Protective Effect of Thiol Compounds Against Cadmium-Induced Vascular Damage to Testis.* (31319)

SAMUEL A. GUNN, THELMA CLARK GOULD AND W. A. D. ANDERSON
Department of Pathology, University of Miami School of Medicine, Miami, Fla.

A previous article(1) referred to various publications which demonstrated that acute destruction of testis resulted from a single subcutaneous injection of cadmium chloride to rats and mice. Necrosis of seminiferous tubules and interstitial tissue was shown to be a secondary result of selective toxicity of cadmium on the vasculature of the testis(2-6). So far, 3 substances have been reported to block the injurious effects of cadmium on the testis, zinc(7-9), selenium(9,10) and estrogens(11). The experiments described here were designed to determine whether sulfhydryl compounds, known to combine with heavy metals, would also protect the testis from cadmium damage.

Materials and methods. Mature male C. D. mice (Charles River Breeding Laboratories, Inc.), 8-12 weeks of age (approximately 40 g) were used. The minimal dose needed to produce necrosis of all seminiferous tubules by 6 days was 0.012 m-mole/kg (0.1 ml of 0.0048 M solution). The characteristic degree of testicular damage produced by this dose, classified numerically as Grade 3, or maximal response, is shown in Fig. 2; compare with the uninjected control, Fig. 1. In studies on degrees of blocking cadmium effect by the various sulfur-containing compounds tested, Grade 2 (Fig. 3) indicated slight protection, Grade 1 (Fig. 4) indicated marked protection and Grade 0 indicated full protection, identical with mice which had not received cadmium.

Two monothiols, cysteine and glutathione, and the dithiol, BAL, were tested for their capacity to protect the testis from damage caused by 0.012 m-mole/kg of cadmium chloride. Two other sulfur-containing compounds, methionine and sodium thiosulfate, were also included in this study. A total of 130 mice was used, 5-10 mice per group. Six days following cadmium injection the mice were killed by chloroform and exsanguination.

Testes were removed, fixed in Bouin's fluid and processed for microscopic study by routine histological techniques. For each mouse 8 cross-sections (4 from each testis) were examined microscopically. The degree of testicular injury for each mouse was classified as a Grade 0, 1, 2, or 3. A mean index of protection was obtained for each treatment group by totalling individual mouse gradings and dividing by the number of mice per group.

Results and discussion. Only the thiols cysteine (Table I) and BAL (Table II) gave complete protection against testicular damage caused by cadmium. Cysteine was completely effective in blocking cadmium when given either 30 minutes before or 30 minutes after the metal; testicular damage was prevented in most mice even when given 3 hours after cadmium. In studies with the light microscope(4,12), no alterations of endothelium of testicular blood vessels have been discerned until 6 hours following cadmium. Although in studies with the electron microscope Chiquoine(4) noted pinocytosis in some testicular capillaries as early as 3 hours, our results with cysteine protection indicated that irreversible damage did not take place until some time after 3 hours following administration of cadmium. When cysteine administration preceded that of cadmium by 3 hours, the thiol offered no protection; this was in agreement with observations that tissue sulfhydryl levels were no longer elevated 2½ hours after cysteine administration(13). In terms of the S:Cd ratio, BAL was more efficient than cysteine in protecting the testis, but had to be given 6 hours before cadmium to exert a block. Although parenterally administered BAL undergoes rapid absorption, metabolism and excretion(14), our data indicated that a time factor is essential for what-

* This investigation was supported by USPHS Research Grant CA-07004-03 from Nat. Cancer Inst.

ever transformations BAL must undergo to produce effective protection of the testis.

Bennett(15) suggested that the role of sulfhydryl groups in regulating capillary permeability should be investigated further. This was based on his own cytochemical demonstrations of mercaptan groups in capillary endothelium(15) and studies of Danielli, Danielli and Mitchell(16) who showed that increased capillary permeability resulting from arsenicals could be prevented by intravenous administration of suitable non-toxic thiols. Zweifach(17) implicated sulfhydryl-dependent systems in inflammatory reactions following local tissue injury. Although sulfhydryl groups may be an essential part of the endothelium of blood vessels in many tissues, this is not to say that they will all be equally vulnerable to heavy metals. Each organ has its own specialized functions and may have its own specialized vascular endothelium that allows specific permeation for its particular needs. It is known that the vascular supply of the testis is not merely a passive conduit for blood, but an active structure involved in the regulation of heat exchange(6). The fact that cadmium evoked an inflammatory response exclusively in one set of blood vessels, those supplying the testis, suggested to us(2) that testicular vascular endothelium may

TABLE I. Comparative Efficacy of Cysteine, Glutathione, Methionine and Sodium Thiosulfate in Blocking Testicular Damage Produced by Cadmium Chloride (.012 m-mole/kg) in C. D. Mice (40 g).

