FURTHER NOTE ON THE EXISTENCE OF A RESPIRATORY NEUROHORMONE

THIS note is a report on the progress of the study of a respiratory neurohormone described in this journal a few months ago.[1]

The respiratory stimulation in atropinized and in eserinized-atropinized dogs by acetylcholine injections occurs following removal of both adrenal glands at the time of experiment or several weeks prior thereto. Similarly, when the liver and abdominal viscera are excluded from the circulation by mass ligation or by clamping the abdominal aorta at the level of the diaphragm, respiratory stimulation results from injection of acetylcholine into the jugular vein. It follows that substances liberated from the adrenal glands or the liver are not necessary for the elicitation of the respiratory stimulation.

It has been repeatedly suggested that nor-epinephrine is a true sympathin. Various doses of this substance, from amounts producing no rise in blood pressure to amounts producing pressor effects up to 150 mm of Hg, have been injected intravenously. Respiratory stimulation was not observed in any of the animals, but, on the contrary, an epinephrine-like apnoea occurred.

In addition to acetylcholine, other choline derivatives—muscarine (nitrous acid ester of choline); mecholyl (acetyl-beta-methylcholine chloride) and doryl (carbamyl choline chloride)—have been assayed for their respiratory stimulating actions in atropinized animals. Muscarine and doryl in doses of 0.15 to 0.5 mgm per kilogram of body weight produced pressor effects and marked stimulation of respiration. Doryl in doses of 0.25 mgm or more per kilogram, produced after a brief stimulation a secondary depression of respiration, as judged by the ineffectiveness of acetylcholine in producing respiratory stimulation during this period. The pressor effect of acetylcholine is not abolished by doryl, but respiratory depression lasts for about twenty minutes. Recovery is gradual. Mecholyl, except in very large doses (2.0 mgm or more), does not produce respiratory stimulation. Atropine does not completely block the vasodilator effect of mecholyl, and because of this, in several animals mecholyl produced a stimulation of respiration due to fall of blood pressure. When this fall was prevented or diminished by the administration of additional atropine, no respiratory stimulation occurred following injections of 0.1 to 2.0 mgm of mecholyl. These experiments show that only choline derivatives with a nicotine-like, *i.e.*, ganglionic stimulant action, possess the property of respiratory stimulation, and that the choline group in itself is not the causative agent.

Nicotine, a ganglionic depressant in large doses, abolishes the respiratory stimulation not only of acetylcholine but also of doryl and muscarine. Ergotamine, which abolishes or reverses the pressor effect of acetylcholine in atropinized animals but does not interfere with the liberation of sympathin as a result of nerve stimulation, does not abolish the respiratory stimulating effect of acetylcholine.

We have already reported on the comparison of the effects of acetylcholine injections into the femoral vein and into the common carotid artery. Repetition of these experiments confirmed the previous observation that acetylcholine chloride, an acid salt, injected into the common carotid artery stimulates respiration in an occasional animal after a latent period of three to seven seconds. Recognizing the possibility of a direct stimulation of the chemoreceptors by the acetylcholine solution, we wish to emphasize that this stimulation is not identical with, and can be differentiated from, the panting response seen following intravenous injection of effective doses of acetylcholine. If effective doses of acetylcholine (0.3 to 1.0 mgm per kgm) are injected into atropinized animals via the common carotid artery one may observe: (*a*) A slight acceleration of respiration about five seconds after injection, and, (*b*) the usual panting response about fifteen seconds later. This sequence is interpreted to mean that the first phase is due either to direct stimulation of the chemoreceptors or to the elaboration of sympathins in the head region, and the second phase to liberation of sympathins following general ganglionic stimulation.

In nine pairs of dogs the attempt was made to transfer this hypothetical respiratory stimulant, by transfusion, from one to the other; the donor receiving atropine and eserine, and some of the recipients atropine. At the beginning of the respiratory response following acetylcholine injection into the donor, from 20 to 30 cc of its blood was rapidly removed from the carotid artery and immediately injected into the femoral vein of the recipient. In five recipients there was no stimulation of respiration following transfusion; in three animals there was a slight respiratory stimulation, and in one there was a marked stimulation of respiration.

Heymans *et al.*,[2] found an immediate and evanescent respiratory stimulation following intracarotid injections of 0.006 to 0.012 mgm of acetylcholine per kgm. We did not observe this phenomenon in all animals. Anitschkow[3] reported that several ganglionic stimulants produced reflex stimulation of respiration by way of chemoreceptors in the carotid body; following transient stimulation these stimulants depressed or paralyzed the chemoreceptors. Anitschkow believes that the chemoreceptors, the cells of the glomus caro-

[1] Koppanyi and Linegar, SCIENCE, 90: 141, 1939.

[2] Heymans, Bouckaert, Farber and Hsu, *Arch. Int. Pharmacodyn.*, 54: 129, 1936.

