Correlation with the Sangamon interglacial meets with several difficulties: cool climatic conditions apparently prevailed throughout the St. Pierre interval. The length of the interval was certainly much shorter than that suggested for Sangamon time (about 100,000 years). For many reasons, it appears from the chronologic point of view that the St. Pierre interval is pre-Wisconsin but post-Sangamon in age. Flint's suggestive speculation (*4*, pp. 284–285) about a possible pre-Wisconsin but post-Sangamon glaciation and an interglacial period deserves mention. Although the present study lends support to Flint's hypothesis, another explanation seems more natural. The advance of the Wisconsin ice, like the recessional process, was not a continuous process but probably was also comprised of several glacial stages separated by nonglacial periods, some of considerable magnitude. It is quite possible that one of these fluctuations is represented by the St. Pierre interval. During the early stages of glaciation, no appreciable depression of the earth's crust (owing to the weight of the ice) would have occurred, and this may well account for the absence of a marine invasion of the St. Lawrence lowland at the beginning of the St. Pierre interval. This concept is also in accord with the stratigraphic observations made in the James Bay lowland. The St. Pierre interval seems to be a correlative of that represented by the nonglacial deposits along the Missinaibi River.

The facts at hand are insufficient to establish a revised chronology of late Pleistocene time in eastern North America.

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6 March 1957

Insect Spiracle as an Independent Effector

Recent studies of the spiracular control of discontinuous respiration in pupal silkworms have shown that the muscle serving the spiracle of *Hyalophora cecropia* is physiologically very different from any muscle heretofore encountered in insects (*1*), a fact confirmed by Case for the cockroach (*2*). The peculiar behavior of the spiracular muscle confers on the spiracular apparatus certain novel properties which are the subject of this report (*3*).

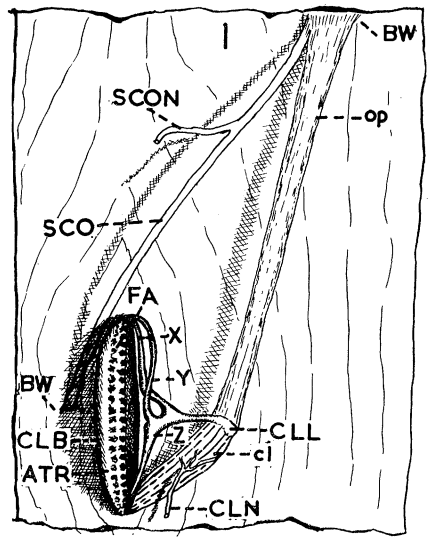

Fig. 1. Pupal spiracular regulatory apparatus as seen from inside the animal. No tracheae are shown. *ATR,* atrium; *BW,* body wall; *cl,* closer muscle; *CLB,* closing bow; *CLL,* closing lever; *CLN,* nerve of closer muscle; *FA,* filter apparatus; *op,* elastic opener; *SCO,* scolopophorous organ; *SCON,* nerve of scolopophorous organ; *X,* dorsolateral closing bar; *Y,* dorsomedian closing bar; *Z,* ventral closing bar.

The aperture of each of the 14 spiracles of a *cecropia* pupa is regulated by a valve. This valve is attached to a chitinous frame consisting of three bars, a bow, and a lever, and is moved by a single closer muscle (Fig. 1). When the muscle relaxes, the elasticity of the chitinous frame and the tension of an elastic ligament which opposes the closer muscle cause the valve to open. The muscle is apparently innervated solely by a nerve from the corresponding segmental ganglion and by a branch of the median nerve of the previous segment. Careful staining of more than 200 preparations with methylene blue and with the silver techniques of Palmgren (*4*) and Golgi (*5*) have failed to reveal nerve cell bodies imbedded in or near the muscle—that is, there is no peripheral ganglion. Histologically, the muscle itself revealed typical striations, a tremendous number of Doyere's cones, and a nerve fiber running under the sarcolemma (Fig. 2).

