antiprotein (or anti-C) remaining in the final supernatant, since only a little over one-third of the total antibody was removed as agglutinin.

7236 C

Glutathione and Methylene Blue in Carbon Monoxide Poisoning.

ROBERT A. BUSSABARGER. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

The experiments of Brooks[1] based upon theory derived from the investigations of Warburg *et al.*,[2] Meyerhof,[3] Heymans and Heymans,[4] Barron,[5] and others, aroused interest in methylene blue as an antidote for carbon monoxide poisoning. Many case reports (Geiger,[6] Bell,[7] Nass,[8] Christoferson,[9] Brown[10]) and some experimental reports (Brooks,[11] Draize,[12] Haggard and Greenberg[13]) have increased the literature upon this subject. Even the contradictory conclusions of Haggard and Greenberg and the concise summary of Henderson[14] have not sufficed to alter the popular conception of methylene blue as an antidote for carbon monoxide poisoning.

In the following experiments, the effect of glutathione upon carbon monoxide poisoning was first investigated because of Brooks' results with a similar oxidation-reduction catalyst, methylene blue. However, the discouragingly negative results soon prompted an investigation of methylene blue.

White rats (100 to 200 gm.) were placed one at a time in a special

1 Brooks, M. M., *Am. J. Physiol.*, 1932, **102**, 145.

2 Warburg, O., *Z. physiol. Chem.*, 1910, **66**, 305; *Biochem. Z.*, 1923, **162**, 518; 1926, **177**, 471; 1930, **227**, 245.

3 Meyerhof, O., *Pflüger's Arch.*, 1912, **149**, 250.

4 Heymans, J. F., and Heymans, C., *Arch. intern. Pharmacodynamie*, 1922, **26**, 443.

5 Barron, E. S. G., *J. Biol. Chem.*, 1929, **81**, 445.

6 Geiger, J. C., *J. Am. Med. Assn.*, 1933, **100**, 1103.

7 Bell, M. A., *J. Am. Med. Assn.*, 1933, **100**, 1402.

8 Nass, J., *J. Am. Med. Assn.*, 1933, **100**, 1862.

9 Christoferson, A. W., *J. Am. Med. Assn.*, 1933, **100**, 2008.

10 Brown, T. C., *Colorado Med.*, 1933, **30**, 302.

11 Brooks, M. M., *Am. J. Physiol.*, 1933, **104**, 139.

12 Draize, J. H., *Science*, 1933, **78**, 145.

13 Haggard, H. W., and Greenberg, L. A., *J. Am. Med. Assn.*, 1933, **100**, 2001.

14 Henderson, Y., *Science*, 1933, **78**, 408.

chamber through which carbon monoxide (produced by the action of hot sulphuric acid upon formic acid, purified by washing with solutions of potassium hydroxide and with water, and made up to 2% by volume in air) was passed at a uniform rate of approximately one-half liter per minute. The animals were removed from the chamber 30 seconds after the disappearance of the righting reflex and immediately injected intraperitoneally with warmed solution. Three solutions were used: (1) Methylene blue: 0.406 gm. of methylene blue U.S.P. Medicinal and 5.24 gm. of dextrose per 100 cc. of solution. (2) Glutathione: 0.60 gm. of glutathione (Eastman Kodak) and 4.00 gm. of dextrose per 100 cc. of solution. (3) Dextrose: 5.42 gm. of dextrose per 100 cc. of solution.

These solutions were freshly prepared before each run. The amount of each solution injected was 1 cc. per 100 gm. of body weight. The time of recovery was taken as the interval between the removal from the chamber and the return of the righting reflex. The time interval between the removal from the chamber and the return of the animal's ability to walk was also noted in an attempt to verify the results of Brooks,[1] though, as Haggard pointed out, this is not a reliable criterion for recovery.

RESULTS

Substance Injected	No. of Animals	Aver. time in chamber (min.)	Aver. time for recovery of righting reflex (min.)	Aver. time for recovery of ability to walk (min.)	Deaths within 5 min. after injection. Not included in preceding aver.
Dextrose	33	4.05 (100%)*	4.97 (100%)	7.85 (100%)	0
Methylene Blue	35	4.10 (101%)	4.67 (94%)	8.30 (106%)	4
Glutathione	15	4.25 (106%)	4.88 (98%)	8.16 (104%)	2

* The average times for dextrose-injected rats were taken as 100% and the other percentages were made on this basis.

It was noted that nearly all of the rats passed through an excitatory stage before collapsing.

The above results show that neither glutathione nor methylene blue injections have an appreciable accelerating effect upon the rate of recovery of the righting reflex in white rats poisoned with carbon monoxide. That such injections may prove to be fatal instead of beneficial was also shown.

Summary. 1. Glutathione injections did not appreciably accelerate recovery of white rats poisoned with carbon monoxide. 2. Methylene blue injections did not appreciably accelerate recovery of white

rats poisoned with carbon monoxide. 3. Methylene blue injections appeared to be detrimental rather than beneficial to white rats suffering from carbon monoxide poisoning.

7237 P

A Thyreotropic Hormone from Pituitary Extracts.*

BORIS KRICHESKY. (Introduced by Bennett M. Allen.)

From the Department of Biological Sciences, University of California at Los Angeles.

The anterior pituitary substance responsible for thyroid hypertrophy and hyperplasia has been attributed to various anterior lobe hormones or to a separate thyreotropic hormone.[1] By heating a pituitary extract (Phyone), the thyreotropic substance has been separated from the contained gonad and growth stimulating substances.†

To determine the effect of heat on the activity of the growth hormone, young mice, 18 to 23 days of age, were used. 172 animals were divided into 5 groups: Group I injected subcutaneously with phyone; group II injected with phyone heated 60°C. 15 minutes; group III injected with phyone heated 80°C. 15 minutes; group IV injected with phyone heated to boiling 15 minutes. Group V was used as controls. All animals were injected with 0.1 cc. extract daily for 5 days. The average increase in body weight in 6 days was as follows: group I, 56%; group II, 55%; group III, 37%; group IV, 34%; controls, 35%. These data indicate that the growth hormone contained is not thermostable and is inactivated at some temperature between 60° and 80°C.

To determine the effect of heat upon the activity of the gonad stimulating hormone, female rabbits, 11 to 12 weeks of age, were used. These were divided into 3 groups of 7 each: group I, injected with 0.5 cc. of phyone, twice daily for 5 days; group II, with

* Aided by a grant from the Board of Research, University of California, under the supervision of Dr. B. M. Allen.

1 Riddle, O., Bates, R. W., Dykshorn, S. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 794. Anderson, E. M., and Collip, J. B., *Ibid.*, 1933, **30**, 680. Crew, F. A. E., and Wiesner, B. P., *Brit. J. Med.*, 1930, **1**, 777.

† Dr. David Klein, Wilson Laboratories, kindly supplied us with the Phyone used in this work.