The Cephalic Hook in Microfilariae of Dipetalonema reconditum in the Differentiation of Canine Microfilariae

THOMAS K. Sawyer,¹ ELLIS F. Rubin,² AND RONALD F. JACKSON³

The specific identification of microfilariae from dogs in the United States usually is made on the shape of the tail, the superficial appearance of the head, and on motility. More critical examination may include the R-cell patterns⁴ and measurements of the microfilariae (Newton and Wright, 1956; Wallenstein and Tibola, 1960; Lindsey, 1961; Sawyer et al., 1963). The present report describes the rediscovery of the cephalic hook in the microfilariae of Dipetalonema reconditum and outlines a staining technique to reveal this hook and to more clearly distinguish the R-cells in the microfilariae of both D. reconditum and Dicrofilaria immitis.

This study was initiated when a cephalic hook was observed on the microfilariae of D. reconditum.
ditum in unfixed blood films stained with brilliant cresyl blue, and the significance of the finding was determined when a search of the older literature led to an illustration of this structure by Fülleborn (1913). The commonly used methods of fixing and staining microfilariae for study usually do not reveal the cephalic hook, a fact which no doubt accounts for the lack of references to this structure in the more recent literature. Accordingly, it was thought worthwhile to bring this morphological characteristic to the attention of other workers and to present a method for demonstrating it. In addition to revealing this hook, the stain provided a method by which the R-cell pattern was easily distinguished.

**METHODS**

Microfilariae of *D. reconditum* were studied from fresh blood samples of seven dogs at NIH, and from formalin-treated sediments from six dogs shipped from other locations (California, Florida, Kansas). The microfilariae from these 13 dogs were identified on the basis of measurements obtained with the modified Knott technique of Newton and Wright (1956) or the saponin–formalin technique described in an earlier study (Sawyer et al., 1963). Microfilariae of *D. immitis* from four other dogs at NIH were identified using the same techniques. Microfilariae of both species were then studied on unfixed blood films stained with brilliant cresyl blue, as follows: (1) Thick blood films from blood taken from marginal ear vein were stored overnight at room temperature. (2) Dehemoglobinized in tap water or saline for 10 minutes or less (depending on thickness of individual films). (3) Films were transferred without drying, to 1:50 dilution of 1% brilliant cresyl blue (both stock and diluted stain are prepared in 0.8% NaCl). (4) Rinsed twice in saline and mounted in saline, cover slip sealed with melted vaseline–paraffin. (5) Films then examined with both high dry and oil immersion objectives.

If desired, such stained slides can be stored in petri dishes lined with moistened paper toweling for subsequent study.

The results obtained with the brilliant cresyl blue stain were compared to those obtained with several routine techniques. Photomicrographs were prepared using both Versaplan (Ansco) and high-contrast (Kodak) sheet film.
**RESULTS**

Unfixed microfilariae of *D. reconditum* on blood films stained with brilliant cresyl blue regularly showed the distinct cephalic hook illustrated by Füllborn (Text-Fig. 1-15b, Figs. 2-5). When the modified Knott technique was employed, the only distinguishing features were the narrow blunt outline of the head, the distinct excretory pore and cell (Figs. 6-7), the “inner body,” and usually the “button-hook” tail. With this technique the microfilariae were fixed simultaneously with the lysing of the blood and there was a pronounced contraction, or folding, of the cephalic hook. Only rarely could the folded hook be seen and then only with the most careful focusing on different levels of the organisms (Figs. 8-9); it certainly would not be readily seen in routine examination. When blood was lysed with saponin instead of formalin, the living unstained microfilariae could not be critically studied because they were too active and frequently were attached to clumps of leucocytes which masked the various structures.
However, as motility decreased with continued exposure to saponin or was arrested by slowly adding 2% formalin, the flexible hook was somewhat retracted but clearly visible in many of these specimens (Figs. 10–12).

As a result, freshly prepared Giemsa-stained thin films and such slides previously prepared from three dogs were carefully examined and reexamined for the presence of the hook, which might have been overlooked in previous studies. As with the formalin-lysed whole blood, the hook could be detected in about one specimen out of every five examined from all three dogs. The hook was poorly stained and difficult to distinguish; it could hardly have been recognized in routine examination.
Microfilariae of *D. immitis* similarly stained with brilliant cresyl blue did not show a cephalic hook. The cephalic structures of this species appeared as a cavity containing a cuticular disc surrounded by flexible lips (Figs. 13–14). A very fine barb appeared to be present on the disc of many but was not always clearly visible. When the Giemsa stain was employed for staining dried blood films, the cephalic features of *D. immitis* were unstained or lightly stained and only the clear cephalic space containing the “rote Mundengebilde” was discernible.