In the intact insect, so far as we can judge, all the spiracles open and close more or less in unison because of the synchronous relaxing of the closer muscles. When the central nerve cord was transected at any level, the spiracles anterior to the cut remained in synchrony with one another, while those posterior to the cut were also synchronous but independent of the anterior set. The central nervous system thus coordinates the spiracles. However, no coordinating center could be found in either the abdomen, thorax, or head, for no group of ganglia was indispensable for coordination. When a segmental ganglion was removed, thus denervating the corresponding spiracular muscle, the muscle remained contracted for more than 90 days, with an occasional uncoordinated opening. The muscle could be induced to relax by exposure to high carbon dioxide or low oxygen tensions, but it contracted again upon return to air. The stimulus for contraction of the closer muscle after it had been deprived of all connections to the central nervous system was not at all apparent, and to clarify this issue we compared closely the behavior of innervated and denervated muscles.

We began by severing the nerve leading from the central nervous system to a single spiracle through carefully placed incisions in the body wall, either close to the abdominal ganglion or close to the spiracular muscle. Spiracles denervated at either site behaved substantially the same, being somewhat less sensitive to carbon dioxide and oxygen than innervated spiracles. We next cut both the scolopophorous organ and the elastic opener (Fig. 1). This failed to interfere with either the coordinated activity of a normal spiracle or the autonomous activity of a denervated spiracle, and thus these two structures appear to play no indispensable role in spiracular activity. This was borne out in experiments with isolated spiracles and bisected pupae.

Spiracles were isolated with only a surround of pupal cuticle supporting the spiracular regulatory apparatus and placed on filter paper moistened with

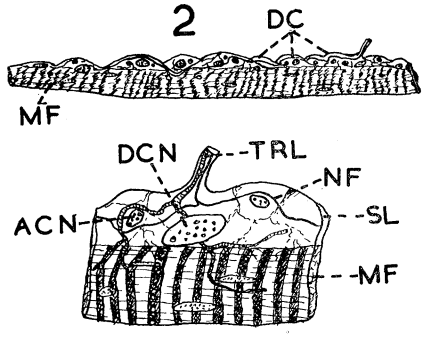

Fig. 2. (Top) Single muscle fiber from a pupal spiracular closer muscle showing the numerous Doyere's cones (×40). (Bottom) Enlargement of a piece of the muscle fiber to show details within one cone. *ACN,* accessory nucleus; *DC,* Doyere's cones; *DCN,* Doyere's cone nucleus; *MF,* muscle fiber; *NF,* nerve fiber; *SL,* sarcolemma; *TRL,* tracheole.

insect Ringer's solution in a glass chamber. When carbon dioxide was added, the isolated spiracles opened; when oxygen was added, they closed. That is, the isolated spiracles behaved in relatively normal fashion for many hours as independent effectors. In hanging drops of a suitable medium (*6*), they functioned for as long as 3 days.

Further experiments were performed on pupae bisected parasagitally in Ringer's solution so that one side had spiracles with intact connections to the central nervous system, while the other side had no connections to the central nervous system. When a stream of carbon dioxide was directed at a spiracle, it opened; when oxygen was applied, it closed. This was true both of spiracles connected to the central nervous system and of those not connected to it.

To see whether carbon dioxide was acting by virtue of its acidity, Ringer's solutions of different *p*H were added to the spiracle. When a spiracle was flooded with Ringer's solution of *p*H 5 or less, it opened; when basic Ringer's of *p*H 9 was added, it closed. Ringer's of intermediate *p*H gave variable results. Both innervated and denervated spiracles responded similarly to changes in *p*H. The *p*H's to which the spiracle responded were extreme compared with the normal *p*H variations expected in the insect. Therefore, it is unlikely that carbon dioxide is acting as an acid. Further evidence that *p*H does not serve as the normal stimulus for the spiracles stems from the fact that spiracles placed in a phosphate buffer at *p*H 7 continued to respond to both carbon dioxide and oxygen.

In all these experiments, the "receptors" sensitive to changes in gas and acidity appeared to be localized only in the region of the spiracle, for when these agents were applied locally to other parts of the pupa, particularly to parts of the central nervous system, the spiracles failed to respond.

