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Curare as a Neuromuscular Blocking Agent in Insects

Abstract. *Topical application of curare produced no effects on electrical activity in single flight-muscle fibers of the fly* Sarcophaga. *However, the intra-abdominal injection of curare induced a neuromuscular block similar to that described for vertebrates. The general refractoriness of some insects to chemical agents may well be due to the method of application.*

The question of the effectiveness of curare as a neuromuscular blocking agent in insects has recently been reopened by the demonstration that solutions containing large doses of curare, when injected into the body of an insect, resulted in a "complete flaccid paralysis" (*1*). Although the conclusion was drawn that this represents a vertebrate-type response to curare—that is, a neuromuscular block—no direct evidence was presented. Since it has long been held that curare is without effect on neuromuscular transmission in insects, this paper reports the results of experiments designed to test the electrical response of a single muscle cell to curare.

The fly *Sarcophaga bullata* Parker was mounted ventral side down on a mound of Tackiwax and fastened to a lucite peg. The peg fitted into the bottom of a lucite chamber filled with a physiological salt solution (*2*). Two fine Ag–AgCl wires were inserted into the thorax and directed downward toward the central thoracic ganglion. Stimuli delivered to this area elicited action potentials in the dorsal longitudinal flight-muscles. The chitin covering the scutellum was excised, thereby exposing the flight muscles. Conventional glass-capillary microelectrodes were used to impale individual fibers for measurement of transmembrane electrical responses.

Curare was administered in two ways: first, by topical application to the surface of the exposed muscle fibers, and second, by intra-abdominal injection. The topical application of curare solution produced no observable effects over a period of 2 hours. Curare as the pure crystalline powder was sprinkled directly onto the surface of

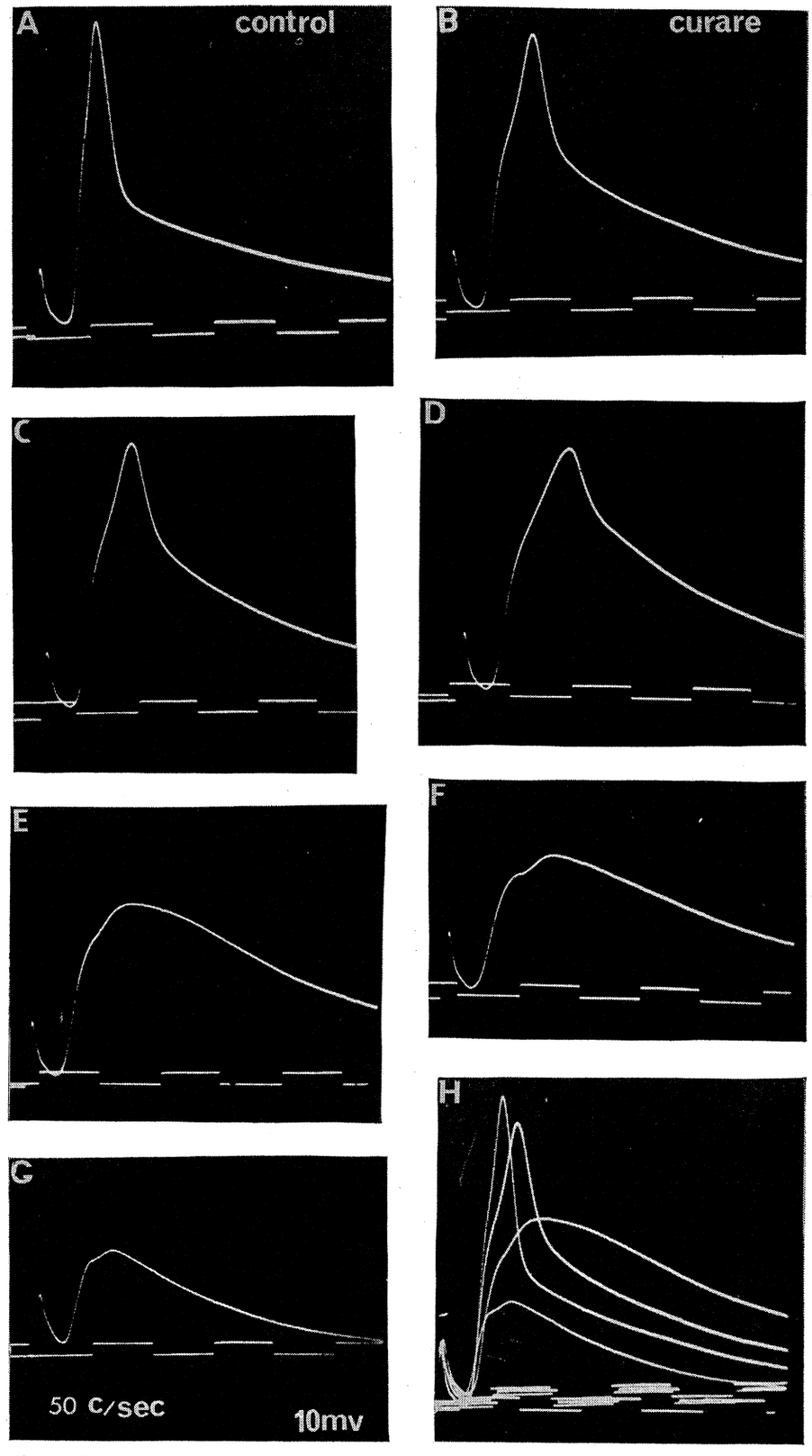

Fig. 1. The effect of curare on the muscle action-potential in a fly, *Sarcophaga bullata* Parker. (A) Control; (B–G) progressive decrement of intracellular response after intra-abdominal injection of curare; (H) composite of A, B, E, and G.

