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.

14391

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.

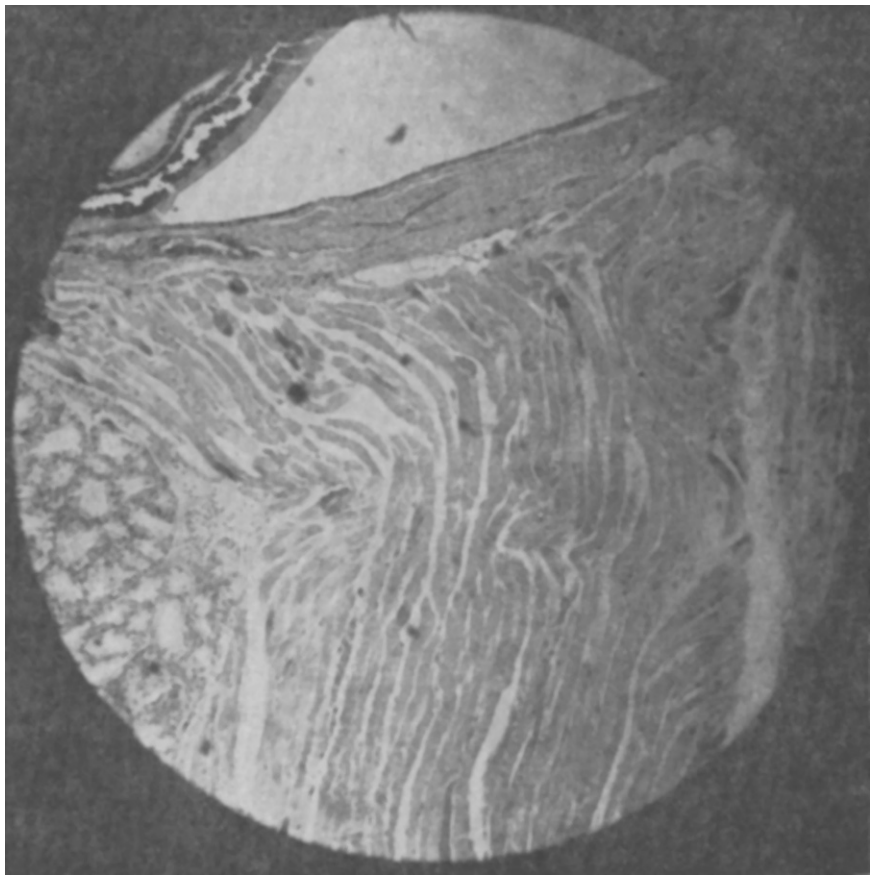

FIG. 1.
Sagittal section through optic nerve sheath of rat poisoned by cyanamide. It shows the encroachment of the engorged harderian gland on the sheath, passive hyperemia of the rim of the lamina cribrosa and disorganization of the ganglionic layers of the retina. Sections of this preparation closer to the midline show complete obliteration of the vaginal space.

tion becomes labored, frequent evacuations are noted and salivation and lacrymation become profuse. With the latter, a moderate mydriasis replaces the initial miosis. Within an hour, the animal lapses into coma with fibrillary twitching and the tearing which continues profusely becomes colorless, slightly mucoid and does not fluoresce in ultra-violet light. Death occurs in from 3 to 6 hours after injection of cyanamide. In the terminal stages of cyanamide "shock" the iris vessels are engorged and the fundus has an unusually reddish reflex. Ophthalmoscopic examination shows papilledema and retinal venous engorgement; the arteries appearing to be collapsed and relatively bloodless.

These findings comprise the ocular manifestations of raised intracranial or intraorbital pressure; there was no systemic evidence of the former but considerable indication of the latter. Heidgen and Barnard[9] in tracing the oculomotor tonus reflex pathway in the rat, found that section of the optic nerve alone would abolish the normally high pupiloconstrictor tone of this animal. Here, obviously, was the key to the cholinergic paradoxical mydriasis; increased intraorbital pressure created by the engorged harderian gland and reflected onto the optic nerve causing a conduction block in the visceral

[9] Heidgen, M. F., and Barnard, R. D., *Am. J. Physiol.*, 1931, **92**, 276.

