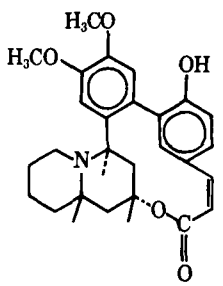
Effects of Certain Nonsteroid Anti-Inflammatory Drugs, Tolbutamide, and Tetrahydroalstonine on Blood Glucose and Carrageenin-Induced Pedal Edema in Rats

ALBIN B. KOCIALSKI*, FRED J. MAROZZI, Jr.†, and MARVIN H. MALONE▲

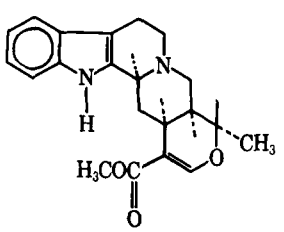
Abstract □ Relative oral potencies for preventing carrageenin-induced pedal edema in rats were, in the order of increasing effectiveness: tetrahydroalstonine, phenylbutazone, tolbutamide, cryogenine, chlorpromazine hydrochloride, tetrabenazine, and indomethacin. Pedal injection of carrageenin elevated blood glucose, and phenylbutazone appeared to be the only drug tested clearly capable of blocking this hyperglycemic effect at an anti-inflammatory dosage. In the absence of carrageenin injection, all of the test agents elevated blood glucose 7–13 hr. after their administration. In rats with alloxan-induced hyperglycemia, orally administered tetrahydroalstonine was effective in reducing elevated blood glucose, as was tolbutamide and subcutaneous protamine zinc insulin. Cryogenine raised blood glucose levels even further, while tetrabenazine did not alter the hyperglycemia significantly.

Keyphrases □ Anti-inflammatory drugs, nonsteroid—effect on blood glucose and carrageenin-induced pedal edema, compared to tolbutamide and tetrahydroalstonine, rats □ Tolbutamide—effect on blood glucose and carrageenin-induced pedal edema, compared to nonsteroid anti-inflammatory drugs and tetrahydroalstonine, rats □ Tetrahydroalstonine—effect on blood glucose and carrageenin-induced pedal edema, compared to nonsteroid anti-inflammatory drugs and tolbutamide, rats □ Blood glucose levels—effect of nonsteroid anti-inflammatory drugs, tolbutamide, and tetrahydroalstonine, rats □ Edema, carrageenin-induced pedal—effect of nonsteroid anti-inflammatory drugs, tolbutamide, and tetrahydroalstonine

While most clinically effective anti-inflammatory drugs are organic acids, Kaplan *et al.* (1) reported that cryogenine, an alkaloid isolated from *Heimia salicifolia* Link and Otto, was effective experimentally for both acute (carrageenin-induced pedal edema) and chronic (adjuvant-induced polyarthritis) inflammatory states. Cryogenine (vertine) has been shown to have some selective CNS effects (2), and tetrabenazine bears some structural resemblance to certain possible metabolic products of cryogenine. Both chlorpromazine (CNS and peripheral effects) and tetrabenazine (negligible peripheral effects) have been shown to be effective against carrageenin-induced inflammation (3); moreover, recent screening in this laboratory indicated that tetrahydroalstonine, an alkaloid isolated from *Vinca rosea* Lynn (*Cantharanthus roseus* G. Don), was also effective (4).



cryogenine



tetrahydroalstonine

Since tetrahydroalstonine has been reported (5) to have some antihyperglycemic activity, and since most of the clinically effective anti-inflammatory agents can modify blood glucose levels, it was of interest to investigate the effect of cryogenine and selected other agents upon blood glucose.