the muscles, and still there were no effects noted. A solution containing 3 mg of curare per milliliter was injected into the abdomen of the fly by means of a 31-gauge hypodermic needle coupled to a microliter syringe. Injection could be carried out without dislodging the recording microelectrode. This technique allowed continuous observation and recording of the curare effect. The complete sequence of events—that is, the control, the treatment with curare, and the recovery—could thus be recorded in a single fiber with the microelectrode undisturbed.

The injection of 0.01 ml of *d*-tubocurarine chloride solution (30 μg) into the abdomen of the fly resulted in a typical vertebrate-type neuromuscular block. The spike-like portion of the action potential gradually diminished in amplitude until only a small, slow, graded-type potential remained. The sequential effects of curare as a neuromuscular blocking agent are illustrated in Fig. 1. Figure 1A shows the normal intracellular response, and B–G show the progressive decrement effected by curare. The resting potential was not affected; it held steady and stable throughout the entire experimental period.

The effectiveness of curare as a neuromuscular blocking agent in insects is apparently related to the route of administration, and this factor may be a reflection of the penetrability of the substance into the target area. The basement membrane, a laminar structure similar to cuticle, invests many insect tissues and is proposed to serve as a diffusion barrier to ions and pharmocologic agents (*3*). The interposition of this tissue structure around the end-plate region as a restrictive factor to the passage of curare molecules appears to be circumvented by the intra-abdominal administration.

However, other possible explanations must be considered. Curare may combine with a substance from the tissues of the abdomen to form a complex intermediate, or it may combine with a component of the circulatory system to produce a substance specifically toxic at the end-plate region.

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Hemoglobin Freiburg: Abnormal Hemoglobin Due to Deletion of a Single Amino Acid Residue

Abstract. *Structural characterization of a new variant of human hemoglobin (adult), designated hemoglobin Freiburg, indicates the deletion of the valyl residue No. 23 from an otherwise normal beta-chain. The formula may be written* $\alpha_2\beta_2^{23\text{val}-0}$. *The abnormal hemoglobin is present with hemoglobin A in the proposita and in two of her three living children, but is not detectable in her parents. We postulate that this variant represents a triplet base deletion which most likely resulted from an unequal crossing-over between two normal beta-chain loci during meiosis in one of the parents of the proposita.*

Chemical and genetic studies of human hemoglobins (Hb) have contributed substantially to modern concepts of molecular biology. The discovery of Hb S by Pauling, Itano, Singer, and Wells (*1*) was the first direct evidence that an abnormal gene could alter the physical-chemical properties of a protein. Ingram's structural characterization of Hb S demonstrated that the genetic change responsible for this abnormal hemoglobin resulted in the substitution of one amino acid residue by another residue at a single site of the protein molecule (*2*). About 50 or more analogous examples of single amino acid substitutions have been observed among other abnormal human hemoglobins (*3, 4*). Evidence has recently been presented that two amino acid substitutions may be present in the β-chain of one abnormal hemoglobin, Hb Cf-Georgetown (*5*). This abnormality may have resulted from two single-point mutations affecting the same gene or from an intragenic equal crossing-over between two different homologous, mutant genes. In addition, evidence of intergenic unequal crossing-over has been obtained from chemical study of Hb Lepore (*6, 7*). We now describe chemical and genetic studies of Hb Freiburg, which is the first example of an abnormal protein in which the deletion of a single amino acid has been recognized. These studies support the theory that intragenic unequal crossing-over accounts for this mutation.

Hemoglobin Freiburg was discovered by Betke and co-workers (*8*) in a German woman in whom they found an associated hemolytic process and mild cyanosis. Figure 1 represents the family pedigree in which the proposita is represented as II-2. Both Hb Freiburg and slight cyanosis were also found in two of her three living children, III-3 and III-4, but the children showed no evidence of hemolysis. The abnormal hemoglobin represents from 27 to 32 percent of the total hemoglobin in the affected individuals. Except for 2.5 to 3.0 percent of Hb A_2, the remaining heme protein appears to be normal adult hemoglobin. A comparison of Hb Freiburg with other hemoglobins by starch-gel electrophoresis (*3*) is shown in Fig. 2. Its electrophoretic mobility is less than that of Hb A, greater than that of Hb S, and very similar to that of Hb F (normal fetal hemoglobin) at *p*H 8.1.

Hemoglobin Freiburg was not detected in the parents of the proposita, I-1 and I-2, nor in her three brothers, II-3, II-4, and II-5, nor in her oldest child, III-1. Her second child, III-2, died as a newborn from a hemolytic disease. Hemoglobin studies were not performed at that time. Analysis of blood groups does not exclude I-1 and I-2 as parents of the proposita, II-2.

The oxygen dissociation curve of blood containing Hb Freiburg is displaced to the left, an indication of greater than normal affinity for oxygen (*9*). This shift was thought to be due, at least in part, to the presence of about 10 percent of the total hemeprotein as methemoglobin. The spectral