The specific function of each part of the spiracular apparatus was studied in preparations in Ringer's solution. Parts of the apparatus were excised individually or in combinations, and the spiracle was stimulated. These experiments revealed that when the spiracular nerve was cut as close to the muscle as possible and the nerve stump carefully dissected out of the muscle, and when all the parts of the apparatus were removed except the naked bars, lever, and closer muscle, the muscle still responded to carbon dioxide and oxygen. It is clear that the autonomy of the spiracle is a function of the closer muscle: the muscle remains contracted without stimulation of the central nervous system, not for hours but for months. Furthermore, since repeated histological study has failed to reveal ganglion cells in or near the muscle, we must conclude that this muscle can contract without any nervous stimulation whatever—that is, the spiracle muscle is spontaneously excitable. This spontaneous excitability is reflected in electric recordings which showed that the denervated muscle continued to produce action potentials. Indeed, even when most of the muscle fibers were cut away, the few remaining fibers continued to produce rhythmic action potentials at a rate of 12 to 15 per second (*7*). In the intact insect, of course, the spiracular muscle is normally controlled by the central nervous system.

From these experiments we have concluded that, while the spiracles are normally coordinated by the central nervous system, they can behave like independent effectors sensitive to carbon dioxide and to oxygen. In the normal as well as in the denervated condition, the receptor mechanism sensitive to these gases seems to be located within the muscle itself. The spiracular muscle, so far as we are aware, is unique among insects and probably among higher animals in exhibiting sustained contractions in the apparent absence of nervous stimulation.

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New Pharmacoconvulsive Agent

In our investigations concerning the anesthetic action of aliphatic fluorinated ethers of low molecular weight (*1*) the anesthetic properties of Fluoromar (R) (trifluoroethyl vinyl ether) were observed. These appeared sufficiently promising to warrant a study of its effect on man (*2*). Further observations among these compounds revealed that perfluorodiethyl ether was devoid of anesthetic activity. On the other hand, hexafluorodiethyl ether ($CF_3CH_2—O—CH_2CF_3$) was found to elicit violent convulsions upon inhalation in many species of laboratory animals.

Hexafluorodiethyl ether is a colorless mobile liquid which emits a mild, pleasant ethereal odor. Its boiling point is 63.9°C, and its specific gravity is $1.41\frac{20°}{4°}$. It is very insoluble in water but soluble in alcohol.

White rats that were exposed to the vapor of hexafluorodiethyl ether in concentrations as low as 30 ppm (wt/vol.) convulsed violently within 30 seconds. There were marked clonic and tonic seizures, and there was some degree of emprosthotonus. The convulsions stopped promptly when the agent was removed from the inspired air. Repeated exposures on subsequent days did not appear to produce injury to the animals, as shown by studies of blood chemistry and by histological examination of the lungs, brain, liver, kidneys, and bone marrow.

The convulsive seizure is readily prevented in the rat, dog, and monkey by Pentothal Sodium, ether, or Fluoromar anesthesia, but not by mephenesin. Under Pentothal anesthesia, hexafluorodiethyl ether caused no change in the pattern of the electrocardiogram and only a slight depression in blood pressure and a mild tachycardia in dogs during the cortical dysrhythmia.

The compound did not produce hypoglycemia in the rabbit. Studies in dogs demonstrated that it did not inactivate cholinesterase.

Electroencephalographic studies in dogs and monkeys under succinylcholine-Pentothal Sodium anesthesia were performed. The inhalation of hexafluorodiethyl ether produced marked cortical dysrhythmia without involvement of the skeletal muscle. The multiple spike and three- to four-per-second slow-wave discharge which first occurs was soon followed by high-voltage multiple spiking similar to that of the myoclonic type *petit mal* epilepsy and to the cerebral pattern elicited by Metrazol injections.

We considered the agent as possibly useful as a pharmacoconvulsive drug for shock therapy in mentally disturbed patients. Among the factors which suggested its use were the apparent harmless nature of repeated exposures to animals, the rapid onset of the seizures, the ease of control of depth and duration of the seizures, and the similarity of the cortical dysrhythmia to that evoked by Metrazol. Accordingly, four patients who were suffering with mental disturbances in which electroshock therapy was indicated were subjected to inhalation of hexafluorodiethyl ether. The agent was placed in a plastic inhaler dispersed on cotton. The inhaler was of the type em-