EXPERIMENTAL

Carrageenin-Induced Pedal Edema—At −24 hr., adult healthy male rats of the Wistar strain[1], formerly maintained with free access to laboratory chow[2] and tap water, were placed on fast in individual cages with wide-mesh screen floors to prevent coprophagy. Tap water was allowed *ad libitum*. At −1 hr., these animals were dosed (5 ml./kg.) with either the test drugs[3] or the 0.25% agar dosage vehicle. Oral administration was selected to avoid the parenteral "counterirritant" effect which can modify carrageenin-induced pedal edema. Right hind-paw volume was measured plethysmographically (3, 6) just after injecting 0.1 ml. of 1% carrageenin[4] in distilled water into the right hind-paw plantar aponeurosis at 0 hr. Paw volume was determined again at +3 and +5 hr., and the percent change from the respective 0-hr. value was used as the experimental parameter.

Blood Glucose Determination—Not less than 0.6 and not more than 0.9 ml. of blood was collected by direct cardiac puncture from the animals at +6 and +12 hr. To permit collection, each rat was anesthetized with 30 mg./kg. of sodium pentobarbital intraperitoneally. Glucose content was determined colorimetrically in the manner of Nelson (7). Tabular means represent not less than seven nor more than 14 determinations at each log time interval.

Alloxan-Induced Diabetes—Young, healthy, male Sprague-Dawley rats[5], which had been maintained with free access to laboratory chow[2] and tap water, were placed in individual containers and fasted 48 hr. with free access allowed to 5% dextrose solution. At the end of this period, 100 mg./kg. of alloxan monohydrate was injected intraperitoneally, using 0.25% agar as the vehicle (5 ml./kg.), and the fast was broken. Animals not manifesting positive glycosuria[6] within 3 days then received a second injection of alloxan monohydrate (160 mg./kg.). If negative glycosuria was still present after another 3-day period, a third injection of alloxan monohydrate (160 mg./kg.) was made. Rats manifesting positive glycosuria for 3 consecutive days were placed on fast at −24 hr. and were injected subcutaneously at 0 time with 10 units/kg. of protamine

[1] Obtained from E. G. Steinhilber Co., Oshkosh, Wis.
[2] Purina.
[3] Sources of the test drugs were: phenylbutazone, Geigy Pharmaceuticals, Ardsley, N. Y.; indomethacin, Merck Sharp & Dohme Research Labs, West Point, Pa.; chlorpromazine hydrochloride, Smith Kline & French Labs, Philadelphia, Pa.; tetrabenazine, Hoffmann–La Roche, Inc., Nutley, N. J.; and tolbutamide, The Upjohn Co., Kalamazoo, Mich. Cryogenine was obtained from Dr. A. E. Schwarting, Division of Pharmacognosy, University of Connecticut, Storrs, Conn., and Dr. M. M. El-Olemy, Department of Pharmacognosy, University of Nebraska, Lincoln, Nebr. The cryogenine (mol. wt. 435.53) used in this study was isolated from Mexican *Heimia salicifolia* and is not the same compound as the tradename product Cryogénine (phenylsemicarbazide, mol. wt. 151.2), a specialty of Lumière of Lyons, France, and distributed by Laboratoires Sarbach of Châtillon, France. Tetrahydroalstonine was provided by Dr. N. R. Farnsworth, Department of Pharmacognosy and Pharmacology, University of Illinois, Chicago, Ill.
[4] Sea Kem Type I, lot 312503, Seaplant Chemical Corp., New Bedford, Mass.
[5] Cox Laboratory Supply, Indianapolis, Ind.
[6] Positive glycosuria was defined as ≥2% glucose in the urine as detected by Clinitest tablets (Ames Co., Inc., Elkhart, Ind.).

