ester hydrolyzing enzyme among the accessory reproductive organs of various animals is reminiscent of similar tissue and species differences in the manufacture of other components of the seminal plasma such as fructose, citric acid, inositol and acid phosphatase (15). The immense rates of arginine ester hydrolysis by some accessory sexual tissues, and the fall in activity induced by castration, adds this arginine ester hydrolyzing enzyme to the already imposing list of chemical accessory sexual characteristics in the male.

Summary. Certain esters of arginine, but not those of a number of other amino acids, are hydrolyzed rapidly by some lobes of the prostate gland of a number of species. Marked differences in the activity of this arginine ester hydrolyzing enzyme in different lobes of the prostate gland of various rodents have been observed. Dog prostatic fluid is extremely rich in this enzyme, whereas the human prostate hydrolyzes arginine esters rather slowly. The activity of the enzyme in rodent prostatic tissue is depressed after castration. Some properties of this enzyme, and its relationship to other proteolytic enzymes in prostatic secretion as well as to the process of semen coagulation are discussed.

1. Gotterer, G., Ginsburg, D., Schulman, T., Banks, J., and Williams-Ashman, H. G., *Nature,* 1955, v176, 1209.

2. Werbin, H., and Palm, A., *J. Am. Chem. Soc.,* 1951, v73, 1382.

3. Warburg, O., and Christian, W., *Biochem. Z.,* 1941, v310, 384.

4. Kunitz, M., *J. Gen. Physiol.,* 1947, v30, 291.

5. Anson, M. M., *ibid.,* 1938, v22, 79.

6. Huggins, C., Masina, M. H., Eichelberger, L., and Wharton, J. D., *J. Exp. Med.,* 1939, v70, 543.

7. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.,* 1934, v56, 658.

8. Lundquist, F., Thorsteinsson, Th., and Buus, O., *Biochem. J.,* 1955, v59, 69.

9. Neurath, H., and Schwert, G. W., *Chem. Rev.,* 1950, v46, 69.

10. Troll, W., Sherry, S., and Wachman, J., *J. Biol. Chem.,* 1954, v208, 85.

11. Sherry, S., and Troll, W., *ibid.,* 1955, v208, 95.

12. Huggins, C., and Neal, W., *J. Exp. Med.,* 1942, v76, 527.

13. Huggins, C., *The Harvey Lectures,* 1947, v42, 148.

14. Walker, G., *Bull. Johns Hopkins Hosp.,* 1910, v21, 182.

15. Mann, T., and Lutwak-Mann, C., *Physiol. Revs.,* 1951, v31, 27.

16. Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.,* 1948, v172, 619.

Received March 5, 1956. P.S.E.B.M., 1956, v92.

Influence on Hepatic Ferritin Systems of Tertiary Amine, G-D 131, with Beneficial Effects in Shock.* (22385)

SILVIO BAEZ, S. G. SRIKANTIA, AND EPHRAIM SHORR.† (With technical assistance of Iris Forbes and Anne Carleton.)

Department of Medicine, Cornell University Medical College, and New York Hospital, N. Y. City.

This report deals with effects and mode of action, in experimental shock, of a group of synthetic agents not previously explored, since they possessed no known properties suggestive of potential value as protective agents in shock. They are tertiary amines, chemically related to β-chloroalkylamines, adrenergic blocking agents, of which N, N-dibenzyl-β-chloroethylamine (Dibenamine) is the prototype. These compounds are structurally altered so that their adrenergic blocking properties have been lost. Several of these compounds were utilized by Nickerson and Gump (1) in their study of the relation between chemical structure and adrenergic blocking activity; others have been synthesized for the current study. This group of compounds was

* Aided by research grants from Josiah Macy, Jr. Foundation, N. I. H., U. S. Public Health Service (Grant H-79), Armour Laboratories, Department of Army and the Postley Fund.

