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POSSIBLE HYGRORECEPTORS IN AEDES AEGYPTI (L.) AND BLATTELLA GERMANICA (L.)

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NINE FIGURES

Within the past few years the probable hygroreceptors in a number of arthropods have been localized and in some instances identified (Blumenthal, '35; Pielou, '40; Wigglesworth, '41; Steiner, '42; Lees, '43; Bentley, '44; Begg and Hogben, '46; Bursell and Ewer, '50; Roth and Willis, '51a, b; Perttunen, '51). Marcus ('48, '49a, b) has described certain sensilla, in moths, termites, and ants, which he claims to be hygroreceptors; however, no experimental evidence is presented to substantiate his views. Gunn and Cosway ('38) failed to localize the position of the humidity receptors in the oriental cockroach *Blatta orientalis*. Thomson ('38) was unsuccessful in his attempts to locate hygroreceptors in the mosquito *Culex fatigans*. Necheles ('25) suggested that "hair-like" appendages of the mosquito act like hair hygrometers, but he did not present any experimental evidence for this view. The present paper deals with experiments performed to localize and identify the hygroreceptors in the German cockroach *Blattella germanica* (L.) and the yellow-fever mosquito *Aedes aegypti* (L.).

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MATERIAL AND METHODS

The olfactometer used for determining the humidity reactions of insects has been described (Willis and Roth, '50). The slightly modified instrument (Roth and Willis, '51b) was used, in which the insects were presented with a choice between two air streams at 27°C., one a low relative humidity and the other a high relative humidity. The cockroaches were presented with a choice between 0% R.H. and 100% R.H. (theoretical values) only. The mosquitoes were presented with a choice between 75% R.H. and 100% R.H.; in addition female mosquitoes were also given a choice between 0% R.H. and 75% R.H.

The adult cockroaches used were reared on Purina dog food; their ages were unknown at the time of the test. The mosquitoes were two to three days old (after emergence) at the time of the test and had been kept supplied with water and honey solution. The sexes in all experiments with both species were tested separately. Mosquitoes were tested in groups of 20 while the roaches were tested in groups of 10.

The index of reaction (I.R.), a measure of the intensity of the humidity response, is the mean excess percentage of position records in the lower humidity of all tests for each experiment. An index for each test was calculated from the formula $100\ (D - W)/N$ (Willis and Roth, '50). An index value of 0% indicates no reaction; a positive index indicates a preference for the lower humidity, and a negative value indicates a preference for the higher humidity.

The method used to localize the positions of the hygroreceptors was to amputate various numbers of antennal segments and observe whether or not the removal of the structures caused a subsequent change in the behavior (i.e., in the index of reaction) of the insects. Antennal segments were cut off with scalpels made from small pieces of razor blades, while the insects were under carbon dioxide anesthesia. After the operations the roaches were placed in cloth-covered beakers containing dog food and a small cotton-stoppered

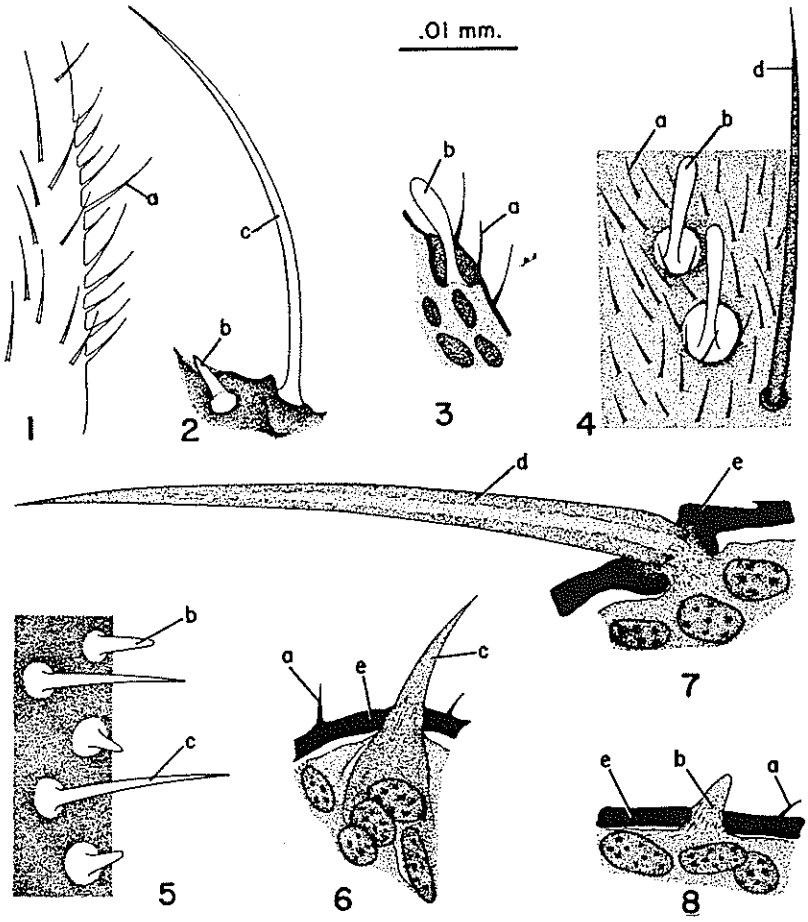
vial filled with water; the insects were tested two to three days after the operations. Following the operations on the mosquitoes they were placed in small jars, supplied with raisins and a moist pad of cotton, and kept in an incubator at 27°C.; these insects were given one day to recover from the operations prior to being tested in the olfactometer.

