position, their average intervals between flicks are 0·94, 5·2, 5·3 and 12 sec. for R.N.W., L.C.T., P.B.W. and R.S.L. respectively. The posture change has little effect on the duration (0·02–0·03 sec.) and magnitude (2–25′) of the flicks. The frequency and character of the blinks (for example, *B*, Fig. 3*L*) are apparently unaffected by the alteration of the subject's position.

The increase in the magnitude of the head movements on sitting up is in accordance with earlier observations[3], and is not surprising in view of the fact that greater muscle tone is associated with the upright than with the supine position. Moreover, for the sitting-up position, the head was not supported at the back as it was in the supine case; it is therefore the more difficult to understand why, in the upright position, the greatest displacement occurs sideways. That a change of posture should have an effect on the eye movements is to be expected; for the extra-ocular muscle tensions are influenced by labyrinth impulses and the stimulation of the otoliths is different for the supine and upright positions. It is not immediately obvious why, in the case of R.W.G.H., change in posture has no marked effect.

I am very grateful to Dr. W. D. Wright and Mr. R. S. Longhurst for recording my observations and for acting as subjects; I wish to express my thanks also to the other subjects: Dr. L. C. Thomson and Messrs. R. W. G. Hunt, P. B. Watt and R. N. Wilson. I gratefully acknowledge the continued financial support of the Medical Research Council.

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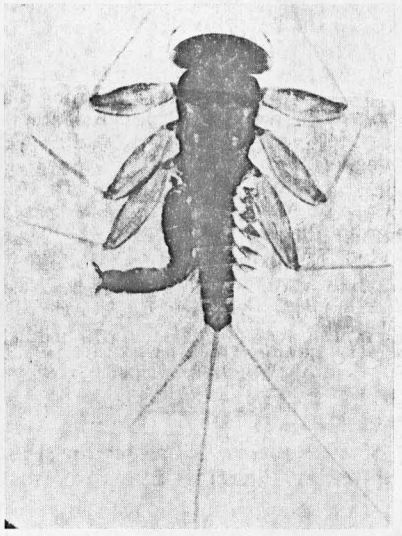
Phoretic Association between *Afronurus* and *Simulium* Species, and the Discovery of the Early Stages of *Simulium neavei* on Freshwater Crabs

During the course of routine sampling of the gravel bottom fauna of the Sagana River (Kenya Colony), at an altitude of 5,865 ft. in connexion with fishery research on trout foods, it was noticed in 1948 that many nymphs of the ephemeropteran *Afronurus peringueyi* (E.-P.) had *Simulium* larvæ and pupæ attached to their backs. Examination of these pupæ by one of us (J. McM.) showed that they were of a species not hitherto described; and this species is not apparently found anywhere else in the river. 49 per cent of all *Afronurus* collected from all parts of the river between 5,900 and 5,600 ft. were found to have *Simulium* larvæ attached; pupæ are much more scarce.

The *Simulium* larvæ are always found in the same position, attached by the anal sucker to the side of the ephemerid nymph just at the base of the coxa of the hind leg, either on the right or left side. In captivity, the *Simulium* larvæ have been seen to move over the surface of the nymph in the typical 'looping' fashion; but they always come to rest in the position described with the head and body pointing backwards over the gills of the nymph (see photograph). Pupation occurs on the dorsum of the nymphal thorax, with the pupal filaments pointing backwards over the body of the host.



Living *Afronurus peringueyi* nymph, with *Simulium* sp. larva attached. × 9

It proved difficult in the laboratory to rear this interesting association; but adults of the *Simulium* species have now been bred out and will be described elsewhere. A most interesting feature of this association, which appears to be a phoresis, is the mutual timing of the various nymphal and larval stages. During the moults of the *Afronurus* nymph, accomplished in the usual manner by splitting along the dorsum, the *Simulium* larva may become detached for a short while until the moult is complete, and then re-attaches itself to the newly moulted nymph in the same position; or alternatively, the *Simulium* larva remains attached to the old nymphal skin, and then transfers itself to the newly moulted nymph when the moult is almost complete.

The *Simulium* larva always pupates after the *Afronurus* has completed its last nymphal moult; when the eclosion of the *Afronurus* sub-imago takes place, and the nymphal skin is finally cast off, the *Simulium* adult also emerges from the pupa. The emergence of both adults takes place in the hours of darkness within an interval of one or two hours. The ovipositing habit of the adult *Simulium* is not known.

The adults of this *Simulium* species which have been bred are so remarkably like the adults of *S. neavei*, the vector of onchocerciasis in the western parts of Kenya Colony, that they may easily be mistaken for it at first glance. Minor differences are, however, apparent, and no true *S. neavei* adults have been caught in the vicinity of the Sagana River. The resemblance, however, is so close that it immediately suggested that the early stages of *S. neavei*, hitherto undiscovered in spite of intensive searches in Kenya and elsewhere, might be found to have this unsuspected habit also.

