been administered suggests that normal tissue level of vitamin had been restored for the time being, even in the most deficient group.

At termination of normal pregnancy, B_6 values are significantly depressed in maternal leukocytes and plasma. In blood of women in last trimester of pregnancy, however, normal average amounts of the vitamin are found although these individuals have a distinct abnormality in tryptophane metabolism which can be readily corrected by administration of pyridoxine(4). This relative pyridoxine deficiency can be demonstrated by the Vit. B_6 load test as described here. From a practical point of view, determination of plasma values 24 hours following administration of Vit. B_6 may be useful in evaluating the B_6 saturation of tissues in clinical subjects.

Summary. A method for determination of pyridoxal phosphate in human plasma is described. The amount of coenzymatically active form of Vit. B_6 was determined simultaneously in blood of normal subjects, delivering women, and in cord blood of their babies. In general, amounts of pyridoxal phosphate in plasma and leukocytes showed parallel changes. They were low in maternal blood, and high in cord blood. Following oral administration of 100 mg pyridoxine hydrochloride, increases were observed in both plasma and leukocytic B_6 values within a few hours. Considerably higher peaks were seen in normal controls as compared to women in last trimester of pregnancy and at time of delivery. The most significant difference between pregnant and nonpregnant subjects was noted in plasma values 24 hours following administration of vitamin. The possible practical application of these findings for evaluation of a latent B_6 deficiency in clinical subjects is suggested.

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Effects of Epinephrine on Toxicities of Several Local Anesthetic Agents. (25516)

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Apparently the opinion is held generally that inclusion of a vasoconstrictor, such as epinephrine, in solution with local anesthetic, such as procaine, affords considerable protection against danger of systemic intoxication by the anesthetic when such solution is injected into soft tissues. This protective action is said to result from slower, more gradual absorption of the anesthetic because of localized vasoconstriction, so that mechanisms for detoxication and elimination of the anesthetic are more nearly able to keep pace with absorption. This, then, prevents development of excessively high blood stream concentration of the toxic agent. Goodman and Gilman(1) state that this is true, although no reference is made to experimental verification. Campbell and Adriani(2) state that systemic toxicity of procaine after infiltration is reduced 30% or more by addition of 1:100,000 epinephrine to the solution, although, no source is given for the information. Sollmann(3) holds a similar view supported by reference to observations by Hatcher and Eggleston(4), Eggleston and Hatcher(5), Zaus and Moody(6), and Beutner, Prusmack, and Miller(7). The conclusions of Hatcher and Eggleston and by Eggleston and Hatcher were based on too few observations, without adequate controls. Since only 2 animals were used, both surviv-

ing, their experimental results hardly justify such conclusion. Zaus and Moody state that their data "rather definitely prove that epinephrine, as used in local anesthetic solutions, is a favorable agent" for it affords protection against procaine. However, their experiments were with direct intravenous infusion of these agents, not with injection into soft tissues. Here, again, the conclusion applied to injection into soft tissues as well, is not justified by published results. Beutner *et al.* found that epinephrine in concentration of 1:50,000 minimized or prevented convulsions in rabbits following intramuscular injection of procaine in doses which, otherwise, caused severe convulsions. But determinations of fatal doses with and without epinephrine were not reported. In contrast Hatcher and Eggleston report that Heineke and Laewen in 1905 observed no appreciable difference in toxicity of procaine on subcutaneous injection into rabbits, with or without epinephrine. Tainter and Throndson(8) found that neither epinephrine (1:10,000 and 1:50,000) nor Neosynephrine (1:2500) caused a decrease in toxicity of procaine on intraperitoneal injection into rats, but a slight though statistically unimportant increase. Cobefrin (1:10,000) caused definite increase in toxicity. Gurd and Sachs (9) very carefully studied the effect of epinephrine on toxicities of cocaine and procaine after subcutaneous injection in mice. They found that relatively low concentrations of epinephrine (1:100,000 or less) had no significant influence on toxicity of either agent, but higher concentrations (1:85,000 for cocaine; 1:50,000 for procaine) significantly increased toxicities of both agents. Investigations by various authors in support of protection contained so little of a positive nature, while investigations which bear directly on the subject received little attention. There is good evidence that appreciable protection is obtained following *intravenous* injection, but this is a different matter. Also, there appears to be no reason to question the widespread belief that inclusion of a vasoconstrictor makes possible satisfactory anesthesia of greater duration with less anesthetic agent than would be possible without the vasoconstrictor. But, this too is a different matter. In view of the confusion which exists with respect to protection following infiltration of local anesthetics into soft tissues, further investigations were considered desirable.

