, g	Kidneys, g	Spleen, mg	Heart, mg	Adrenals, mg	Thymus, mg	Testes, g
			Males						
I	100	—	3.81	.85	190	378	15.7	32.6	1.43
II	200	—	3.66	.83	160	336	14.7	26.1	1.34
III	400	—	4.32	1.01	200	378	20.5	34.7	1.30
IV	100	+	3.48	.59	150	275	8.6	74.9	.89
V	200	+	3.70	.76	180	299	10.4	72.6	.99
VI	400	+	3.38	.58	150	277	9.8	64.9	.81
VII		+	3.44	.62	160	250	11.1	61.9	.93
VIII		—	4.01	1.18	240	435	25.2	47.5	1.17
			Females						
			Liver, g	Kidneys, g	Spleen, mg	Heart, mg	Adrenals, mg	Thymus, mg	Ovaries, mg	Uterus, mg
I	100	—	4.24	.85	180	438	22.6	25.1	45.0	103.1
II	200	—	4.34	.98	260	361	19.4	32.2	25.2	28.4
III	400	—	4.27	1.10	270	389	24.3	47.7	31.0	49.4
IV	100	+	3.29	.64	180	296	19.4	82.2	39.4	114.6
V	200	+	3.69	.72	270	386	15.8	84.0	42.3	144.3
VI	400	+	3.55	.71	200	282	17.6	105.6	41.7	186.7
VII		+	2.91	.63	170	262	18.6	104.8	30.5	141.4
VIII		—	4.47	1.24	270	551	27.5	44.4	32.7	43.4

zation of any increased level of vitamin by the animal might have obscured the fact that under the influence of the drug, more vitamin was being made available to the animal. The recent work of Barnes *et al.*(14) indicates that coprophagy may be the mechanism by which the animal obtains increased vitamins synthesized in the cecum or large intestine rather than by direct absorption. This factor is under investigation.

Work has been completed in our laboratory in which chlorpromazine HCl either inhibited or potentiated activity of yeast hexokinase depending on concentration of drug. These observations, to be reported, indicate that the drug may be acting to protect the animal at some points in its metabolic function from loss or wastage of nutrients and energy.

The fact that low levels of the drug are active as stimulants, to 'growth' similar to findings with a number of other stimulants(15), and that these effects are observed in a deficient diet but not in a complete diet make this phenomenon closely similar to that with antibiotic as a 'growth' enhancing agent(16).

Summary. At very low dietary levels, chlorpromazine has a favorable effect on growth and metabolism of Vit. B_6-deficient rats. These animals gained more weight, had more nearly normal organ weights, and excreted less creatine and phosphates than did the pyridoxine-deficient controls.

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Plasma Unesterified Fatty Acid Concentration in Fetal and Neonatal Life.* (25330)

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During late stages of pregnancy the human fetus rapidly builds up fat stores in adipose tissue. High respiratory quotients during this period suggest catabolism of carbohydrate or synthesis of fat, or both(1). Decrease in respiratory quotient to approximately 0.7(2) and loss of weight usually seen in first few days of life suggest that these lipid stores are drawn on for energy needs in neonatal life. Utilization of lipid is not surprising since glycogen stores are not overly abundant in the newborn(2) and since protein catabolism can account for only a fraction of caloric requirement during first 48 hours of life(3). Recent studies on metabolic activity and physiological significance of plasma unesterified fatty acids (UFA) in adult mammals indicate that UFA originate chiefly in adipose tissue and appear to be the major form of transported lipid immediately available for oxidation by peripheral tissues(4). The plasma level of UFA is decreased under conditions that promote oxidation of carbohydrate, and increased when carbohydrate is less readily available for energy needs(4). These considerations led us to investigate metabolism of UFA in fetal and neonatal life. Our report describes plasma levels of UFA in sheep fetus and in newborn man and sheep during first few hours of life. Plasma UFA concentrations in mothers were also measured concomitantly.

Methods. Studies in sheep. In sheep the uterus can be opened and umbilical vessels cannulated without apparent disturbance of the placental function(5). Cesarean sections under chloralose anesthesia were performed on 4 crossbred sheep within a few days of term. The fetuses were delivered and immediately immersed without interruption of placental circulation in bath of Ringer's solution maintained at 39°C. Polyethylene catheters were placed in tributaries of the umbilical artery and vein and threaded into main channels. A catheter was also placed in carotid artery of the mother. Serial blood samples were obtained simultaneously from these 3 sources over 1 to 3 hr. period. In one case the fetus was delivered successfully from the incubating bath and serial blood samples were obtained from the umbilical artery for 6 hours. *Studies in man.* Mothers of infants received a variety of analgesic and anesthetic agents during labor. Single blood samples were obtained from the umbilical vein and artery of 9 newborn infants and from an antecubital vein of their mothers. All samples were taken within 2 min. after delivery and before separation of the placenta (Group 1). In an additional 8 cases, samples were taken from either the umbilical vein or artery of the infant and from the antecubital vein of the mother 1 to 7 min. after delivery (Group 2). Single blood samples were also obtained from the internal jugular vein of 9 newborn infants 1 to 6½ hr after birth (Group 3).§ These samples were

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