Observations on Pharmacology of the Anticholinesterases Sarin and Tabun. (21155)

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The following account summarizes observations made in late 1947 and early 1948 on the pharmacology of sarin and tabun, potent anticholinesterase nerve gases, which, according to Holmstedt(1), were first synthesized by Schrader and were once considered by German military authorities as chemical warfare agents.* Wood, and Grob and Harvey have discussed certain aspects of the action of such anticholinesterase compounds elsewhere(2,3). The structure of sarin and tabun is compared in Fig. 1 with di-isopropylfluorophosphate

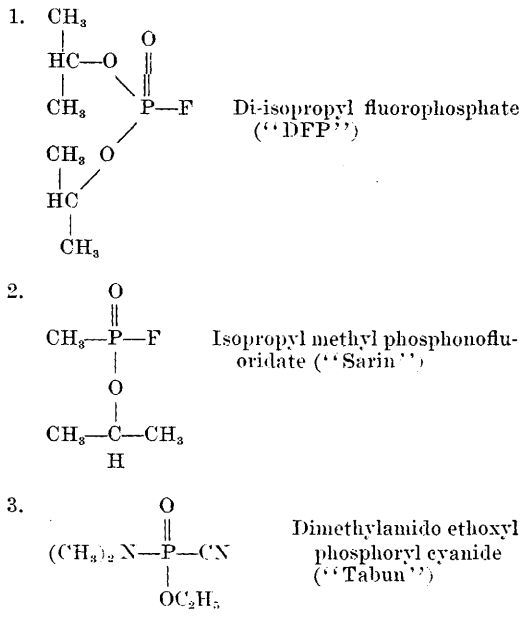

FIG. 1.

(DFP), another well known anticholinesterase compound. The compounds were dissolved in propylene glycol, and were administered in this form intravenously in all experiments summarized in this report.†‡

The following experiments were performed to define the mechanism of toxic action and lethal outcome in cats and dogs.

1. *Signs of poisoning.* After intravenous administration of 20 μg of sarin per kilo§ to cats and dogs, the animals became apprehensive and showed signs of respiratory distress in 15 to 30 seconds. The respiratory rate became somewhat accelerated, while the respiration became deep and apparently labored. Within the next 15 to 30 seconds, cyanosis began to appear, and approximately 30 seconds later, cyanosis became extreme. In experiments uncomplicated by anesthesia,‖ increasing signs of excitement occurred simultaneously and, particularly in cats, severe convulsions supervened after a period of marked unrest, variable in duration, interspersed with intervals of aimless, fearful running about. Miosis under these conditions was not seen, although under anesthesia miosis was seen before cyanosis appeared. Generally, intense salivation became evident at the time that severe respiratory difficulty (gasping) appeared. In cats, pilomotor activity was frequently seen.¶

* Earlier publication was not possible. Since completion of the work described here, Holmstedt(1), and De Candole(4) have published reports on tabun and sarin respectively, which include many of the observations presented here.

† The compounds were prepared in other laboratories of the Chemical Corps.

‡ In general, the effects of sarin and tabun were qualitatively similar in the dog and cat, sarin being from 5 to 10 times more potent; hence, only the effects of sarin are described in this report.

§ All doses here reported are per kilo of body weight.

‖ Since the central nervous system (CNS) effects of DFP may be suppressed by anesthesia, some observations on symptoms and signs of poisoning were required in unanesthetized animals to determine whether convulsant CNS effects of sarin and tabun exist to an extent presenting a problem in treatment of poisoning.

¶ In the monkey, excitement and convulsions were so fleeting as to appear negligible, and the animals passed quickly into a state of unconsciousness, flaccidity, and cessation of respiration. (Unpublished, recent observations, Loomis and Krop)