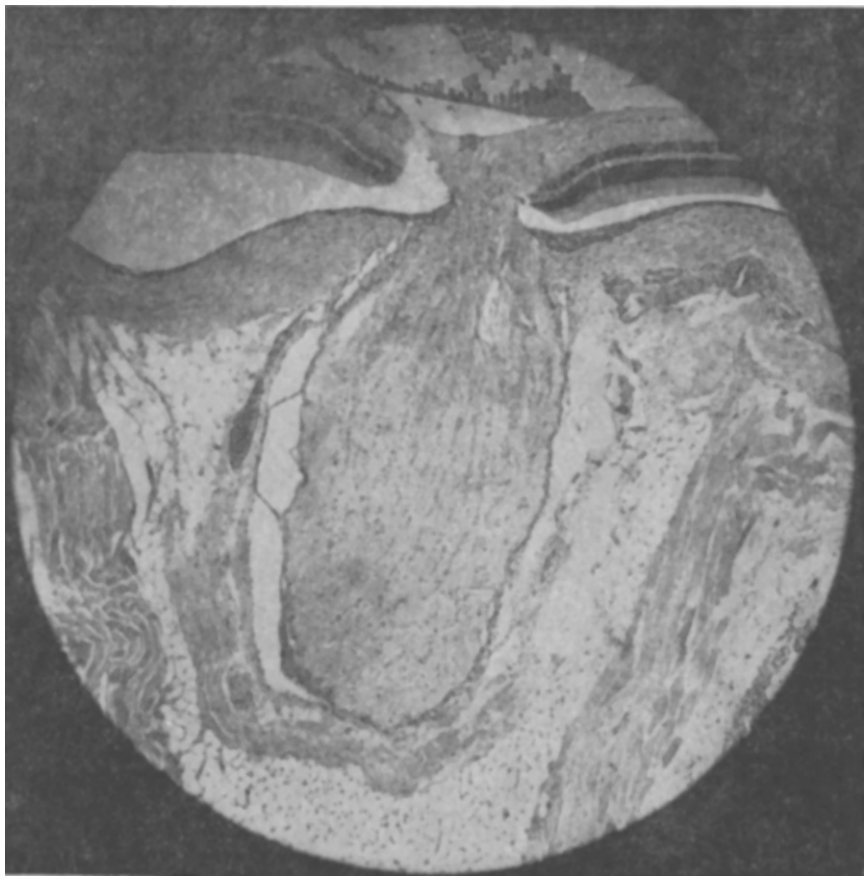

FIG. 2.
Midsagittal section through optic nerve head of atropinized rat poisoned by cyanamide. The essential normality of all structures indicates that the changes shown in Fig. 1 are probably not a specific effect of cyanamide (carbimid) but due rather to cholinergesis.

efferent oculomotor fibres. Histologic section of the intraorbital portion of the nerve, excised with its contiguum during the later stages of cyanamide intoxication, shows, in addition to the complete investment of this portion of the nerve by the intumescent gland, the passive hyperemia distal to it. (Fig. 1).

None of the 5 animals atropinized (0.3 mg/kilo) immediately after administration of cyanamide showed any evidence of raised intraorbital pressure. Mydriasis, of course, is immediate and complete but lacrymation is absent. (The preservation of the vaginal space around the optic nerve of one of these animals is shown in Fig. 2). The coma of such an animal is as profound as that exhibited by the non-atropinized, cyanamide intoxicated ones. There is, however, no fibrillary twitching, bronchorrhea or lacrymation indicating that these are the cholinergic manifestations of the poison. One can not account for the extreme prostration of cyanamide intoxication—actually resembling in its degree that of surgical shock—on the basis of cholinergesis. While the latter seems to be the main toxicologic feature of cyanamide absorption in man, severe cases of poisoning in humans[10] also results in prostration to the shock level. A further curious propensity of human cyanamide intoxication is its aggrava-

[10] Mellinghoff, E., and Thomas, D., *Deutsch. Med. Wchnscrft.*, 1939, **65**, 1636.

tion by alcohol imbibition. The parasympatheticomimetic effects of acetylcholine are also potentiated by alcohol[11] and the pharmacologic similarity of cyanamide to the parasympathetic hormone along with certain distinctive chemical features of the former permits the postulation that choline esterase is a heme compound and even enables us to assign to it a structure similar to that of the iron-porphyrin complex in ferrihemoglobin. For cyanamide, like cyanide, combines with the iron of ferriheme in either or both of two distinct types of linkages; (A), the polar covalent or ferrideheme linkage and (B), by coordination; the hemochromogen linkage. (A) appears to be the pharmacodynamically important linkage since the heme compounds as they exist physiologically are already in the (B) form and the amino acids, while they are suitable hemochromogen formers (Bertin-Sans and deMontessier[12]) have little pharmacologic effect. Thiamine, which does coordinate with ferriheme[13] and which potentiates acetylcholine, does not act on the esterase.[14] The ferrideheme formers, on the other hand are all very active pharmacologically and both on the basis of this activity and their chemical behavior, may be separated into two categories:

1. Those which form ferridehemes with a very low degree of dissociation, *e.g.*, azide, cyanide, fluoride, fulminate and hydrosulfide. These are predominantly tissue asphyxiants through their affinity for the ferriheme component of the cytochrome system. If cholinergesis attends their advent in the organism it is masked by their more rapid and dramatic toxic effects.*

2. Those which differ quantitatively from (1) by their larger ionic diameter and the higher degree of dissociation of the ferridehemes which they form. Cyanamide, cyanate nitrite and thiocyanate make up the group and while they have some tissue asphyxiant action they are mainly depressor drugs.