Material and methods. It has long been recognized that local anesthetics undergo changes within the animal, these changes being referred to as "detoxication." Brodie, Lief, and Poet(10) demonstrated that in the human some extra-hepatic mechanism, probably associated with blood, is responsible for the greater part of detoxication of procaine. Kalow(11) identified this mechanism, so-called "procaine esterase," as plasma or serum cholinesterase (pseudocholinesterase). It has been recognized also that rate of detoxication of even a single agent varies greatly from one species to another. Thus, Aven, Light and Foldes(12) found that mouse serum is much more active in hydrolyzing procaine than that of most other commonly available laboratory animals, and that it approaches, but does not equal, human serum. Unfortunately, procaine appears to be the only agent investigated with respect to rates of hydrolysis in sera other than the human. However, on the basis of present evidence, it appears that the mouse is the preferred species for many investigations dealing with toxicity of local anesthetics. The ready availability of large numbers of uniform stock is an additional advantage. Accordingly, male white mice of 20-30 g body weight were used. Five representative anesthetic agents were selected. Three of these, procaine, tetracaine, and piperoceaine are esters, and are subject to hydrolysis by human plasma esterases though at different rates (Kalow). The other agents, lidocaine and dibucaine, are amides, and are not hydrolyzed at measurable rates by human plasma esterases. If vasoconstriction at site of injection slows absorption of the anesthetic agent, then one would anticipate greater protection by a vasoconstrictor against agents which are hydrolyzed rapidly, and less protection against those which are hydrolyzed slowly or not at all. With exception of lidocaine, all anesthetic solutions were prepared from hydrochloride crystals. A crystalline preparation of lidocaine hydrochloride was not readily available, so commercial 2% solution was used. Con-

TABLE I. Effects of Epinephrine on Toxicities of Several Local Anesthetic Agents.

Agent	Epinephrine conc. × 1000	LD_{25}	$LD_{50} \pm$ S.E.	LD_{75}	Slope $(\log mg)^{-1}$
Procaine	0	616*	695 ± 17	785	12.8
	1:500	316	602 ±122	1149	2.40
	1:100	314	539 ±104	924	2.88
	1:50	399	453 ± 26	765	4.77
Piperocaine	0	371	615 ± 84	1021	3.07
	1:500	481	706 ± 54	1035	4.06
	1:100	320	441 ± 39	608	4.83
	1:50	365	447 ± 27	546	7.80
Tetracaine	0	34.6	40.6 ± 2.1	47.7	9.6
	1:500	40.8	51.0 ± 4.0	58.7	8.5
	1:100	56.8	64.8 ± 2.8	74.1	11.6
	1:50	69.0	76.6 ± 4.0	89.8	9.8
Lidocaine	0	113	163 ± 16	235	4.24
	1:500	163	185 ± 6.3	210	12.2
	1:100	178	195 ± 6.2	214	16.8
	1:50	153	198 ± 14	256	6.04
Dibucaine	0	24.6	25.4 ± .28	26.2	48.9
	1:500	23.4	24.7 ± .44	26.1	27.9
	1:100	25.5	26.6 ± .36	27.8	36.2
	1:50	24.8	28.1 ± .96	31.7	12.5

* All doses expressed as mg/kg of hydrochloride.

centrations were such that a constant volume of 0.01 ml/g body weight was injected. All injections were made beneath the skin of lumbar region of the back. Epinephrine was added to solutions to produce concentrations as indicated in Table I. Dose-response curves were determined by at least 3 points, usually 4 or 5. As a rule, 15 to 25 animals were used in each group; but in a few only 10. LD_{50}'s and standard errors were calculated by minimum normit chi-square(13).