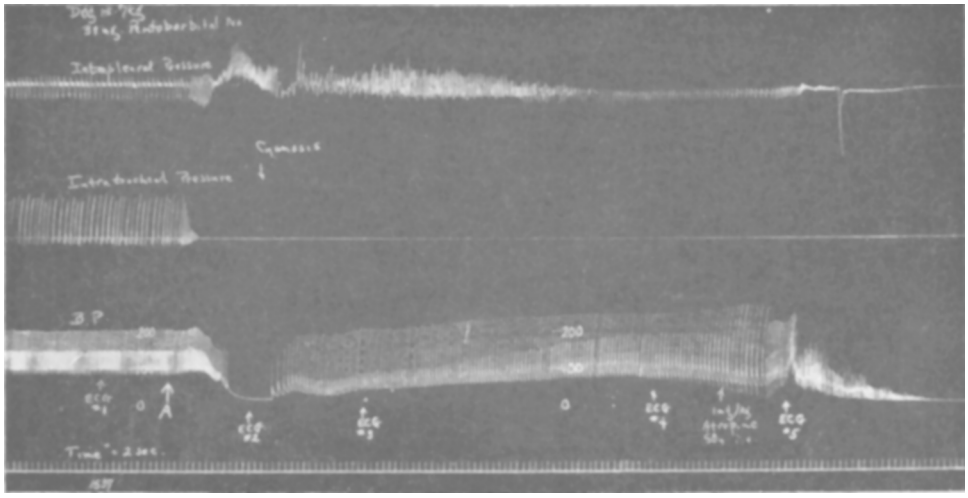

FIG. 2. Dog anesthetized with sodium pentobarbital. Changes in caliber of bronchial tree measured by simultaneous intrapleural and intratracheal pressure by means of rubber membrane tambours. Blood pressure recorded by means of a rubber membrane calibrated manometer.

Defecation and urination occurred. Death commonly followed in 5 minutes or less; often, animals surviving the first 5 minutes of poisoning ultimately recovered, showing general weakness for long periods. Prior to death, and starting with the excitement and convulsive phenomena, skeletal muscular twitchings appeared. These continued for some minutes after the convulsive seizures, and after respiratory and cardiac activity ceased. Death appeared to be primarily asphyxial in some instances and primarily cardiovascular in others; and in some cases, failure of both seemed to coincide.

These observations suggested that an obstruction of the respiratory tract plays an important part early in the picture of poisoning by these compounds. Indeed, at a time when the animals were capable of executing vigorous respiratory movements and the heart beat was palpable, cyanosis was observed to be extreme. Since phonation or stridor were absent, severe respiratory tract obstruction below the larynx was suspected. Considering the rapid development of the respiratory embarrassment, the hypersecretion of fluid in the respiratory tract did not appear to be the dominant or decisive factor in the presumed respiratory obstruction despite the eventual appearance of intense salivation in consequence of the known "para-sympathomimetic" properties of anticholinesterase agents. The more likely explanation for early respiratory tract obstruction resulting in cyanosis appeared to lie in the response of the bronchial, and perhaps other intrapulmonary, smooth musculature after intravenous administration of these compounds. Considering these facts, the following experiments were performed.

2. *Action of sarin on respiration.* Intrapleural and intratracheal pressure changes below the larynx were recorded in anesthetized (pentobarbital sodium) cats and dogs by means of tambours of requisite sensitivity by the method of Jackson. Simultaneously, a continuous record of carotid arterial blood pressure was made and electrocardiograms were taken at frequent intervals (Fig. 2). Fifteen to 20 seconds after intravenous injection of 10 μg of sarin, respiration became moderately increased in depth and rate. Intrapleural pressure between respiratory cycles tended to approach positive (atmospheric) pressure, while inspiratory (negative) and expiratory (positive) became greater, reflecting greater muscular effort applied to the thorax in intake and expulsion of air. During the succeeding 15 seconds or so, the intratracheal pressure changes in each respiratory cycle—a measure of the tidal volume—declined very rapidly, in 30 seconds became absent, and simultaneously cyanosis became severe. This

"bronchoconstriction" was seen most consistently and completely in the dog. Vigorous inspiratory and expiratory efforts (intrapleural pressure changes) continued for several minutes, or until death or reversal of effects by an antidote.** Evidence for "bronchoconstriction" was also obtained in anesthetized, curarized dogs under positive pressure artificial respiration, *viz.*, a progressive rise in intratracheal and a progressive fall in intrapleural pressures during each inflation cycle in consequence of sarin action.

3. *Action of sarin on the cardiovascular system.* Thirty to 60 seconds after intravenous injection of 10 to 20 μg of sarin per kilo, the arterial blood pressure record, taken with a calibrated rubber membrane manometer, showed a sharp, moderate rise in systolic pressure followed by a marked fall accompanied by slowing of the heart. Cardiac arrest often occurred, requiring ½ to one minute for "escape" of the heart to resume a rate of 20 to 30 beats per minute until death of the animal or relief of the bradycardia by an antidote. Despite this bradycardia, the mean arterial blood pressure often tended to rise above pre-poisoning levels during this period prior to death. The onset of the severe bradycardia was very commonly associated with the decline in the "tidal volume," but often bradycardia did not appear until the reduction in tidal volume was well advanced.

The electrocardiogram showed an initial slowing with normal rhythm, followed by progressive prolongation of the P-R interval and ultimately complete block, with disappearance of the P wave, when the ventricular rate became 20 to 30 per minute; the T wave showed inconstant changes in direction and degree, and the QRS complex became lengthened with frequent "notching" of the R spike. The electrocardiogram often continued at a low rate after the arterial pressure and respiration failed.