The antennae of both species of insects were studied as whole mounts and in histological sections. For whole mounts insect heads were treated with 20% KOH, dehydrated, cleared in xylene, and mounted in balsam. The heads of teneral adults were fixed in Bouin's, sectioned by the paraffin method, and stained with Delafield's haematoxylin and eosin.

RESULTS

Descriptions of the antennae. In both the mosquito and cockroach, the antennae were found to be necessary for the humidity response. The antennae and associated sensilla of both species of insects are described below.

Aedes aegypti. The antennae of the sexes differ markedly in mosquitoes. In both sexes the antenna is made up of 15 segments: a scape (segment 1), pedicel (segment 2), and 13 flagellar segments. Roth ('48) has described the antenna of the male. Each of the first 11 flagellar segments has a subapical or nearly medial whorl of long, stout, rigid, thick-walled fibrillae. The first flagellar segment bears scales, minute microtrichia, and several long thick-walled setae. Flagellar segments 2 to 11 are more or less similar to the first except that they are smaller, and larger portions of them are unpigmented and unsclerotized. The last two antennal segments differ markedly from the preceding segments in that they are considerably elongated and darkly pigmented, possess many thin-walled trichoid sensilla and a few small peg organs (fig. 2), and the whorls of thick-walled fibrillae are basal; flagellar segments 1 to 11 do not possess thin-walled sensilla. The sense cells of the thin-walled sensilla are intraepidermal. The microtrichia are merely cuticular



Figs. 1 to 8 Sensilla in *Aedes aegypti* and *Blattella germanica.*

1 to 4 *Aedes aegypti.*

1 Portion of the 5th flagellar segment of the male antenna (from whole mount).

2 Portion of the last segment of the male antenna (from whole mount).

3 Section through a basiconic sensillum on the last palpal segment of the female.

4 Surface view of a portion of the last segment of the female palpus (from whole mount).

5 to 8 *Blattella germanica.*

5 Surface view of a portion of the 10th antennal segment of the male antenna.

6 Section through a trichoid sensillum.

7 Section through a sensillum chaeticum.

8 Section through a basiconic sensillum.

Symbols: a = microtrichia; b = peg; c = trichoid sensillum; d = sensillum chaeticum; e = cuticle.

outgrowths and no innervation to them was seen in the histological preparations.

The antennae of the female differ considerably from those of the male in that each of the 13 flagellar segments bears many thin-walled trichoid sensilla and an occasional small peg organ, both structures similar to those found on the last two segments of the male's antenna (fig. 2). In addition to the thin-walled sense organs, the first flagellar segment of the female bears small, slender microtrichia and thick-walled setae near the apex; each of the other flagellar segments has several long, stout setae around the base of the segment. Except for a small unpigmented area around the base of flagellar segments 2 to 13 (i.e., at the joints), each of the segments is darkly pigmented. Smith ('19) figures only basiconic sensilla near the tip of the antenna of *Culex pipiens*.

