factor decrease following infusion of isotonic saline. This is to be expected in a parallel type of circuit. In a circuit of the series type, a decrease in resistance would result in an increased Q. The studies here presented are of a preliminary nature and do not permit a judgment as to the contributory rôle of the various anatomical elements lying along the current path to the total result. A rough analysis of the probable relative effects of the extracellular and intracellular phases has been presented for the particular case where isotonic saline is injected intravenously. It is possible that vascular factors also intervene. Studies along these lines are now in progress.

I am indebted to A. N. Mayers for assistance during part of this investigation.

11384

Action and Toxicity of Vitamin B_6 Hydrochloride.

C. G. WEIGAND, CHARLES R. ECKLER AND K. K. CHEN.

From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis.

In view of the increasing interest in vitamin B_6[1-4] as a nutritional accessory, the present investigation was undertaken in order to determine the degree of potency and limits of toxicity. All experiments were carried out with crystalline vitamin B_6 hydrochloride, m.p. 212°C (corrected) with effervescence.

General Properties. Vitamin $B_6 \cdot HCl$ is easily soluble in water. Its aqueous solutions are acid in reaction. A 1% solution has a pH of 2.44. *In vitro* a quantity of 4 mg caused hemolysis of sheep's washed erythrocytes, but if it was previously neutralized, no laking took place. Obviously the hemolytic effect was due to the acidity. When a 1% solution of $B_6 \cdot HCl$ in the amount of 0.5 cc was injected both subcutaneously and intramuscularly into 3 rabbits, practically no irritation occurred, but when 0.1 cc of the same

1 György, P., *Nature*, 1934, **133**, 498; *J. Am. Chem. Soc.*, 1938, **60**, 983.

2 Fouts, P. J., Helmer, O. M., and Lepkovsky, S., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 4; *Am. J. Med. Sc.*, 1940, **199**, 163.

3 Spies, T. C., Bean, W. B., and Ashe, W. F., *J. A. M. A.*, 1939, **112**, 2414.

4 Kark, R., Lozuer, E. L., and Meiklejohn, A. P., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 97.

solution was injected intracutaneously, congestion was evident for more than 4 days in 2 out of 3 animals.

Potency. In addition to physical and chemical characterization, crystalline vitamin B_6 may be assayed biologically. For this purpose, young rats of 21 to 23 days of age were fed a ration composed of sucrose 67%, vitamin-free casein 20%, McCollum's salt mixture No. 185 4%, Crisco 3%, liver filtrate free from B_6 2%, cod liver oil 2%, and agar 2%. In addition, each animal received daily doses of 20 γ each of thiamin chloride and riboflavin. Towards the end of 6 to 8 weeks, these rats developed typical signs of B_6-avitaminosis—acrodynia and inhibition of growth. They were then treated individually with single doses of various size by mouth, and examined daily for 2 weeks. In a group of 60 rats, it was found that a dose of 40 to 60 γ cured acrodynia in an overwhelming majority of the rats within 14 days. Meanwhile, there was a decisive increase in their body weights. These results are comparable to those of Reedman, Sampson, and Unna.[5] For testing the potency of a new lot of $B_6 \cdot HCl$, it has been the practice of this laboratory to determine the median curative dose for acrodynia (CD_{50}), and compare it with the CD_{50}, determined simultaneously, of the purest lot which is being preserved as a standard.

Acute Toxicity. By intravenous injection, the median lethal dose (LD_{50}) of $B_6 \cdot HCl$ in mice was found to be 545.3 ± 42.9 mg per kg, and that in rats 657.5 ± 18.3 mg per kg. The data are shown in Table I. Tonic and then clonic convulsions occurred, and death followed rapidly. Animals either completely recovered or succumbed within 5 minutes after injection. No after effects were noted in the surviving rats. The acute toxicity of $B_6 \cdot HCl$ in rats

TABLE I.
Toxicity of Vitamin $B_6 \cdot HCl$ in Mice and Rats by Intravenous Injection.

Animal	Conc. of solution, %	Dose, mg per kg	No. died / No. used	$LD_{50} \pm$ Standard error, mg per kg
Mice	1	300	0/5	545.3 ± 42.9
		400	1/5	
		500	1/5	
		600	3/5	
		700	3/3	
Rats	5	500	0/3	657.5 ± 18.3
		600	1/5	
		650	4/10	
		700	4/5	

[5] Reedman, E. J., Sampson, W. L., and Unna, K., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 112.

by subcutaneous and oral administration has been estimated by Unna and Antopol.[6]

Repeated Administration. Five mice were injected by the tail vein with daily doses of 100 mg per kg except Saturdays and Sundays for 2 weeks. All of them gained weight during the course of medication. Upon sacrifice, no pathological changes were detected.

In Men. Twelve young male adults consented to take $B_6 \cdot HCl$ by different routes as shown in Table II. The drug was dispensed in capsules for oral use, but made into 2.5% solution for injection purposes. Briefly, it may be stated that no ill effects were observed when $B_6 \cdot HCl$ was given either by mouth or by intravenous injection in the dosage of 100 to 200 mg. Pain uniformly occurred when the drug was injected intramuscularly, perhaps due to its

TABLE II.
Action of Vitamin $B_6 \cdot HCl$ in Men by Various Routes of Administration.