On the assumption of a ferrihemoglobin type of structure for choline esterase, the depressor activity of these drugs might be explained. Ferrideheme formation with any of the members of category (2) would prevent the labile valence change of the iron by which the activity of many of the heme compounds is conditioned. That cyanamide inhibits the decomposition of cetyl pyridinium chloride by ferriheme has been shown.[13] There remains to be explained on the basis of this assumption the cholinergic effects of physostigmine and of choline; the latter not being a ferrideheme former. Likewise to be explained is the anti-esterase potentiation of acetylcholine by ethanol.[11] The case of choline may be one of enzymatic synthesis of acetylcholine. Physostigmine may be a ferrideheme former through acid dissociation of its secondary imine hydrogen; spectroscopic studies designed to demonstrate such a combination with ferrihemoglobin have been inconclusive; the pigment is partially denaturated and the alkaloid, itself, becomes colored in solution. One cannot rely on spectroscopic evidence for the negative case in this connection, because fluoride does not modify the color of cetyl pyridinium ferrihemochromogen but does, like cyanamide, inhibit its disruption. A spectroscopically indistinctive combination with ferrihemoglobin is that of ethanol; the latter having anti-esterase activity.[11] Non-ferrideheme combinations with the iron of ferrihemoglobin of iminazolyl groups and of neutral moieties (ammonia, ethanol) have been described.[16,17] Coryell and Stitt[17] report a significant change in the magnetic susceptibility from that of ferrihemoglobin in its ethanol derivative. This change would portend an alteration in the fer-

[11] Ettinger, G. H., Brown, A. B., and Megill, A. H., *J. Pharm. and Exp. Therap.*, 1941, **73**, 119.

[12] Bertin-Sans, H., and deMontessier, J., *C. R. Soc. de Biol.*, 1892, **114**, 923.

[13] Barnard, R. D., *J. Am. Pharm. Assn.*, in press.

[14] Byer, J., and Harpruder, K., *J. Pharm. and Exp. Therap.*, 1940, **70**, 328.

* Choline esterase is inactivated by fluoride ion *in vitro* (Nachmansohn[15]).

[15] Nachmansohn, D., *C. R. Soc. de Biol.*, 1939, **130**, 1065.

[16] Russell, C. D., and Pauling, L., *Proc. Nat. Acad. Science*, 1939, **25**, 517.

[17] Coryell, C. D., and Stitt, F., *J. Am. Chem. Soc.*, 1940, **62**, 2951.

riheme prosthetic nucleus and were it to occur in one of the components of a delicately poised enzyme system might modify its activity.

The only other suggestion as to the chemical nature of choline esterase has resulted from the investigations of Nachmansohn and Lederer.[18] They concluded that its activity was conditioned by a sulfhydryl group because of its inactivation by heavy metal ions; treatment with reduced glutathione was followed by reactivation. The same facts, however, can be cited in support of the ferriheme theory of its constitution. Ferrihemoglobin forms well defined compounds with each of the ions of the b family of the heavy metals of Groups I and II of the periodic system[19] (these incidentally also form insoluble sulfides) and the metalloferrihemoglobin combination is broken by its treatment with any reducing agent.

The hypothesis of the ferriheme nature of choline esterase and its corollary, that the enzyme may actually be a cytochrome, peroxidase, catalase, ferrihemoglobin and/or myomethemoglobin will have its scrutiny hampered by the universal distribution of iron compounds in living tissues.

[18] Nachmansohn, D., and Lederer, N., *Bull. Soc. Biol. Chim.*, 1939, **21**, 797.

[19] U. S. Patent No. 2,296,377 (assigned to Armour and Company).

14392

Antibacterial Filtrates from Cultures of *Aspergillus flavipes.*

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The production of a powerful, non-toxic antibacterial substance by *Penicillium notatum* has stimulated search in many quarters for other molds showing similar behavior. In addition to the work with *Aspergillus flavus* already reported,[1] the author has examined about 30 molds. Most of them were aspergilli, obtained from the collection of Dr. Charles Thom. A number have shown antibacterial activity of varying degree, but one, *Aspergillus flavipes,* has been of particular interest. As this work has been interrupted, it seems appropriate to record the results at this time.

The mold was designated in the Thom collection "175.4303.46-Texas soil." As received on an agar slant there was a heavy, tight mycelial growth which could be broken with difficulty, and spores for transfer were not easily obtained from this part of the growth. However, a light sandy area of sporulation was present at the tip of the slant where it was thinnest. This type of growth persisted in subcultures on slants, but by growing on Czapek-Dox agar in a Petri dish an even, sandy growth with ample sporulation was obtained. The growth at room temperature is colorless for about 3 days, then takes on a light brown sandy appearance as sporulation develops, and after 4 or 5 days a characteristic camphor-like odor is noticed. This odor has been developed only on solid media.

Little or no antibacterial activity was noted when the mold was grown on Czapek-Dox solution, but it appeared when a protein digest was used. With this medium (Tryptone-Difco) the best results were noted without any adjuvants at all, and sugar was definitely deleterious. The best medium of all, however, consisted solely of corn steep liquor, in the concentration of 5 or 10 ml to 100 ml of water. There was no definite advantage in the

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[1] White, E. C., and Hill, J. H., *J. Bacteriology*, 1943, **45**, 433.