Blattella germanica. It was difficult to determine the number of segments comprising the antennae of roaches because old individuals rarely have their full complement of segments; roaches may bite off the tips of their own antennae (cf. Gunn and Cosway, '38). The largest number of segments comprising any one antenna was 91. There are three types of processes on the antennal segments: (1) large, thick-walled, pigmented setae or sensilla chaetica (fig. 7); (2) thin-walled, sharply-pointed, trichoid sensilla (figs. 5, 6); and (3) some small, thin-walled, peg organs (basiconic sensilla) usually interspersed among the trichoid sensilla (figs. 5, 8). The trichoid sensilla are more numerous than the peg organs. The sense cells of the thin-walled sensilla (2 and 3, above) are intraepidermal, and it is very difficult to distinguish between the sensory and epidermal nuclei. The numbers of sensilla on antennal segments 3 to 13 are shown in table 1. It will be seen that whereas the thick-walled setae are present on segments 3 to 13 in both sexes, the thin-walled sensilla are not. In the females, thin-walled sensilla are absent from segments 3 to 8 but are found in small numbers on segments 9 to 11. They become more numerous on segment 12 and are present in fairly large numbers on all of the remaining flagellar seg-

ments. In contrast, the thin-walled sensilla of the male are absent from segment 3, present in very small numbers on segments 4 and 5, and are found in larger numbers on segments 6 and beyond. The difference between the sexes in antennal segments 10 to 13 is shown in figure 9.

The humidity behavior of B. germanica and A. aegypti. The undesiccated, unstarved German cockroach gave a dry

TABLE 1

Number of sensilla on antennal segments 3 to 13 of Blattella germanica

SEGMENT NUMBER	MEAN [1] NUMBER OF SENSILLA ± STANDARD ERROR			
	Sensilla chaetica		Thin-walled sensilla [2]	
	Males	*Females*	*Males*	*Females*
3 [3]	9.0 ± 0.7	11.8 ± 0.7	0	0
4	5.8 ± 1.2	4.7 ± 0.3	0.4 ± 0.2	0
5	7.4 ± 0.6	4.9 ± 0.5	2.7 ± 0.8	0
6	7.5 ± 0.3	6.2 ± 0.1	9.2 ± 1.4	0
7	9.2 ± 0.7	6.6 ± 0.2	9.1 ± 1.4	0
8	9.9 ± 0.9	8.8 ± 0.5	11.5 ± 1.0	0
9	12.4 ± 0.3	11.2 ± 0.4	10.4 ± 1.2	0.02 ± 0.1
10	12.2 ± 1.0	11.5 ± 0.4	15.3 ± 1.6	1.4 ± 0.3
11	11.5 ± 0.6	13.2 ± 0.4	25.6 ± 0.7	4.4 ± 0.6
12	13.4 ± 0.7	16.5 ± 1.0	33.8 ± 1.4	11.9 ± 1.3
13	13.8 ± 0.8	19.1 ± 1.0	33.6 ± 1.9	14.6 ± 1.6

[1] The sensilla on both antennae of each of 5 males and 5 females were counted.

[2] The numbers of these sensilla were determined by counting the round clear areas which indicate the insertions of the sense hairs (see fig. 5); the sensilla are for the most part trichoid but some of them are peg organs.

[3] Segment 3 is the first segment of the flagellum. Segment 1 is the scape and segment 2 the pedicel.

reaction (i.e, preferred the lower humidity) when presented with a choice of 0% and 100% R.H. The effect of the removal of various numbers of antennal segments on the index of reaction is shown in table 2. In the females the response was eliminated when only 12 antennal segments remained on each antenna, while a significant response was obtained when 13 segments were left on the antenna. In the males the response was eliminated when only 10 seg-

ments remained on each antenna, while a significant response was obtained when 11 segments were left on the antenna.