Subject No.	Age	Dose, mg: Oral	Dose, mg: Intramuscular	Dose, mg: Intravenous	Reactions noted
1	22	100			None
			50		"Sore" at site of injection for 1½ hours
				100	None
2	42	100			"
3	35	100			"
4	40	100			"
5	24	100			"
			50		"Aching" for 15 min
				100	None
6	33		50		"Burning" for 15 min
				100	None
7	28		50		"Sore" for 2 hrs
				100	None
8	30		50		"Sore" for 15 min
				100	None
9	28		50		"Aching" for 2 hrs
				100	None
10	21			200	"
11	29			200	"
12	21			200	"

[6] Unna, K., and Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 116.

acidity. In 3 out of 6 cases, it lasted as long as 1½ to 2 hours. No other toxic manifestations were noted.

Other Effects. Casual observations were made on cats' blood pressure and respiration. Doses of 100 mg did not alter the pulse nor the respiratory rate; nor did they change the height of carotid pressure. Appropriate concentrations (1:8000) of $B_6 \cdot HCl$ caused brief inhibition of isolated rabbit's small intestines with prompt recovery, and contraction of the isolated guinea pig's uterus. The response here was not due to a low pH, because controls with the same acidity did not reproduce these results.

Summary. 1. Vitamin $B_6 \cdot HCl$ is acid in reaction which may be responsible for certain irritating effects in body tissues. 2. A method of bioassay has been described based upon the curing of rats' acrodynia. 3. The acute toxicity of $B_6 \cdot HCl$ in mice and rats by intravenous injection has been determined. 4. Mice can tolerate repeated doses of 100 mg per kg, given intravenously, without pathological

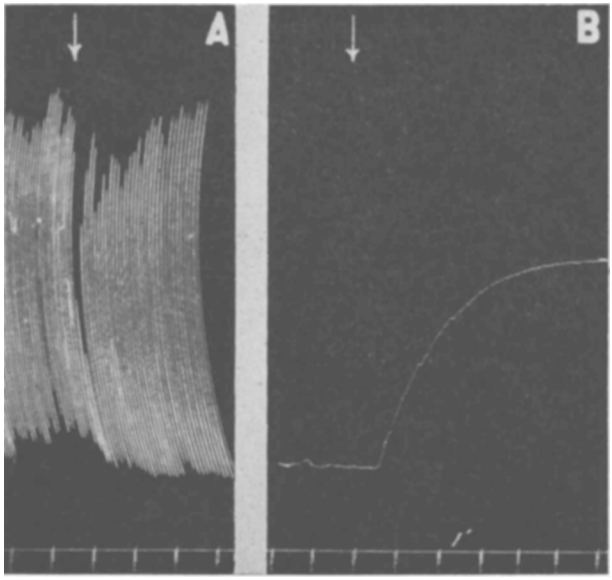

FIG. 1.

Action of Vitamin $B_6 \cdot HCl$ on Isolated Smooth Muscle Organs.

A. The peristaltic movements of a strip of a rabbit's small intestines immersed in Tyrode's solution at 38°C. At arrow, 0.5 cc of 2.5% solution of $B_6 \cdot HCl$ was applied (making the concentration 1:8000). There was a brief inhibition followed by prompt recovery.

B. The myogram of a horn of a virgin guinea pig's uterus immersed in Tyrode's solution at 38°C. At arrow, the same amount of $B_6 \cdot HCl$ as above was added. It resulted in a contraction.

changes. 5. No ill effects occur in men when $B_6 \cdot HCl$ is administered orally or intravenously in the dosage of 100 to 200 mg. Pain occurs when the drug is injected intramuscularly. 6. In the concentration of 1:8000, $B_6 \cdot HCl$ inhibits isolated rabbit's small intestines, but contracts the isolated guinea pig's uterus.

11385

Effects of Subcutaneous Injection of Estrogen upon Skeleton in Immature Mice.

CHARLES J. SUTRO. (Introduced by H. L. Jaffe.)

From the Laboratory Division, Hospital for Joint Diseases, New York City.

It is known that the subcutaneous administration of estrogen produces osteosclerosis in immature mice.[1] Hitherto, however, detailed roentgenographic and histologic studies explaining the mode of development of the osteosclerosis have been lacking. This paper reports such an investigation.

The study was conducted upon 66 immature mice, some of the C_3H strain and some from stock colony of the Rockland Farms. The experimental subjects received subcutaneous injections of estradiol benzoate in sesame oil* each week for a certain number of weeks. The control animals received injections of sesame oil for corresponding periods of time.

At the expiration of the total period allotted for the experiment, the animals were roentgenographed and autopsied. The skeletal tissues [femur, tibia, humerus, vertebral column, calvarium, ribs, pelvis, foot and jaw (including incisors)] were fixed in Helly's fluid, decalcified in 5% nitric acid, embedded in paraffin and stained with hematoxylin and eosin. The soft tissues were likewise prepared for histologic examination.

Table I summarizes the organization of the experiments.

The control mice received weekly injections of sesame oil.

Roentgenographic and Gross Pathologic Findings: Roentgenographic examination reveals that in immature mice osteosclerosis, caused by the formation of new bone in the medullary cavity, especially in the lower end of the femur and upper end of the tibia,

1 Gardner, W. U., and Pfeiffer, C. A., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 678; *ibid.*, 1938, **38**, 599.

* Progynon-B was generously supplied by Schering Corporation.