† Deceased Jan. 6, 1956.

selected on the basis of our analysis of mechanisms by which related adrenergic blocking agents provide high degree of protection in experimental shock which we(2) and other workers(3,4) have described. We observed two distinct actions of Dibenzyline: one, resulting from adrenergic blockade, was the blunting of the usual extreme peripheral vasoconstrictive response to hemorrhage or trauma, which affords a better perfusion of vital splanchnic organs throughout the shock syndrome(5); the other was a specific effect on the ferritin (VDM) regulating mechanisms of the liver(6). At low dosage levels *in vivo*, and by brief exposure to it *in vitro*, Dibenzyline inhibits normal anaerobic formation of vasoactive ferritin and also preserves the aerobic ferritin-inactivating system from deterioration ordinarily resulting from prolonged hepatic hypoxia. These observations bear on the concept of shock advanced by us (7), which would assign a major role in determining the outcome to the liver and, specifically, to the hepatic systems regulating metabolism of ferritin. These systems are believed to contribute to irreversibility of shock syndrome when they deteriorate to such a degree, from prolonged hypoxia, that the liver loses its capacity to inactivate the hepatic vasodepressor, ferritin, aerobically and despite restoration of aerobic conditions by transfusion, continues to elaborate this vasoactive agent, with its potentially unfavorable influence on the dynamics of the splanchnic terminal vascular bed. With Dibenzyline, our observations were compatible with this concept; but a more direct test of this hypothesis would be provided by agents which act only on hepatic ferritin systems, in the same manner as Dibenzyline, but without its additional adrenergic blocking action. It seemed reasonable to look for such compounds in the group, described above, which preserved certain chemical features of active adrenergic blocking agents but had lost this pharmacologic property.

The present report concerns the actions of N - (2-chloroethyl) - N-(cyclohexylmethyl)-ethylamine hydrochloride (G-D 131), whose loss of adrenergic blocking action was attributed by Nickerson and Gump(1) to saturation of the benzene ring, the unsaturated homologue being highly active.

Methods. Female rats of Wistar strain, weighing 120 to 150 g, were used. For *in vitro* studies, rats were killed by a blow on the head, liver removed immediately and sliced as for microrespiratory studies. The slices were placed in Erlenmeyer flasks containing Ringer-phosphate medium, pH 7.4, 5 volumes of medium to one gram of tissue. G-D 131 was added 2 μg/ml (10 μg/g liver) before incubating at 37.5° C in oxygen for 10 minutes. Slices from the same liver, treated similarly except for omission of the drug, served as controls. After incubation the media were discarded and the slices washed twice with Ringer-phosphate. For studies of anaerobic VDM formation, fresh Ringer-phosphate, in the same (1:5) proportion was added to the slices which were then incubated in N_2 for 90 minutes. Then the clear supernatant liquid was bioassayed by the rat mesoappendix method(8). The same slices were then reincubated, to test their ferritin-inactivation capacity, in O_2 in Ringer-phosphate containing known amount of vasoactive ferritin (usually 0.001 μg ferritin N/ml). After 2 hours the media were bioassayed as above. *Traumatic shock* experiments were carried out by the method of Noble and Collip(9) in unanesthetized rats, using 715 rotations of the drum at 44 r.p.m. 200 μg G-D 131‡/100 g body wt were given intravenously 60 or 180 minutes before drumming. A parallel set of saline-injected controls was drummed the same day. *Hemorrhagic shock* was induced under light anesthesia by graded bleeding, controlled by self-infusion apparatus(10). G-D 131 (80 μg/100 g body wt in 0.2 ml saline) was given intravenously one hour before start of experiment. Controls received 0.2 ml saline, while another series were given 20 μg Dibenzyline/100 g body wt i.v. at the same time interval. Heparin was present in saline and blood reservoir system in all experiments. Blood pressure was maintained at

‡ Kindly supplied by Dr. Mark Nickerson, Univ. of Manitoba and Dr. William Gump, Givaudan-Delawanna Corp.

or above 70 mm Hg for the first hour, at 50 mm Hg during the following hour and 30 mm Hg for remaining 2 hours. At end of the 4 hour period blood remaining in reservoir was slowly force-infused. Direct microscopic observation of terminal vascular bed in the mesoappendix was carried out throughout the hemorrhagic procedure in a number of animals(8). Ability to restrict VDM release to anaerobiosis and the capacity to inactivate ferritin aerobically were examined in livers removed from a number of rats at the end of the 4 hour hypotensive period. For this purpose liver slices were incubated aerobically in Ringer-phosphate for one hour, or in Ringer-phosphate containing vasoactive ferritin for 2 hours. Media were then bioassayed by rat test.