Table I—Relative Anti-Inflammatory Evaluation of Selected Drugs against Carrageenin-Induced Acute Pedal Edema in Rats

Drug Administered	Oral Dosage, mg./kg.	Mean Percent Change in Paw Volume, ml. — Without Carrageenin: +3 hr.	p	+5 hr.	p	With Carrageenin: +3 hr.	p	+5 hr.	p
Agar vehicle	—	−11.2(4.0)[a]	—	−10.9(3.7)	—	+91.0(3.7)	—	+89.6(2.4)	—
Phenylbutazone	125.0	+2.3(2.4)	<0.01[b]	−3.5(2.1)	>0.05[b]	+41.7(5.6)	<0.001[b]	+49.2(4.3)	<0.001[b]
Indomethacin	12.5	−1.0(1.4)	<0.05	−2.6(1.2)	<0.05	+54.1(4.3)	<0.001	+56.3(3.6)	<0.001
Chlorpromazine hydrochloride	100.0	−1.2(1.2)	>0.05	−0.6(1.6)	<0.05	+44.0(2.8)	<0.001	+54.2(2.7)	<0.001
Tetrabenazine	37.5	+1.9(1.2)	<0.005	+1.8(1.4)	<0.005	+53.7(1.7)	<0.001	+60.7(3.8)	<0.001
Cryogenine	100.0	−1.6(1.7)	<0.05	−4.3(1.5)	>0.10	+51.8(4.6)	<0.001	+55.1(4.6)	<0.001
Tetrahydroalstonine	125.0	+4.4(0.9)	<0.001	−1.7(1.5)	<0.05	+58.2(3.6)	<0.001	+71.4(3.0)	<0.001
Tolbutamide	100.0	−2.2(1.4)	>0.05	−0.3(1.5)	<0.05	+75.6(4.2)	<0.025	+80.1(3.1)	<0.025

[a] Figures in parentheses indicate the calculated standard error of the listed mean. [b] Indicates the level of significance relative to corresponding values in animals receiving only the agar vehicle orally.

Table II—Relative Blood Glucose Levels after Oral Drug Treatment in Rats with and without Pedal Injection of Carrageenin

Drug Administered	Oral Dosage, mg./kg.	Mean Blood Glucose, mg.% — Without Carrageenin: +6 hr.	p	+12 hr.	p	With Carrageenin: +6 hr.	p	+12 hr.	p
Agar vehicle	—	77.5(2.1)[a]	—	77.4(3.8)	—	93.0(2.2)	—	103.2(3.2)	—
Phenylbutazone	125.0	86.0(3.6)	>0.05[b]	101.3(3.0)	<0.001[b]	75.1(1.6)	<0.001[b]	90.6(1.8)	<0.01[b]
Indomethacin	12.5	97.8(1.9)	<0.001	97.5(2.7)	<0.001	100.4(1.8)	<0.025	110.3(3.7)	>0.10
Chlorpromazine hydrochloride	100.0	102.5(4.9)	<0.001	114.0(3.9)	<0.001	125.0(8.7)	<0.001	120.5(18.4)	>0.25
Tetrabenazine	37.5	94.9(2.5)	<0.001	100.2(3.8)	<0.001	93.2(2.6)	>0.50	88.8(1.7)	<0.001
Cryogenine	100.0	100.4(3.0)	<0.001	107.8(2.6)	<0.001	104.6(2.4)	<0.005	108.3(4.3)	>0.25
Tetrahydroalstonine	125.0	83.4(2.3)	>0.05	94.5(2.5)	<0.005	92.0(1.7)	>0.50	101.0(3.3)	>0.50
Tolbutamide	100.0	100.0(3.3)	<0.001	104.7(3.0)	<0.001	92.7(3.9)	>0.50	101.9(4.7)	>0.50

[a] Figures in parentheses indicate the calculated standard error of the listed mean. [b] Indicates the level of significance relative to corresponding values in animals receiving only the agar vehicle orally.

zinc insulin (40 units/ml.) or were dosed orally (5 ml./kg.) with the test drugs or the 0.25% agar dosing vehicle.