When females of *A. aegypti* were given a choice between 0% and 100% R.H., their behavior was very erratic; the in-

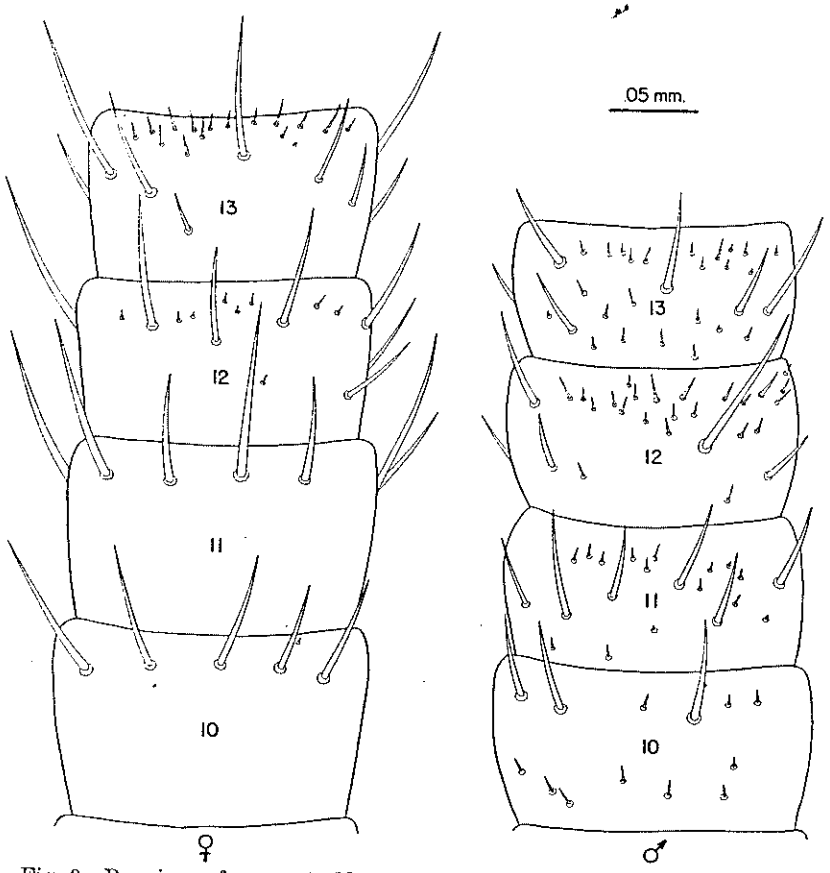


Fig. 9 Drawings of segments 10 to 13 of the antennae of *Blattella germanica*. Note the difference in distribution of the small thin-walled sensilla (among the large, thick-walled sensilla chaetica) between the sexes. Numerals indicate segment numbers.

sects were almost continually in flight and it was impossible to make visual counts of their distribution. Thomson ('38) found that *Culex* avoided both high and low humidities which may explain the erratic behavior of the females of *A. aegypti;*

the insects in attempting to avoid both the 0% and 100% relative humidities were almost constantly in motion. However, males of *A. aegypti* showed a dry reaction when given a choice between 0% and 100% R.H. with an I.R. of 47 ± 2.8% (4 tests). When presented with a choice of 75% and 100% R.H., both sexes showed a dry reaction. The effects of amputation of various antennal segments on the index of re-

TABLE 2

Effects of symmetrical amputations of the antennae at different levels, on the humidity reactions of Blattella germanica

NUMBER OF SEGMENTS REMAINING ON EACH ANTENNA	INDEX OF REACTION ± STANDARD ERROR WHEN THE INSECTS WERE GIVEN A CHOICE BETWEEN 0% AND 100% RELATIVE HUMIDITY		NUMBER OF TESTS FOR EACH SEX
	Males	*Females*	
75 ± 1.4[1] ♂	66 ± 7.2	...	5
70 ± 0.9 ♀	...	67 ± 7.8	5
13	37 ± 5.5	26 ± 2.3	5
12	30 ± 7.5	9 ± 9.3	5
11	23 ± 8.0	8 ± 5.5	5
10	3 ± 6.4	4 ± 2.9	5
1	9 ± 6.0	−1 ± 3.1	5

[1] Mean ± standard error based on counts of the segments of all the roaches tested (i.e., 100 male antennae and 100 female antennae). Those insects which had antennae that measured 10 mm or more in length were selected for these tests; these are considered to be the control group. After testing the humidity response of the insects they were killed, their antennae mounted in glycerine, and counts were made of the number of segments comprising each antenna.

action, when these latter humidities were employed, are shown in table 3. In both sexes hygroreceptors apparently are located on the antennae. The humidity response was eliminated when all of the flagellar segments were removed, but a significant response was elicited when only the first flagellar segments were left on the antennae. Two other experiments, in addition to those shown in table 3, were performed in which females were given a choice between 0% and 75% R.H. Unoperated females (control) when presented

with these two humidities gave an I.R. of $-54 \pm 1.5\%$ (5 tests)showing a preference for the higher humidity. When the flagellar segments from both antennae were removed (5 tests), the response was eliminated (I.R. of $2 \pm 1.7\%$).