Treatment	No. of inj	Time of inj	M soln inj	Total dose, m-mole/kg	Ratio total S:Cd	Testicular effect Mean protection Full	Partial	None
None	—	—	—	—	—			3.0
Cysteine*	1	30 min before Cd	.30	6.0	500:1	.0		
	1	30 min after Cd	.30	6.0	500:1	.0		
	2	30 min before and 30 min after Cd	.15	6.0	500:1		.7	
	1	30 min before Cd	.15	3.0	250:1			2.6
	1	30 min after Cd	.15	3.0	250:1			2.1
	1	3 hr before Cd	.30	6.0	500:1			2.2
	1	3 hr after Cd	.30	6.0	500:1		.8	
Glutathione*	1	30 min before Cd	.30	6.0	500:1		1.2	
	1	30 min after Cd	.30	6.0	500:1		1.0	
	2	30 min before and 30 min after Cd	.15	6.0	500:1		1.5	
Methionine*	2	30 min before and 30 min after Cd	.30	12.0	1000:1			3.0
Sodium thiosulfate†	5	Every 2 hr starting 4 hr before Cd	.24	6.0	500:1			3.0

* Each injection .8 ml intraperitoneally (aqueous solution).
† Each injection .1 ml intraperitoneally (aqueous solution).

TABLE II. Efficacy of Various Dose-Time Sequences of BAL* in Blocking Testicular Damage Produced by Cadmium Chloride (.012 m-mole/kg) in C. D. Mice (40 g).

No. of inj	Time of inj	No. ml each inj	Total dose, m-mole/kg	Ratio total S:Cd	Testicular effect Mean protection Full	Partial	None
2	30 min before and 30 min after Cd	.2	1.00	42:1			2.4
2	6 hr before and at time of Cd	.2	1.00	42:1	.0		
2	*Idem*	.1	.50	21:1			3.0
1	6 hr before Cd	.2	.50	21:1	.0		
1	*Idem*	.1	.25	11:1			2.8
1	3 hr before Cd	.2	.50	21:1			2.6
1	*Idem*	.1	.25	11:1			2.8

* Injections of .1 M solution in peanut oil given subcutaneously.