Results. The results shown in Table I are at variance with the view that inclusion of a vasoconstrictor reduces systemic toxicity. With only one agent, tetracaine, of the 5 studied, was this found to be true. Early in the investigation it became apparent, especially with procaine, that inclusion of epinephrine may lead to a marked change in slope of the dose-response curve. Not only the LD_{50}'s for various agents and their mixtures with epinephrine, but also the slopes and doses for responses at both 25% and 75% mortality levels are therefore included in the Table. With procaine there appears to be no significant reduction in toxicity at any response level or with any concentration of epinephrine with the possible exception of the LD_{75} obtained with 1:500,000 epinephrine. On the contrary, there is a strong suggestion of increased toxicity with increase in concentration of epinephrine, statistically significant at the LD_{50} level with 1:50,000 epinephrine. With piperocaine, also, there is no evidence of protection but rather the suggestion of an increase in toxicity under most conditions. With both lidocaine and dibucaine there is evidence of a slight reduction in toxicity under certain conditions, but this is of doubtful importance.

Summary. With only one agent (tetracaine) of the 5 studied, was addition of epinephrine accompanied by a highly significant reduction in systemic toxicity. With the 4 remaining agents toxicity was not altered appreciably under most conditions, although procaine containing 1:50,000 epinephrine seems more toxic than procaine alone.

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Adrenal Sensitivity to ACTH as a Function of Time after Hypothalamic Lesion and after Hypophysectomy.* (25517)

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The usually available criteria for endogenous discharge of ACTH are indirect and are based on corticotropic stimulation of the adrenal cortex. When studying hypophysiotropic activity of preparations of corticotropin releasing factor (CRF) in animals with "effective" hypothalamic lesions, one should therefore know the sensitivity of the adrenals to ACTH. Considered "blocked" in their ACTH discharge when they show no evidence of adrenocortical stimulation upon exposure to stress, these animals might simply be less sensitive to ACTH than normal. Adrenocortical sensitivity to ACTH has been studied at various time intervals ranging from 24 hr to 60 days after stereotaxic placement of a lesion in the median eminence and demonstration of its effectiveness to inhibit stress-induced ACTH release. For comparison, adrenal sensitivity to ACTH was also studied in hypophysectomized animals.

Materials and methods. A. *After hypothalamic lesion.* Two hundred sixty animals (rats, male, body weight 230-250 g, Holtzman Farms, Houston) were used. A lesion of the bulbar part of the median eminence was made (Fig. 1), using a high frequency generator, the electrodes being placed with a modified Krieg-Johnson stereotaxic instrument. Brains of *all* animals were preserved at autopsy and a description of lesions from frozen sections is available for each animal. Eighteen to 19 hours after placement of the lesions, all animals were tested for block of stress-induced ACTH discharge as previously described(1). Only animals with "effective" lesions (60 out of 260) were saved for experiments reported here. Twenty-four hours after lesioning, 3 groups of animals were administered 0.5, 1.0, 1.5 mU ACTH USP Reference Standard (I.V., in 0.2 ml of 0.01 N HCl in saline). Adrenocortical response to 1 mU ACTH USP Reference Standard administered as above was investigated at 2, 4, 7, 9, 20, 30 and 60 days following placement of hypothalamic lesion. Persistence of inhibition of stress-induced ACTH-release(3) was reevaluated 6 hours before injection of ACTH in all animals used after fourth post-lesion day. In all cases adrenocortical stimulation produced by ACTH was assessed by measurement of peripheral plasma free corticosteroids 15 minutes after injection of the solution of adrenocorticotropin(2,3). The animals were then sacrificed and weight of adrenal glands recorded.

B. *After hypophysectomy.* One hundred fifty-six animals (rats, male, body weight 175-200 g, Holtzman Farms) were hypophysectomized. Three groups were administered 0.25, 0.5, 1.0 mU ACTH USP Reference Standard (as above), 24 hours later. Animals of other groups were similarly injected with 1 mU

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