Results. Properties of G-D 131. Absence of adrenergic blocking properties, reported by Nickerson and Gump(1), was confirmed in the rat for amounts up to 200 μg/100 g body wt, the maximal amount administered in present experiments. At this dosage level, it was also without anticholinergic action.

Dibenzyline, in amounts which provide excellent protection in shock (*i.e.* 5 μg or more /100 g body wt), depresses reactivity to topical epinephrine of muscular capillaries of the normal rat; whereas G-D 131, at dosage levels up to 200 μg/100 g body wt was without effect on epinephrine reactivity, vasomotion or any other characteristic of blood flow in the mesoappendix of rats examined immediately, or up to 3 hours, after administration of drug. G-D 131 was tested for antibiotic activity§ and had none in concentrations of 50 and 250 μg against 8 organisms (*Staphylococcus aureus, Streptococcus* (Group A)*-C-203, Proteus vulgaris, Escherichia coli, Salmonella, Klebsiella pneumoniae, Clostridium welchii, Pseudomonas aeruginosa*). In the method employed Aureomycin is effective for all these organisms at concentration of 50 μg and for *Cl. welchii* at 3 μg.

Protection of hepatic ferritin mechanisms against anaerobic deterioration by brief exposure to G-D 131 in vitro. Our studies of *in vitro* action of Dibenzyline(6) on normal liver ferritin mechanisms suggested that similar experiments might be used to select, from this large series of tertiary amines, those most likely to be of value in shock experiments. Table I summarizes the results obtained with G-D 131 in *in vitro* studies of livers removed from normal rats. In agreement with our earlier results control liver slices released vasoactive ferritin into the medium during anaerobic incubation(11); under similar conditions, those briefly exposed to G-D 131 did not do so. Control liver slices, exposed to anaerobiosis, uniformly failed to inactivate added ferritin on subsequent aerobic incubation, while those briefly exposed, before anaerobiosis, to G-D 131 retained their normal ferritin-inactivating capacity. Thus, despite chemical changes which deprive it of adrenergic blocking activity, G-D 131 resembles Dibenzyline in its specific influence on ferritin regulating mechanisms of liver, and it therefore seemed an appropriate agent for evaluating the relation of these ferritin mechanisms to recovery from shock.

TABLE I. *In Vitro* Effect of G-D 131* on the Hepatic Ferritin Systems.

VDM Activity	Treatment of liver slices after exposure to G-D 131: N_2 x 90 minutes	N_2 x 90 minutes, then O_2 + ferritin x 120 minutes
Strong	● ● ● ●	● ● ● ● ● ● ● ●
Moderate	● ● ● ●	● ● ●
Mild	● ●	○
Neutral	○ ○ ○ ○ ○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○ ○ ○ ○
	○ = G-D 131	● = Controls

* Liver slices (1 g) incubated 10 min. at 37.5°C with G-D 131 (10 μg) in 5 ml Ringer PO_4 medium in 100% O_2. Slices washed 2 times and reincubated as above.

G-D 131 in traumatic shock. The percent survivals of controls and rats treated with G-D 131 at 60 or 180 minutes before rotation in the drum, are given in Table II. Although the small difference between controls and

§ By Drs. R. M. McCune, Jr. and P. Dineen at Cornell University Medical College.

TABLE II. Effect of Intravenous Pretreatment with G-D 131 on the Outcome of Drum Trauma in the Rat.

Treatment	Saline	200 μg G-D 131/100 g body wt	
Time of inj.	−60 to −180 min.	−60 min.	−180 min.
No. of rats	94	28	93
" " rotations	715	715	715
" surviving at 24 hr	41	15	78
% survival at 24 hr	44	53	84

those treated 60 minutes before shock is not significant, when G-D 131 is given 3 hours before drumming the survival is almost doubled, 84% as compared with 44% in controls.