Each rat was anesthetized with 30 mg./kg. of sodium pentobarbital intraperitoneally, and blood samples were collected at intervals of +0.5, 3.5, 6, 12, 24, and 48 hr. by orbital sinus puncture (8) using heparinized microhematocrit tubes[7]. Three to four drops of blood were allowed to flow from the open end of the tube before removing it from the sinus. Alternate eyes were used at each successive time interval. A 0.2-ml. amount of whole blood was added to 1.8 ml. of 2% sodium fluoride in 0.9% saline. This amount allowed for replicate determinations of glucose using a micromethod based upon the procedure of Hoffman (9) designed for the AutoAnalyzer (potassium ferricyanide–ferrocyanide oxidation–reduction reaction)[8]. Each tabular mean represents the mean glucose of not less than 10 and not more than 14 experimental animals. All data in this paper were analyzed using the analysis of variance methods of Bliss and Calhoun (10).

RESULTS AND DISCUSSION

Plethysmographic Studies—In carrageenin-treated animals, all mean paw volumes at +3 and +5 hr. showed significant increases ($p < 0.001$) over the respective 0-hr. mean volumes, thus indicating the presence of pedal inflammation and edema in all groups (Table I). Preliminary screening had been conducted to ascertain the active anti-inflammatory dosage ranges for the test compounds, so the statistically significant anti-inflammatory responses for each test agent were expected. All dosages produced roughly equivalent anti-inflammatory responses, although the effect with 100 mg./kg. of tolbutamide was somewhat less than expected. Even though these are one-level comparisons, the test drugs can be ranked in order of decreasing anti-inflammatory effectiveness (mg./kg.) as follows (+3-hr. comparison): indomethacin, tetrabenazine, chlorpromazine hydrochloride, cryogenine, tolbutamide, phenylbutazone, and tetrahydroalstonine.

[7] Catalog No. A-2930, Clay-Adams, Inc., New York, N. Y.
[8] Technicon Method File No. N-9a, Technicon Instrument Corp., Chauncey, N. Y.

In animals not exposed to injection with carrageenin, all paw volumes at +3 and +5 hr. showed no significant change from their respective 0-hr. readings, except for the agar controls (Table I). This slight but significant decrease ($p < 0.05$) in paw volume due to agar was totally unexpected. This decrease might be due to dehydration from a laxative effect of the orally administered agar vehicle (12.5 mg./kg. of agar); however, no evidence of diarrhea was seen and all drug-treated animals (also receiving the agar vehicle) showed no significant change in paw volume when compared with their respective 0-hr. readings. Apparently, treatment with any drug at an effective anti-inflammatory dosage neutralizes this unusual "agar effect."

Blood Glucose Determinations—Preliminary screening indicated that intracardiac blood sampling as described here could not be conducted prior to +5 hr. since such collections affected the development of the carrageenin-induced edema. It had been hoped that blood sampling and plethysmographic measurements of paw volume could be conducted concurrently.

While not shown in Table II, mean blood glucose at 0 time in a separate control group of 12 rats fasted for 18 hr. was determined to be 73.8 mg.% ($SE = 2.0$); this value was not significantly different ($p > 0.20$) from the experimental value of 77.5 mg.% observed at +6 hr. (Table II) for the group receiving only agar and no carrageenin treatment. Blood glucose in animals receiving agar only and with carrageenin treatment was significantly higher at both time intervals (Table II) than the blood glucose in animals receiving agar only and no carrageenin (+6 hr., $p < 0.001$; +12 hr., $p < 0.001$). This would appear to indicate that carrageenin-induced pedal edema is a stressful treatment, having as one parameter the elevation of blood glucose. Moreover, the animal handling associated with plethysmographic measurements of foot volume and with blood sampling can eventually contribute to an elevation of blood glucose since +24- and +48-hr. estimations of blood glucose for animals receiving only agar and no carrageenin treatment (89.1 ± 2.2 and 95.7 ± 2.9 mg.%, respectively) were statistically higher ($p < 0.05$) than the tabular values noted at +6 and +12 hr.