TABLE 3

Effects of symmetrical amputations of the antennae at different levels, on the humidity reactions of Aedes aegypti

NUMBER OF FLAGELLAR SEGMENTS REMAINING ON EACH ANTENNA	INDEX OF REACTION ± STANDARD ERROR WHEN THE INSECTS WERE GIVEN A CHOICE BETWEEN 75% AND 100% RELATIVE HUMIDITY		NUMBER OF TESTS FOR EACH SEX
	Males	*Females*	
13 (control)	64 ± 1.4	51 ± 4.6	5
12	55 ± 1.0	...	5
11	48 ± 6.5 [1]	...	5
5	40 ± 5.7	47 ± 2.2	5
3	...	36 ± 2.9	5
2	33 ± 2.4	30 ± 2.4	5
1	14 ± 2.2	27 ± 2.0	5
0	1 ± 1.4	1 ± 2.5	5

[1] One experiment (5 tests) was performed in which the last two antennal segments were removed and, in addition, the stout fibrillae from the remaining flagellar segments were removed with a camel's hair brush (Roth, '48, p. 319); these insects gave an I.R. of $39 \pm 3.5\%$ which is not significantly different from the response of the males which lacked the last two segments but had the fibrillae intact.

DISCUSSION

Frings and Frings ('49) noted that when both labial and maxillary palpi were removed from water-satiated German roaches, the insects could no longer find sugar solutions. However, if the roaches were thirsty they recognized water ". . . apparently through some water-vapor sense." This water-vapor sense has been shown, in the present experiments, to be localized in the antennae. In *B. germanica* the differences in behavior between the sexes can be correlated with the distribution of the thin-walled sensilla. Thus in the males the humidity response was elicited when 11 or more segments remained on the antennae while the females required 13 segments to give a significant response. Actual counts of the

sensilla have shown that the thin-walled sense organs on the basal antennal segments are more numerous in the male than in the female; in the female they are, for the most part, completely absent from the first 10 segments. The insects failed to respond as a result of the removal of certain segments even though some trichoid and basiconic sensilla remained on the antennae (on segments 10–12 in the female and segments 5–10 in the male). This lack of response does not necessarily eliminate these thin-walled sense organs as hygroreceptors for one possible explanation is the assumption that the number of sensilla that remain on the antennae is less than that required to mediate a response (cf. Pielou, '40; Roth and Willis, '51b). However, if this is the case, then the threshold number of sensilla required to mediate a response is less for the female than for the male (between 18–32 thin-walled sensilla on each female antenna and between 59–84 on each male antenna; see table 1).

The gradual reduction in the index of reaction with the progressive removal of antennal segments of the yellow-fever mosquito and the elimination of the response upon removal of the flagella of both antennae show that the humidity response is dependent upon the presence of the flagellar segments of the antennae in both sexes. Frings and Hamrum ('50) studying the contact chemoreceptors of this species stated that if the antennae of the female possess water-vapor receptors at all, they are not the sole loci of these receptors. Their method of testing (local stimulation of mosquitoes mounted on wax blocks) was quite different from that used by the present workers. The response (reaching with the proboscis for a moist object brought nearby) used by Frings and Hamrum, however, was reported to be somewhat difficult to interpret at times because of other confusing responses given by the females.