G-D 131 in hemorrhagic shock. Data obtained on effects of pretreatment with Dibenzyline or G-D 131 on outcome of hemorrhagic shock are presented in Table III. The shock was profound and resulted in survival of only 27% in controls. The recovery rate with G-D 131 was 77%, and 63% with Dibenzyline.

During the first few hours of the hemorrhagic experiment, direct observations of blood flow in mesoappendiceal vessels disclose a close resemblance between controls and G-D 131 treated rats. However, as hypotension is prolonged, in control animals there is progressive deterioration of circulation, with decline in reactivity to topical epinephrine, loss of vasomotion and eventual stagnation of blood in the capillary bed. In treated animals a compensatory type of peripheral vascular behavior persists throughout the experiment, with preservation of enhanced reactivity to epinephrine, and vasomotion, and with good flow throughout the capillary bed. In Dibenzyline-treated rats the originally depressed reactivity to topical epinephrine is rapidly replaced by compensatory type of behavior persisting throughout the syndrome.

TABLE III. Comparison of the Effect of Dibenzyline and G-D 131 on the Outcome of Hemorrhagic Shock in the Rat.*

Inj. into tail vein	Controls, .2 ml saline	Dibenzyline, 20 μg/100 g body wt	G-D 131, 80 μg/100 g body wt
No. of animals	22	19	22
Max blood lost† (% body wt)			
Avg	4.5	4.5	4.1
Range	3.5–5.3	3.3–5.5	3.5–5.0
Uptake of blood‡ (% body wt)			
Avg	1.0	.5	.5
Range	.0–3.6	.1–1.0	.0–1.8
% survival at 24 hr	27	63	77

* Both agents inj. 60 min. before bleeding.

† Total blood loss required to bring blood pressure to 30 mm Hg level.

‡ Amount of blood spontaneously taken up from reservoir to maintain blood pressure at 30 mm Hg for 2 hr.

TABLE IV. Effect of Pretreatment with G-D 131* on Hepatorenal Vasoactive Factors in Blood and Liver after Four Hours of Hemorrhagic Hypotension.†

		Blood .5 ml.	Liver slice incubation O_2 x 60 min.	Liver slice incubation Plus ferritin in O_2 x 120 min.
VEM	Mild	○		
VEM	Neutral	○ ○ ○ ○ ● ●	○ ○ ○ ○ ○ ○	○ ○ ○ ○ ○ ○ ○
VDM	Mild	●	●	●
VDM	Mod.	○ ○	○ ● ●	●
VDM	Strong	● ● ● ●	● ● ● ●	● ● ● ● ●
		○ = G-D 131		● = Controls

* G-D 131 80 μg/100 g body wt was given into tail vein 60 min. before hemorrhagic procedure.

† At or above 70 mm Hg for first hr, at 50 mm Hg for second hr, and at 30 mm Hg for following 2 hours.

The status of the hepatic ferritin regulating systems at end of the standard 4 hour hemorrhagic hypotensive period was investigated in another series of rats (Table IV). In contrast to liver slices from control shocked rats, which now released vasoactive ferritin in oxygen and had completely lost their aerobic ferritin-inactivating capacity, livers from G-D 131 treated animals behaved as do normal unshocked livers, releasing no ferritin aerobically and inactivating added vasoactive ferritin. Bioassays of blood samples drawn at end of the hemorrhagic experiment were generally confirmatory of findings in livers; blood from treated animals usually contained no VDM while the opposite was true of controls. From these results it is apparent that protection afforded by G-D 131, against deterioration of the ferritin-inactivating systems during *in vitro* hepatic hypoxia, is also exerted in the living animal during hemorrhagic hypotension.