In the animals not subjected to carrageenin, all drug treatments significantly elevated blood glucose levels (relative to the agar controls) at both time intervals, with the exception of phenylbutazone and tetrahydroalstonine at +6 hr. At the doses tested, a drop

Table III—Relative Blood Glucose Levels after Drug Treatment in Alloxan-Diabetic Rats

Drug Administered	Oral Dosage, mg./kg.	Mean Blood Glucose, mg.% +0.5 hr.	+3.5 hr.	+6 hr.	+12 hr.	+24 hr.	+48 hr.
Agar vehicle	—	108.9 ± 7.6[a]	139.4 ± 14.3	134.3 ± 16.5	154.6 ± 20.2	103.8 ± 4.2	139.9 ± 7.2
Protamine zinc insulin	—[b]	125.9 ± 10.3 (>0.10)[c]	56.8 ± 9.0 (<0.001)	54.7 ± 7.8 (<0.001)	41.3 ± 9.0 (<0.001)	37.1 ± 5.7 (<0.001)	34.5 ± 3.4 (<0.001)
Tolbutamide	100.0	89.2 ± 5.7 (>0.05)	100.7 ± 5.8 (<0.025)	111.3 ± 3.2 (>0.10)	119.8 ± 8.1 (>0.10)	115.8 ± 3.4 (<0.05)	101.8 ± 7.4 (<0.005)
Tetrahydroalstonine	125.0	77.7 ± 4.9 (<0.005)	145.1 ± 10.0 (>0.50)	157.6 ± 22.3 (>0.25)	145.9 ± 22.8 (>0.50)	75.1 ± 8.6 (<0.01)	78.2 ± 11.8 (<0.001)
Cryogenine	100.0	148.4 ± 21.8 (>0.10)	236.0 ± 20.0 (<0.001)	224.8 ± 17.6 (<0.005)	189.4 ± 28.8 (>0.25)	115.5 ± 18.3 (>0.50)	132.0 ± 13.0 (>0.50)
Tetrabenazine	37.5	99.8 ± 9.6 (>0.25)	121.6 ± 9.8 (>0.25)	113.1 ± 6.1 (>0.25)	131.8 ± 7.1 (>0.25)	103.3 ± 4.6 (>0.50)	123.2 ± 7.5 (>0.10)

[a] Tabular figures indicate the mean ± 1 *SE* of the mean. [b] Dosage is 10.0 units/kg. administered subcutaneously. [c] Figures in parentheses indicate the observed significance, *p*, relative to the respective agar control values.

in blood glucose was not observed with either tolbutamide or tetrahydroalstonine and tolbutamide was clearly hyperglycemic in action.

In the rats subjected to carrageenin, only phenylbutazone appeared to be effective in reducing the hyperglycemic response seen in the controls; the blood glucose level was actually lower than that seen in the noncarrageenin animals treated with the same dose of phenylbutazone. Chlorpromazine hydrochloride treatment clearly accentuated the hyperglycemia at +6 and +12 hr., while indomethacin and cryogenine appeared to have a qualitatively similar but less dramatic effect at +6 hr. The unusually large standard error associated with the +12-hr. blood glucose estimation for chlorpromazine hydrochloride can be explained by the fact that the noted hyperglycemic effect was wearing off in six of the 12 test animals at this time period. Tetrahydroalstonine and tolbutamide did not significantly alter blood glucose at either time interval, and tetrabenazine was without effect at +6 hr. The apparent lowering of blood glucose by tetrabenazine at +12 hr. is discounted as a true pharmacologic effect since it was recorded 13 hr. after actual drug administration (+12 hr. after carrageenin administration).

Effects in Alloxan-Diabetic Rats—The hyperglycemic response of tolbutamide in fasted normal rats and in animals stressed with carrageenin-induced pedal edema was interesting since tolbutamide is a clinically effective antihyperglycemic agent in patients with an inadequate number of functional β-cells in the pancreas. Therefore, a study was undertaken in alloxan-treated rats to see whether there would be some differences of effect between tolbutamide, tetrahydroalstonine, and the previously undocumented effects of cryogenine and tetrabenazine. Effective anti-inflammatory dosages were used for each, and protamine zinc insulin was used as a reference agent.

