produce any significant biochemical changes *per se*. Similarly, alterations in blood volume as determined by hemoglobin estimations were not related to these changes. Control experiments with adrenaline (0.25 mg per kg) produced the same elevation of the blood sugar in the resistant as in the normal animal.

The differences in blood and tissue chemistry shown by the normal and the resistant rat in response to trauma seem to indicate that the resistance which develops as a result of repeated traumatization, involved a stabilization of metabolic processes which are markedly upset in the non-resistant traumatized animal.

Summary. Biochemical changes in the blood and tissues of fed normal rats, and of rats made resistant to trauma, have been studied following the subjection of these animals to trauma in a revolving drum.

NPN rose significantly in the normal, but remained unchanged in the resistant rat.

Blood sugar, and blood and muscle lactic acid, rose in both the normal and resistant, but the difference between the two groups was highly significant. Pyruvic acid rose significantly in the normal, but showed no change in the resistant animal.

While whole blood and serum PO_4 rose 100% in the normal, Ca remained unchanged. There was no change in PO_4 in the resistant rat.

Values for Na and K of blood and muscle, and for liver, heart, and skeletal muscle glycogen showed non-significant differences between normal and resistant animals.

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Drug Prophylaxis against Lethal Effects of Severe Anoxia: VI. Neostigmine Bromide and Diphenylhydantoin.*

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In a preliminary survey of effects of autonomic agents on mortality of mice exposed to acute anoxic anoxia, it was noted that large doses of physostigmine and certain other cholinergic drugs tend to protect vs. anoxia while large doses of neostigmine methylsulfate do not do so and instead appear to be harmful. It was thought advisable to study this unexpected result in more detail.

Neostigmine bromide was administered intraperitoneally (ip.) or orally to 240 mice in doses listed in Table I, 30-60 min. before exposure to anoxia. The higher doses were within the lethal range: 5/20 mice treated with 0.3 mg/kg ip., 1/20 treated with 0.5 mg/kg orally, and 3/20 treated with 0.3 mg/kg ip. + diphenylhydantoin ip. died before exposure to anoxia.

Diphenylhydantoin Na was also administered to 140 mice, 110 of which had received neostigmine a few minutes before this treatment. All injections of dilantin were of 100 mg/kg ip. and were given approximately 30 minutes before exposure to anoxia. This agent has been shown by Hoff and Yahn[2] to be of value in protecting rats vs. acute anoxia and was used in the present study to test its effects in mice and also to determine whether or not a prophylactic synergy results from combination with neostigmine therapy.

Anoxia was induced by a standard method previously described.[3] Treated mice together with an equal number of control mice were exposed with adequate ventilation in a decompression chamber for 10 minutes to a reduced atmospheric pressure corresponding to an ap-

* This study was supported in part by a grant-in-aid from Hoffmann-LaRoche, Inc., Nutley, N.J.

1 Emerson, G. A., and Van Liere, E. J., *J. Lab. and Clin. Med.*, 1943, **28**, 700.

2 Hoff, E. C., and Yahn, C., *Federation Proc.*, 1943, **2**, 22.

3 Emerson, G. A., and Van Liere, E. J., *J. Lab. and Clin. Med.*, 1943, **28**, 689.

TABLE I.
Influence of Neostigmine Bromide and Na Diphenylhydantoinate on Lethal Effects of Acute Anoxia.

Neostigmine bromide		Dilantin Na		Mortality ratios		
Route	mg/kg	Route	mg/kg	Treated mice	Control mice	p
Intraper.	0.1	—	—	12/30	13/30	—
"	0.3	—	—	9/15	10/20	—
Oral	0.1	—	—	4/20	9/20	>0.05
"	0.2	—	—	2/20	9/20	<0.05
"	0.3	—	—	11/20	10/20	—
"	0.5	—	—	4/19	2/20	—
Intraper.	0.1	intraper.	100	2/30	13/30	<0.005
"	0.3	"	100	4/17	10/20	>0.05
Oral	0.2	"	100	0/40	27/40	<0.005
"	0.3	"	100	2/20	10/20	<0.05
—	—	"	100	3/30	13/30	0.005

proximate altitude of 10,000 feet, after which the pressure was further reduced at a rate corresponding to an elevation in altitude of approximately 1,000 ft/min. Exposure was usually terminated at a reduced pressure under which about half of the control mice died.

Results are noted in Table 1. Mortality ratios signify the number of mice dying during anoxia / the total number of mice exposed. Statistical treatment of the results by the exact factorial method[4] yields the values noted for p in Table 1, or the hazard that the results are due solely to chance. It may be seen that the most significant prophylaxis was obtained with combined treatment; and particularly that all of 40 mice given 0.2 mg/kg of neostigmine bromide by mouth + dilantin ip. survived anoxic conditions killing 27/40 controls exposed simultaneously.