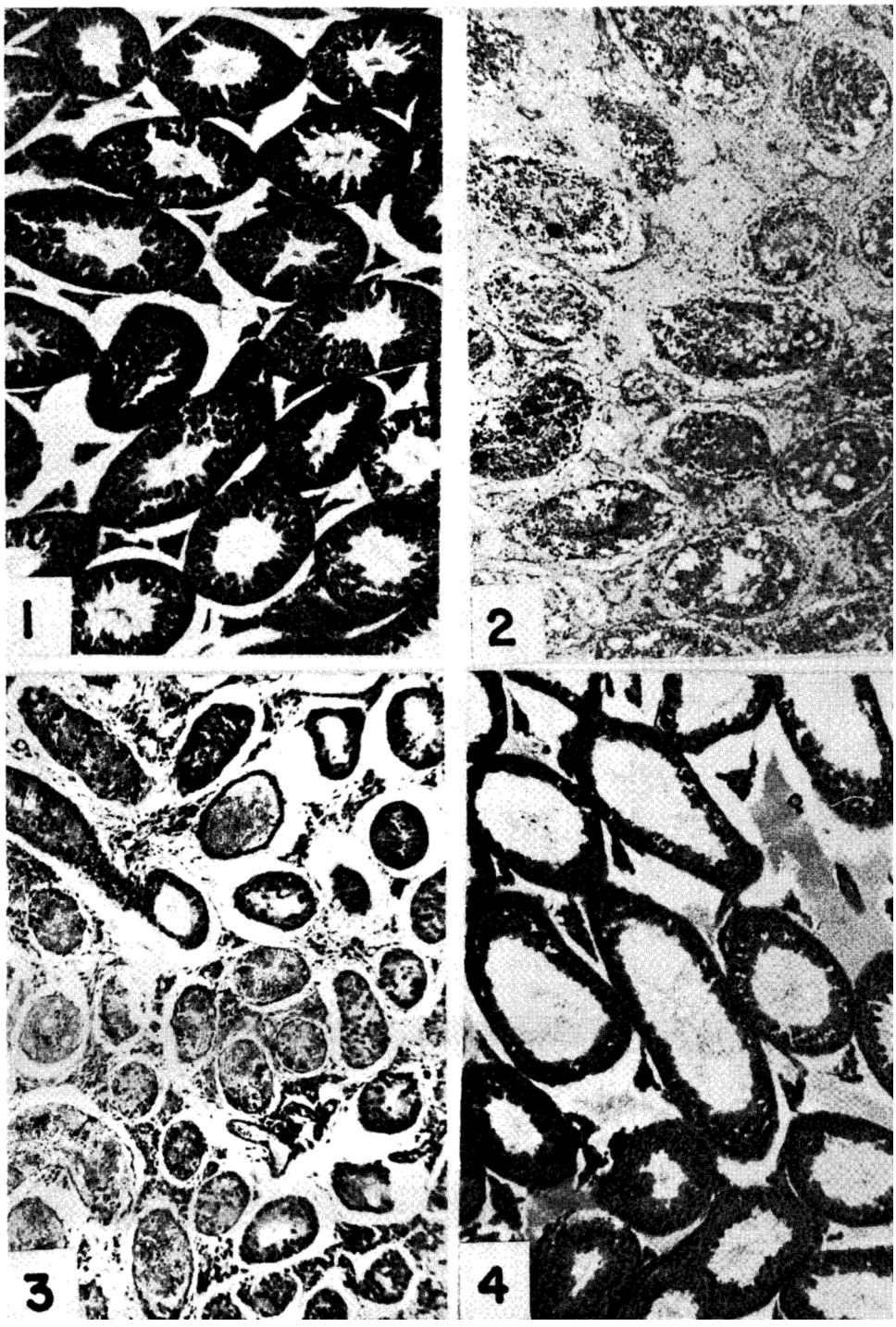

FIG. 1. Testis of control mouse. H & E × 70.

FIG. 2. Testis of mouse with maximal response to cadmium, Grade 3. All testicular elements, tubules and interstitial tissue, have undergone complete necrosis. H & E × 70.

FIG. 3. Testis of mouse, showing slight protection from cadmium, Grade 2. Most tubules and interstitial tissue have undergone necrosis. A few tubules show the presence of germinal

epithelium, but no active spermatogenesis. H & E × 70.

FIG. 4. Testis of mouse showing marked protection from cadmium, Grade 1. Inter- and intra-tubular edema are present with dilatation of tubules. There is no apparent damage to any of the tubular epithelium. H & E × 70.

have biochemical properties unique from blood vessels of other organs. Parizek(7), aware that the most widely accepted theory of cadmium toxicity was due to blocking sulfhydryl groups, dismissed thiol involvement as an explanation of testicular damage since such a strong sulfhydryl inhibitor as mercuric chloride did not affect the testis. Earlier Simon, Potts and Gerard(18) pointed out, however, that some sulfhydryl-containing enzymes were not affected by cadmium in concentrations which completely inhibited others and that not all heavy metals capable of sulfhydryl-binding shared the drastic effects of cadmium. These authors suggested that specific steric features of protein structures might account for both these findings. The results of this paper, demonstrating that the testis could be protected from cadmium by thiols, reopens the possibility that cadmium may exert damage to testicular vasculature by interference with essential sulfhydryl groups.

Summary. Vascular damage to mouse testis caused by subcutaneously administered cadmium chloride (0.012 m-mole/kg) was prevented by cysteine (6 m-mole/kg) when the thiol was administered 30 minutes before, 30 minutes after or 3 hours following cadmium. Glutathione (6 m-mole/kg) was less consistent, and methionine (12 m-mole/kg) and sodium thiosulfate (6 m-mole/kg) offered no protection whatsoever. Testicular damage was completely blocked by BAL (0.5 m-mole/kg) when administration of the dithiol preceded that of cadmium by 6 hours.

1. Gunn, S. A., Gould, T. C., Anderson, W. A. D., J. Reprod. Fertil., 1965, v10, 273.

2. ———, Am. J. Path., 1963, v42, 685.

3. Mason, K. E., Brown, J. A., Young, J. O., Nesbit, R. R., Anat. Rec., 1964, v149, 135.

4. Chiquoine, A. D., ibid., 1964, v149, 23.

5. Niemi, M., Kormano, M., Acta Path. Microbiol. Scand., 1965, v63, 513.

6. Waites, G. M. H., Setchell, B. P., J. Endocrinol., 1966, v34, 329.

7. Parizek, J., ibid., 1957, v15, 56.

8. Gunn, S. A., Gould, T. C., Anderson, W. A. D., Arch. Path., 1961, v71, 274.

9. Mason, K. E., Young, J. O., Brown, J. E., Anat. Rec., 1964, v148, 309.

10. Kar, A. B., Das, R. P., Proc. Nat. Inst. Sci. India, 1963, v29, B (suppl.), 297.

11. Gunn, S. A., Gould, T. C., Anderson, W. A. D., Proc. Soc. Exp. Biol. and Med., 1965, v119, 901.

12. Kar, A. B., Das, R. P., Acta biol. med. germ., 1960, v5, 153.

13. Calcutt, G., Connors, T. A., Elson, L. A., Ross, W. C. J., Biochem. Pharmacol., 1963, v12, 833.

14. Randall, R. V., Seeler, A. D., New Eng. J. Med., 1948, v239, 1004.

15. Bennett, H. S., Anat. Rec., 1951, v110, 231.

16. Danielli, J. F., Danielli, M., Mitchell, P. D., Biochem. J., 1947, v41, 325.

17. Zweifach, B. W., Functional Behavior of the Microcirculation, Charles C Thomas, Springfield, 1961.

18. Simon, F. P., Potts, A. M., Gerard, R. W., Arch. Biochem., 1947, v12, 283.

Received April 1, 1966. P.S.E.B.M., 1966, v122.