In the female *A. aegypti* the humidity behavior can be correlated with the presence of the thin-walled sensilla which are found on all of the 13 flagellar segments. It has been shown that in some arthropods two types of receptors may be pres-

ent which mediate a wet and a dry reaction respectively. These two types of receptors may be located on different parts of the body of the animal (Bursell and Ewer, '50; Begg and Hogben, '46; Roth and Willis, '51b) or on the same appendage (Roth and Willis, 51a, b). Since both the dry reaction (when the mosquitoes were given a choice between 75% and 100% R.H.) and the wet reaction (choice between 0% and 75% R.H.) were eliminated when the flagellar segments of the female antennae were removed, receptors for mediating a dry and a wet reaction are located on the antennae. Whether the receptors for mediating both the dry and wet reactions in mosquitoes are the same is unknown. Thin-walled, basiconic sensilla, which differ in size and shape from those found on the antennae (cf. figs. 2–4), are present on the palpi of females of *A. aegypti.* These palpal sensilla apparently are not involved in hygroreception since mosquitoes with intact palpi did not react to the choice of humidities after the flagella of the antennae had been removed. In *T. castaneum* the maxillary palpi bear peg organs which differ in appearance from those on the antennae and which mediate a dry reaction; the antennal receptors in this species mediate a dry or a wet reaction depending on the physiological state of the insect (Roth and Willis, '51b).

The humidity reaction of the males of *A. aegypti* cannot be correlated with the distribution of the thin-walled sensilla since these sense organs are found only on the last two segments. However, that the last two segments take part in hygroreception is indicated by the fact that there was a significant reduction in the intensity of the reaction when these two segments were removed. It is unlikely that the large thick-walled fibrillae which form the whorls are hygroreceptors. Removal of practically all of the antennal fibrillae plus the last two segments resulted in a response which was not significantly lower than that shown by insects which had their last two segments removed but with their fibrillae left intact; if the fibrillae were hygroreceptors one would expect a significant reduction in the response after their

removal. Aside from the trichoid and basiconic sensilla on the last two segments of the male antennae there appear to be no other sense organs of the chemoreceptive type which might be considered as hygroreceptors. The minute spicules (microtrichia), so numerous on flagellar segments 1 to 11, are outgrowths of the exoskeleton and no innervation to them was seen.

No receptors have been found to be associated with the humidity reaction of land isopods. Waloff ('41) suggested that in these arthropods loss of water through the thoracic appendages and the consequent concentration of the body fluids might initiate the humidity reaction. In this connection Edney ('51) has shown that in isopods the rate of water loss from the pleopodal area is much greater than from other parts of the body. Lees ('43) could not associate the humidity response of wireworm larvae with any particular type of sense organ though it was shown that the antennae, and maxillary and labial palpi were necessary for the normal response. Lees suggested that the humidity reaction was initiated by loss of water through these head appendages, particularly through the unsclerotized regions, and that local changes in osmotic pressure might be sufficient to stimulate the sensory nerves directly. Perhaps a similar sort of mechanism is involved in the response of the males of *A. aegypti;* it will be recalled that large portions of flagellar segments 2 to 11 are thin, unpigmented, and unsclerotized, and it is possible that water could be lost or gained through these thin membranous areas of the segments. Though the first flagellar segment of the male is largely pigmented and sclerotized, a small zone near the apex (below the whorl of fibrillae) is unpigmented and unsclerotized (Roth, '48, fig. 6 A): males with only this segment remaining on each antenna gave a weak but significant reaction. It might be argued that the mechanism of loss of water through unsclerotized portions of the antenna could also apply to the females, in which case the thin-walled sensilla need not be involved. However, the only unsclerotized areas on the female antenna are com-

paratively very small and are present only at the joints of the segments; when one flagellar segment was left on each antenna of the female, the insects gave a response which was twice as intense as that of similarly operated males, even though the thin, unsclerotized portions of the female antennae had been eliminated.

SUMMARY

1. The sensilla on the antennae of males and females of the German roach, *Blattella germanica,* and the yellow-fever mosquito, *Aedes aegypti,* are described. Possible hygroreceptors in these insects have been localized through studies of the humidity reactions of the insects in an olfactometer, together with morphological examinations of the antennae.

2. The antennae in both species of insect were shown to be the sites of the water-vapor sense. The humidity reactions of both sexes of *B. germanica* and females of *A. aegypti* can be correlated with the distribution of the thin-walled sensilla (trichoid and/or basiconic) on the antennae.

3. The humidity reaction of the males of *A. aegypti* cannot be correlated with the distribution of thin-walled sensilla. It is suggested that the humidity reaction, in this case, is initiated by the loss or gain of water through the thin, membranous, unsclerotized portions of the antennal segments.

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