Previous results[1] with neostigmine methylsulfate showed a slight, statistically insignificant prophylactic effect of ip. injection of 0.1 mg/kg and a definitely harmful effect of ip. injection of 0.5 mg/kg. The present results indicate a more marked prophylactic effect of oral doses of neostigmine bromide below the lethal range, with probable statistical significance at a dose of 0.2 mg/kg. With larger oral doses, however, this protective effect disappears.

Diphenylhydantoin has a definite prophylactic effect vs. anoxia in mice as well as rats, and is more effective in mice than ethanol, amytal or pentobarbital.[5] The terminal stage of anoxia in mice is usually convulsive and convulsant agents in the main do not reduce mortality.[6] It is probable that diphenylhydantoin exerts its preventive action in mice through its anticonvulsant properties, so that application of the present findings to man is not justified. Neostigmine, in large dose, acts as a convulsant; this may explain the lack of prophylactic effects above a certain optimum dose. Further, excessive salivation occurs in mice given large doses of neostigmine, especially by the ip. route; this also may play a part in increasing mortality under the physiologic handicaps of severe anoxia.

As an incidental finding, diphenylhydantoin appears to antagonize in part the lethal toxicity of neostigmine even when administered after considerable absorption of neostigmine has occurred. All deaths occurring before exposure to anoxia in mice treated with both agents were accompanied by convulsions and could be attributed to toxicity of neostigmine.

Summary. Prophylactic effects of diphenylhydantoin vs. anoxia in rats are confirmed

[4] Loewenthal, L. J. A., and Wilson, W. A., *Brit. Med. J.*, 1939, **2**, 110.

[5] Emerson, G. A., Van Liere, E. J., and Morrison, J. L., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 376.

[6] Emerson, G. A., and Van Liere, E. J., *Arch. internat. Pharmacodyn.*, 1940, **64**, 239.

for mice also. Oral administration of 0.2 mg/kg of neostigmine bromide to mice reduces mortality in severe anoxia but larger doses are not prophylactic. Together, the 2 agents prevented death of any of 40 mice exposed to anoxic conditions simultaneously killing 27 of 40 untreated mice.

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Cholinergic Porphyrin Lacrymation and Paradoxical Mydriasis in the Rat. Possible Hame Nature of Choline Esterase.

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Waddell[1] first reported the pilocarpine mydriasis in the rat and attributed it to peripheral parasympathetic depression. Koppanyi and Sun[2] ascribed the paradoxical response to a ganglion paralysant action. Barnard, Tyllas and Mizock[3] could not relate the anomalous pilocarpine response to corneal anesthesia but they noticed and failed to report that acetylcholine gave the same response. Both pilocarpine and acetylcholine would provoke the secretion of "bloody tears" in a certain number of rats, particularly younger ones. Cytologic examination of the tears failed to show erythrocytes and sections of the harderian gland excised at the height of the response showed neither the expected hyperemia nor rhexis. Repeated pilocarpine administrations over a period of weeks appeared to exhaust the mechanism and this was attributed to a traumatic fibrosis but here again histologic examination of the gland failed to bear out the assumption.

The discovery of the fluorescence of this structure[4] and of the porphyrin nature of the staining material on the snouts of rats with vitamin B complex component deficiencies[5] and that the harderian gland was a porphyrin excreting organ[6] and the source of the staining material[7] redirected attention to the phenomenon of reddish tears during cholinergesis in the rat particularly since Hodge and Goldstein[8] reported as "bloody tears" the secretion observed after toxic doses of choline. A reexamination of the lacrymal secretion of rats poisoned by choline, by acetylcholine, by pilocarpine, and by cyanamide, revealed no other pigment than neutral amorphous protoporphrin.

During the course of the study with the last named drug, the nature of the paradoxical mydriasis was elicited. In doses of from 200 to 400 mg per kilo, cyanamide evokes a parasympatheticomimetic response in the rat, much more protracted in its course than that of pilocarpine, the animal surviving for several hours in coma. This protraction allows for a thorough observation of the ophthalmic findings in sustained cholinergesis. Immediately after the intraperitoneal injection of cyanamide, there is weakness and fibrillary twitching; during this stage a definite miosis is evident. In about 15 minutes, the respira-

[1] Waddell, J. A., *J. Lab. and Clin. Med.*, 1926, **12**, 232.

[2] Koppanyi, T., and Sun, K. H., *Am. J. Physiol.*, 1926, **78**, 358.

[3] Barnard, R. D., Tyllas, H. A., and Mizock, S. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 691.

[4] Strong, L. C., and Figge, F. H. J., *Science*, 1941, **94**, 331.

[5] Smith, S. G., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 691.

[6] Grafflin, A. L., *Am. J. Anat.*, 1942, **71**, 43.

[7] McElroy, J. W., Salmon, K., Figge, F. H. J., and Cowgill, G. R., *Science*, 1941, **94**, 467.

[8] Hodge, H. C., and Goldstein, M. R., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 281.