4. *Duodenal activity.* Activity of the duodenum of anesthetized cats and dogs was observed utilizing the hydraulic system of Krop and Loomis(5). Duodenal activity is extremely susceptible to the influence of these compounds, responding with increased tonus and rhythmicity to doses producing no discernible effects on respiration or circulation. Doses producing respiratory and circulatory effects resulted in intense spastic contraction of the duodenum generally within one to 2 minutes.

5. *Effects of atropine.* Control of the "muscarinic" and of certain central nervous system effects of di-isopropylfluorophosphate by atropine has been reported elsewhere(6-8). One tenth to one mg of atropine sulfate intravenously given prophylactically or after administration of sarin in the amounts here reported prevented or abolished the effects of sarin described in the foregoing. Particularly striking was the abolition of the excitement and convulsions in the cat. Although the excitatory effects on the nervous system, and the effects on respiratory obstruction and circulation were controlled by atropine to a life-saving degree when given after doses of sarin described here, death from larger doses of sarin was not prevented presumably due either to a paralytic action of sarin on the central nervous system or peripheral neuromuscular block of the respiratory apparatus (6). Occasionally, atropine precipitated fatal ventricular fibrillation when given late in the course of poisoning.

Discussion. Sarin and tabun exert marked "muscarinic" and "nicotinic" effects in cats and dogs in very small doses, presumably by virtue of inhibition of cholinesterases(1). The observations reported here suggest that the principal mechanisms of lethal action of these phosphate esters given intravenously to the cat and dog center on the lung, the circulation and the central nervous system. Any of these 3 effects alone appeared sufficient to result in a lethal outcome. The ventilatory resistance and the attendant cyanosis which develops early in poisoning appears to be chiefly due to contraction of the smooth musculature of the lower respiratory tract, *i.e.*, "bronchoconstriction," including possibly other intrapulmonary smooth musculature. These are simi-

** Recently, similar effects have been observed by us in unanesthetized dogs by means of a body plethysmograph (for respiratory tidal volume) and an esophageal balloon (to reflect intrapleural pressure changes), the latter being a technic applicable to similar problems in man.

lar to the effects of choline esters and other parasympathomimetic drugs described by others(9). The pattern of intrapleural pressure changes during the respiratory cycle indicated that in some instances, early in the course of poisoning, air may be trapped in the lung producing an "asthmatic" condition. That "bronchoconstriction" should occur following inhalation of high concentrations of sarin and tabun vapor seems certain, since this route of administration closely resembles intravenous administration in many respects, and provides for contact of pulmonary smooth musculature with high concentrations. Hypersecretion of fluid in the lower respiratory tract, though undoubtedly occurring, can hardly account for the great rapidity with which ventilatory resistance develops and with which it is relieved by atropine under the conditions of our experiments. The transient cardiac arrest and subsequent bradycardia could in themselves account for cyanosis in the early stages of poisoning, since the blood might not be circulated through the lung at a rate adequate to maintain a degree of hemoglobin oxygenation required to prevent cyanosis and tissue hypoxia. Convulsive seizures produce fatigue and by themselves prevent adequate pulmonary ventilation. Although the successful treatment of sarin and tabun poisoning rests fundamentally on the prevention of anoxia, the simultaneous control of all 3 effects described here must be achieved. For example, barbiturates prevent the excitatory and convulsive actions effectively, but "bronchoconstriction" is unaffected and death may be asphyxial nonetheless; similarly, if the cardiovascular effects could be counteracted without relieving ventilatory resistance, asphyxia would nevertheless also develop.

Artificial respiration by conventional methods (manual) may not be relied upon to bring about the recovery of an animal severely poisoned by these agents, even if depressed blood circulation could be disregarded, so long as severe "bronchoconstriction" persists. With a positive pressure method adequate for the task, there can be no assurance that patches of "emphysema" will not be blown into some portions of the lung leaving patches of "atelectasis" in others; further, secretions may be blown into the lung. Moreover, artificial respiration alone, no matter how effectively the alveoli may be ventilated thereby, will not wholly relieve the marked vascular stasis consequent upon the bradycardia. The aim in treatment should therefore be to assure an airway patent functionally, to assure that the circulation is not impeded, and to prevent the marked stimulation (convulsions and attendant interference with artificial respiration) and subsequent depression of the central nervous system. These requirements appear to be met within limits by readily tolerable doses of atropine, thus providing physiological conditions optimal for treatment of poisoning from these and other compounds similar in action such as the insecticide tetraethylpyrophosphate.

Summary. The phosphate esters sarin and tabun, potent anticholinesterase compounds, exert profound effects on the respiration, circulation, central nervous system and gastrointestinal tract of experimental animals.

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