Discussion. A tertiary amine, N-(2-chloro-

ethyl)-N-(cyclohexylmethyl)-ethylamine hydrochloride (G-D 131), related structurally to Dibenamine type of adrenergic blocking agent, but altered so that adrenergic blocking properties are lost, shares with Dibenzyline the property of modifying the ferritin regulating mechanisms of the liver. *In vitro,* brief exposure to 10 μg/g of tissue results in inhibition of the usual anaerobic release of vasoactive ferritin (VDM) from normal liver slices and in preservation of the aerobic hepatic ferritin-inactivation mechanism despite prolonged anaerobiosis. *In vivo,* pretreatment of rats with 200 μg of G-D 131/100 g body wt almost doubles survival rate in drum shock, and administration of 80 μg/100 g (approximately 1/100 of the LD_{50}) results in protection against hemorrhagic shock equal to that provided by Dibenzyline which had hitherto proved to be the most effective protective agent. Liver ferritin mechanisms, examined in G-D 131 treated rats following hemorrhagic procedure, compare favorably with those of normal unshocked rats, releasing no ferritin aerobically and retaining normal ferritin-inactivation capacity, in contrast to livers of control rats which now release ferritin aerobically and are unable to inactivate added ferritin.

Since G-D 131 is devoid of observable direct effects on behavior of terminal vascular bed of mesoappendix of the normal rat, maintenance of compensatory peripheral vascular activity throughout hemorrhagic shock procedure would not appear to be attributable to a direct influence of the drug, but may be a consequence of its action in preventing release into the blood stream of deleterious factors from the liver.

The failure of G-D 131 to protect against drum shock unless injected 3 hours before trauma (see Table II) appears paradoxical in view of our previous finding(12) that it exerts a protective effect in hemorrhagic shock when administered as late as 90 minutes after start of procedure. However, one of the differences between the two shock procedures may be related to this time factor; the drastic hemorrhagic hypotension may be assumed to become maximally harmful only after it has been maintained for several hours while in drum trauma the entire stress is imposed within the 15 minute period of rotation in the drum. The possibility is being investigated that a lapse of some time is required for G-D 131 to exert its maximum effect on the hepatic ferritin systems *in vivo.* A comparable time requirement has been noted with Aureomycin in drum shock(13)—the protection exerted is greater when this agent is injected 2 hours rather than one hour before trauma.

Summary. These observations with G-D 131 are regarded as support for our concept that deterioration of the hepatic ferritin mechanisms brings about consequences of major importance for ability of the animal to survive a standardized shock procedure.

1. Nickerson, M., and Gump, W. S., *J. Pharmacol. and Exp. Therap.,* 1949, v97, 25.

2. Baez, S., Zweifach, B. W., and Shorr, E., *Fed. Proc.,* 1952, v11, 7.

3. Wiggers, H. C., Ingraham, R. C., Roemhild, F., and Goldberg, H., *Am. J. Physiol.,* 1948, v153, 511.

4. Remington, J. W., Hamilton, W. F., Boyd, G. H., Jr., Hamilton, W. F., Jr., and Caddell, H. M., *ibid.,* 1950, v161, 116.

5. Zweifach, B. W., Baez, S., and Shorr, E., *Fed. Proc.,* 1952, v11, 177.

6. Baez, S., Srikantia, S. G., Forbes, I., and Shorr, E., *ibid.,* 1956, v15, 396.

7. Shorr, E., Zweifach, B. W., Furchgott, R. F., and Baez, S., *Circulation,* 1951, v3, 42.

8. Zweifach, B. W., *In Methods in Medical Research* (V. R. Potter, ed.), 1948, v1, 131, Year Book Publishers, Chicago.

9. Noble, R. L., and Collip, J. B., *Quart. J. Exp. Physiol.,* 1942, v31, 187.

10. Baez, S., Zweifach, B. W., Pellon, R., and Shorr, E., *Am. J. Physiol.,* 1951, v166, 658.

11. Shorr, E., Zweifach, B. W., and Furchgott, R. F., *Ann. New York Acad. Sc.,* 1948, v49, 571.

12. Srikantia, S. G., Baez, S., Carleton, A., and Shorr, E., *Fed. Proc.,* 1956, v15, 177.

13. Baez, S., and Shorr, E., *Shock and Circulatory Homeostasis* (H. D. Green, ed.), Tr. Fifth Conf., Josiah Macy, Jr. Foundation, New York, 1955, in press.

Received March 5, 1956. P.S.E.B.M., 1956, v92.