As shown in Table III, significant hyperglycemia (108.9 mg.%) was noted in the agar control rats at +30 min. and this hyperglycemia persisted throughout the 48-hr. test period. A separate group of 12 alloxanized rats was also tested at −24 hr., and the mean blood glucose was documented at 328.7 ± 10.6 mg.%. After 23 hr. of fasting, the mean blood glucose for these same animals was recorded as 117.7 ± 5.1 mg.% (this latter value would be equivalent to a −1-hr. reading for the animals in Table III).

The characteristic hypoglycemic action of protamine zinc insulin was observed, with the onset of effect beginning after +30 min. and persisting through the +48-hr. test period. Tetrabenazine was without significant effect on blood glucose while cryogenine dramatically accentuated the existing hyperglycemia from +0.5 through +6 hr. This accentuation of effect clearly was absent by +24 hr. Tetrahydroalstonine appeared to produce a triphasic response of a rapid-onset hypoglycemia, a recovery, and a delayed-onset hypoglycemia associated with the +24- and +48-hr. determinations. At the anti-inflammatory dosage used here, tolbutamide did not produce a dramatic hypoglycemic effect but was significantly hypoglycemic at +3.5 hr. Like tetrahydroalstonine, tolbutamide appeared to have some delayed hypoglycemic effect at +48 hr.

Since a triphasic response for tetrahydroalstonine had not been previously reported (5), and since it could not have been detected in the previous experiment (Tables I and II), and because such a response is unusual, another experiment was conducted to confirm or to reject this finding. Results are summarized in Table IV using alloxanized rats of the same breed and sex and using the same experimental techniques as in Table III. Tetrahydroalstonine definitely appears to have a triphasic response pattern at this dosage level. It would appear that the sustained tertiary effect of tetrahydroalstonine should be investigated further for possible utility in the treatment of diabetes, while cryogenine should be avoided in diabetic patients.

Table IV—Effect of 125 mg./kg. of Tetrahydroalstonine Orally on Blood Glucose in Alloxan-Diabetic Rats

Sampling Time, hr.	Mean Blood Glucose, mg.% Agar Vehicle	Tetrahydroalstonine	*p*
+0.25	106.1 ± 4.2[a]	122.3 ± 5.4	<0.05
+0.5	113.3 ± 4.3	94.7 ± 4.8	<0.01
+1.0	105.9 ± 4.5	92.3 ± 4.5	<0.05
+2.0	116.0 ± 4.0	138.5 ± 10.9	>0.05
+4.0	123.0 ± 4.9	159.0 ± 10.2	<0.005
+12.0	132.2 ± 5.6	136.3 ± 14.7	>0.50
+24.0	121.3 ± 5.1	84.0 ± 4.8	<0.001
+48.0	128.1 ± 3.9	75.9 ± 8.8	<0.001

[a] Tabular figures indicate the mean of 10–14 animals ± 1 *SE* of the mean.

SUMMARY

An attempt was made to document both a stress-induced parameter (hyperglycemia) and an inflammation-associated parameter (carrageenin-induced pedal edema) in one experiment. Pedal edema is best recorded at the +3- and +5-hr. time intervals, but blood sampling during this period interfered with the validity of such measurements. Carrageenin-induced pedal edema elevated blood glucose values at +6 and +12 hr., and phenylbutazone premedication appeared to be effective in preventing this response at a dosage also effective in reducing pedal edema formation. At one or both of the +6- and +12-hr. sampling times, chlorpromazine hydrochloride, indomethacin, and cryogenine accentuated carrageenin-induced hyperglycemia while tolbutamide, tetrahydroalstonine, and tetrabenazine were essentially without effect. In rats tested concurrently but not subjected to pedal edema, all of the orally administered test drugs elevated blood glucose at one or both of the +6- and +12-hr. sampling times. At an anti-inflammatory dosage level, tetrabenazine did not affect the hyperglycemia produced by alloxan in rats, while cryogenine definitely accentuated the hyperglycemic response. At an anti-inflammatory dosage level, tolbutamide was just detectable as mitigating alloxan-induced hyperglycemia, while tetrahydroalstonine displayed a triphasic action of transient hypoglycemia, recovery, and a subsequent sustained hypoglycemic effect. While the present study did not attempt to arrive at mechanisms of action, the hyperglycemic effect of cryogenine and the delayed onset hypoglycemia of tetrahydroalstonine in diabetic rats appear worthy of further investigation.