In sediments of *D. immitis* microfilariae from blood treated with the modified Knott technique the head was wide and tapered but details of the lips and cuticular disc were obscured; in these preparations the barb was never seen. In contrast to specimens of microfilariae of *D. reconditum* similarly treated, the excretory pore and cell were not distinct and the tail was wide for a greater portion of its length. Unlike the microfilariae of *D. reconditum*, the living unstained *D. immitis* microfilariae in saponin-treated blood were not usually attached to cell clumps or to the glass slide, and they rarely moved from the microscopic field.

Further examination of unfixed blood films stained with brilliant cresyl blue revealed that the R-cells of both *D. immitis* and *D. reconditum* were large and distinct in most of the specimens on any given slide. The complete group of four cells was detected in approximately 90% of the specimens of *D. immitis* (Fig. 15) but, due to the tendency of *D. reconditum* microfilariae to coil during drying, these cells could be readily distinguished in only about 50% of these microfilariae. Furthermore, the “clear cephalic space” described from routine films is not really clear. With the brilliant cresyl blue stain, distinct hook muscle cells which have been described by Taylor (1960b) can be readily seen in this area, particularly those of *D. reconditum* microfilariae.

**DISCUSSION**

The cephalic hook illustrated by Fülleborn (1913) and the hook muscle cells described by Taylor (1960b) are readily seen in almost every unfixed microfilaria of *D. reconditum* stained with brilliant cresyl blue. In methyl alcohol- or formalin-fixed preparations the cephalic structures are so shrunken that the presence of the hook can rarely be detected and the muscle cells are never seen. Since the microfilariae of *D. reconditum* are usually few in number and in the present series many slides were negative or with rarely more than two or three microfilariae per slide, a concentration technique is desirable. Fortunately, the hook may be readily seen in unstained microfilariae in saponin-lysed blood. Curiously enough, subsequent formalin fixation of microfilariae in saponin-treated blood does not appear to produce the same shrinkage of the cephalic hook which results when the modified Knott technique is employed.

No hook was seen in any of the *D. immitis* microfilariae, but a very fine barb was seen in the cephalic end of some of them. What relation, if any, this barb bears to the cephalic hook on *D. reconditum* microfilariae, or to the cephalic hook described in young embryos of both *D. immitis* and *Litomosoides carinii* developing *in utero* (Taylor, 1960a), is not evident at present.

The value of the cephalic structures in the identification of filarial parasites has received little attention apparently because routine fixing and staining methods may obscure these features. The illustrations by Fülleborn (1913) clearly indicate that microfilariae described in recent years should be reexamined employing unfixed specimens stained with vital dyes, or when possible by more specialized procedures as phase contrast microscopy (McFadzean and Smiles, 1956), ultraviolet microscopy (Taylor, 1960b, 1960c), and agar mounts (Taylor, 1960b; Esslinger, 1962). Recently, a large cephalic hook on microfilariae of *Brugia pahangi* was discovered by means of the agar pad (Esslinger, 1962) which was not included in earlier descriptions. Of seven species of *Brugia* (Ash and Little, 1964) the cephalic structures have been described only for *B. pahangi* (Esslinger, 1962) and *B. malayi* (Taylor, 1960b). Nelson described distinct hooks, or lancets, on the developing first-stage larvae (from insects) of *Dipetalonema mansoni-bahri* (1961) and *D. reconditum* (1962) which were detached with the first ecdysis. This structure on the developing larvae of *D. mansoni-bahri* described by Nelson (1961) appears
to be identical to the hook that we have seen on microfilariae of *D. reconditum* in fresh blood. This structure may be present but unrecognized in many species of microfilariae.

Because other unrecognized filarial infections may be present in dogs in the United States (Rothstein, 1961; Lindsey, 1961), it is important that microfilariae be examined carefully using various techniques. From the results obtained with both fixed and unfixed blood sediments and blood films, it is evident that morphological distinctions among microfilariae may be incomplete when only one method of examination is employed.

**Summary**

A staining technique for microfilariae using brilliant cresyl blue is presented which clearly demonstrates the cephalic hook of *Dipetalonema reconditum*, a morphological feature which is not readily discernible with the commonly used methods of fixing and staining. Microfilariae of *Dirofilaria immitis* stained by this method did not reveal a cephalic hook, but a fine barb was sometimes seen. The R-cells of both species were more distinct when stained with brilliant cresyl blue than when stained with the routine Giemsa stain. This staining technique offers a method for examining some of the morphological features of microfilariae that are often poorly stained and overlooked. The presence of the cephalic hook in microfilariae of *D. reconditum* and the absence of such a hook in those of *D. immitis* may offer yet another tool for the differential diagnosis of canine filariae.

**Literature Cited**