[3] Anitschkow; *Ibid.*, 55: 61, 1937.

ticum, are the equivalents of sympathetic ganglia or chromaffin cells. If so, it is not surprising that acetylcholine and other ganglionic stimulants excite these structures directly. Dautreband and Maréchal[4] and Wispelaere[5] studied the respiratory stimulating action of various choline derivatives. Wispelaere apparently errs in his statement that mecholyl is a respiratory stimulant, since his tracings reveal that the slight respiratory stimulation which he obtained is due to the steep fall of blood pressure.

We say, in conclusion, that we have shown the existence of a neurohormonal mechanism involving the excitation of the chemoreceptors in the carotid sinus region which, in turn, causes reflex stimulation of respiration. This is a mechanism which may be involved in respiratory stimulation not dependent upon increase in the alveolar carbon dioxide pressure or increase of fixed acids in the blood. In this connection the following paragraph is interesting:[6]

> . . . The pressure of carbon dioxide in the alveolar air is raised during severe exercise, as might be expected, otherwise there would be no stimulus to increase the ventilation; the amount of oxygen lack is usually insufficient to produce it. It has been shown, however, that administration of carbon dioxide in the inspired air can produce a greater rise in the alveolar carbon dioxide pressure than is ever produced in exercise; nevertheless, the ventilation rate is much less than that produced by even moderate exercise. It would appear, therefore, that besides the increase in alveolar carbon dioxide pressure, some factors, as yet unrecognized, may take part in increasing the ventilation rate during exercise.

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SARCOMATA AND CARCINOMATA INDUCED IN COTTONTAIL RABBITS BY METHYLCHOLANTHRENE[1]

THE advantages of the rabbit as a host in which to investigate cancer have become apparent as the result of the study of the carcinoma that follows Shope's virus-induced papilloma in both the domestic (Oryctolagus) and cottontail (Sylvilagus) rabbit.[2] As part of our investigation of this papilloma-to-carcinoma sequence, we attempted to produce malignant tumors in cottontails by the use of methylcholanthrene, a highly potent carcinogenic agent in mice. To this end, we brought the carcinogenic hydrocarbon into prolonged contact with epidermal and mesodermal tissues, with the result that both carcinomata and sarcomata have been produced. Since the rabbit has been generally regarded as comparatively refractory to the action of carcinogenic agents, we have thought it desirable to make our positive results immediately available to the numerous workers engaged in cancer research. Our findings will appear in detail in forthcoming papers. Meanwhile, it should be emphasized that the present note is concerned only with those of our animals which received methylcholanthrene (in its vehicle) alone, *i.e.*, we are not including herein any animals which received both carcinogenic agent and virus.

The cottontail rabbits studied fall into two groups. In Group A, 1 gm of methylcholanthrene was given to each of eleven animals. The carcinogenic agent was injected into each of four sites, two subcutaneous and two intramuscular, in amounts of 250 mg in 1 ml of tricaprylin. This pure saturated triglyceride was used because of Fieser's suggestion[3] that it was desirable to administer carcinogenic hydrocarbons in a solvent with known chemical characteristics. Of the eleven rabbits, six survived for 175 days or more, the rest dying of intercurrent infection or injury. Of the six survivors, five had soft-tissue sarcomas and one an epidermoid carcinoma. The diagnosis was established in each instance by autopsy and histopathological examination. The epidermoid carcinoma, which was discovered on the 176th day, developed at a site of subcutaneous injection. The sarcomata, which were present in rabbits autopsied on from the 225th to the 295th day after injection, were multiple. Metastases had occurred in three of the five animals.

Eight rabbits comprised Group B. Each animal received from thirty-five to forty-two applications of 1 per cent. methylcholanthrene in benzene on the inner aspect of each ear. This treatment extended over a period of from 166 to 220 days. When autopsied from 228 to 362 days after the first application, seven of the eight animals were found to have developed epidermoid carcinomata. In five of the seven rabbits, metastases had occurred—to the lymph nodes in two, and to both the nodes and lungs in three.

The findings described in the present note clearly show that methylcholanthrene is an effective agent for inducing sarcomata and carcinomata in the cottontail rabbit. Its action on the tissues of the domestic rabbit and other hosts is under investigation.

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[4] Dautreband and Maréchal, *C.R. Soc. Biol.*, 113: 76, 1933.

[5] Wispelaere, *Arch. Int. Pharmacodyn.*, 56: 363, 1937.

[6] Winton and Bayliss, "Human Physiology," 2nd edition, p. 181–182.

[1] This investigation has been aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

[2] For related bibliography, see Peyton Rous, "The Harvey Lectures," Williams and Wilkins Company, 1936, p. 74–115.

[3] Louis F. Fieser, *Am. Jour. Cancer*, 34: 37–124, 1938.