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* Present address: Pesticides Regulation Division, Environmental Protection Agency, Washington, DC 20250

† Present address: Gillette Medical Evaluation Laboratories, Rockville, MD 20850

▲ To whom inquiries should be directed. Present address: Department of Physiology-Pharmacology, School of Pharmacy, University of the Pacific, Stockton, CA 95204

Effects of Ascorbic Acid Deficiency on Kinetics of Drug Hydroxylation in Male Guinea Pigs

A. E. WADE▲, BETTY WU, and PAUL B. SMITH

Abstract □ The effects of ascorbic acid (vitamin C) deficiency on components of drug-hydroxylating systems in guinea pig liver were investigated. Although the liver weight–body weight ratio was increased, the concentration of microsomal protein was markedly less in ascorbic acid-deficient guinea pigs. This was reflected in a decrease in aniline and hexobarbital hydroxylation reactions when calculated on a unit of liver weight; however, when analyzed per unit of microsomal protein, ethylmorphine demethylase activity was unaffected. The K_m's for these substrates, as well as the ethyl isocyanide difference spectra, were unchanged, indicating that no qualitative changes had occurred in the enzymes responsible for their metabolism or in the cytochrome P-450. Aniline metabolism per unit protein was depressed by ascorbic acid deficiency, as was the content of cytochromes P-450 and b_5. The return of function by a single injection of ascorbic acid given 1–24 hr. prior to decapitation was not frequently observed. Induction with sodium phenobarbital was not blocked by this dietary deficiency state.

Keyphrases □ Ascorbic acid deficiency—effect on kinetics of drug hydroxylation, guinea pig liver □ Drug hydroxylation kinetics—effect of ascorbic acid deficiency, guinea pig liver

The duration and intensity of action of many drugs are largely determined by the speed at which they are metabolized in the body (1). The rate of such drug metabolism may be altered by drug pretreatment, hormones, or the nutritional status of the animal. Increased rates appear to be due to an increased concentration of microsomal enzyme or to the increased affinity of the enzyme for the substrate. Past studies

Table I—Effect of Ascorbic Acid Deficiency on Body Weight, Liver Weight, and Hepatic Microsomal Protein Content of Guinea Pigs

	Days on Diet	Control	Ascorbic Acid Deficiency	p
Terminal body weight, g. ± *SE*	12	340.1 ± 15.6 (15)	281.8 ± 11.6 (15)	<0.01
	18	389.3 ± 6.8 (4)	302.2 ± 20.8 (4)	<0.05
Liver weight g. ± *SE*	12	13.13 ± 0.79 (15)	13.13 ± 0.59 (15)	>0.05
	18	13.95 ± 1.24 (4)	13.40 ± 1.32 (4)	>0.05
$\frac{\text{Liver weight}}{\text{Body weight}} \times 100$	12	3.85 ± 0.12 (15)	4.68 ± 0.15 (15)	<0.01
	18	3.59 ± 0.18 (4)	4.42 ± 0.21 (4)	<0.05
Protein concentration[a], mg./g. liver ± *SE*	12	22.97 ± 1.03 (6)	16.78 ± 0.79 (6)	<0.01
	18	29.25 ± 2.28 (4)	15.53 ± 0.33 (4)	<0.01

[a] 105,000×*g* microsomal pellet. Number in parentheses is number of animals in group.