It was known that *S. neavei* could be eliminated from an infested area by dosing the rivers and streams with DDT emulsion, a fact indicating that the early stages were to be found in such rivers and streams. One of us (J. McM.) therefore proceeded to Ngoina, near Kericho (Kenya Colony), in February 1950 in order to carry out further investigations. This area is a focus of onchocerciasis, and *S. neavei* adults are very numerous.

Catches made over a period of years indicated that *S. neavei* adults seem to prefer to live near the smaller rivers and streams, and accordingly work was begun on a small river passing through heavily wooded country, and having many small cascades and waterfalls in its course. Large numbers of the younger stages of various insects, as well as crabs and fish, were examined during a period of two weeks for evidence of such an association, with negative results. However, searches in two of the larger rivers, the Kipsonoi and the Sondu, soon produced evidence that there is a definite association between fresh-water crabs (*Potamonautes* (*Potamon*) sp.) and a *Simulium* sp. As many as ten larvæ have been found attached to the carapace of one crab, and the maximum number of pupæ observed was three. Adults bred from these pupæ appear to be *S. neavei*, and the male and pupa will be described in detail at a later date; the pupal respiratory organ has eight long slender breathing filaments, equal in length to the cocoon. The female has already been described by Roubaud.

The association between crabs and this *Simulium* species coincides with the distribution of *S. neavei* adults, whereas that between *Afronurus* and *Simulium* species seems to occur at a higher altitude well outside the *S. neavei* belts. Only one specimen of *Afronurus* collected in the Kipsonoi was found to have a pupa attached, and not more than twelve were collected in the Sondu during a period of six days; but in the higher Kiptiket River, 20 miles away and some 300 ft. higher, twenty nymphs with pupæ attached were collected during a period of two hours.

The crabs concerned seem to prefer to live in the rockier parts of the rivers, such as cascades, and no specimens have yet been caught on mud or other soft river-bed material.

Simulium–Afronurus associations have now been found in several Kenya rivers, and the whole problem is still the subject of study in all its aspects. Dr. G. Marlier, of I.R.S.A.C., Belgian Congo, has informed one of us (V. D. v. S.) *in litt.* that he has found similar *Simulium–Afronurus* associations in rivers in the Kivu Province of the Congo, apparently two species of *Simulium* being involved; Dr. Marlier is publishing a note on this subject elsewhere.

In conclusion, we wish to thank Mr. Hugh Copley, fishwarden, Kenya Colony, and Dr. T. Farnworth Anderson, director of Medical Services, Kenya Colony, respectively, for permission to publish this joint note.

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Sulphamic Acid as a Test for Nitrate-reducing Bacteria

SULPHAMIC acid (aminosulphonic acid) decomposes nitrites quantitatively[1] with evolution of nitrogen gas according to the equation

$$NH_2.SO_3H + MeNO_2 \rightarrow MeHSO_4 + H_2O + N_2.$$

The reaction, which is very specific and strongly catalysed by hydrogen ions, may be used for the removal of interfering nitrites, for example, when testing for nitrates[2], or in water analysis as part of the Winkler method for dissolved oxygen determinations, where nitrites will cause serious error unless removed. For this latter purpose, according to Cohen and Ruchhoft[3], sulphamic acid possesses distinct advantages over sodium azide.

It seemed worth while investigating whether sulphamic acid had any value in bacteriology as a reagent for detecting nitrites in the nitrate-reduction test, for which it would appear it has not hitherto been used. Preliminary experiments to ascertain the limits of sensitivity of the reagent were carried out using a solution of sulphamic acid prepared according to the directions of Cohen and Ruchhoft (*loc. cit.*). This is a 4 per cent solution in 20 per cent v/v sulphuric acid. Standard solutions of nitrite were prepared from silver nitrite. The following technique was used. 1 ml. of standard nitrite was pipetted into a small test tube (7·5 cm. long by 8 mm. internal diameter). The tube was slightly inclined and 1 ml. of the sulphamic acid solution was pipetted down the side. By this method water blanks showed no bubble formation. In the test solutions gas bubbles were readily seen in concentrations of nitrite down to 5 p.p.m. (expressed as nitrogen).

One hundred cultures of catalase-positive cocci were tested for nitrate reduction both by the sulphamic acid technique outlined above and also by the usual modification employing the Griess–Ilosva reagents (sulphanilic acid and α-naphthylamine in acetic acid solution). All the cultures were examined by one test before the second test was applied. In this way, the result of one test on an individual culture was not known before applying the second test, thus avoiding any prejudgment of the result. The records showed that in each of the sixty-two cultures in which a positive result was noted with the Griess–Ilosva reagents, evolution of gas was observed with the sulphamic acid solution. No gas bubbles were seen in the remaining cultures.

A full account of the work and a critical survey of the two methods will be published elsewhere.

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April 20.

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Effect of Aspartic Acid on Growth of Plant-Virus Tumour Tissue

RECENTLY several workers have concluded that, under certain conditions, amino-acids can be assimilated intact by yeasts[1,2]. White and Munns[1] conclude that aspartic acid can be used as a source of nitrogen by yeast, and also that the carbon skeleton can be used if fermentable sugars are present (probably as energy sources). They found that when aspartic acid is added to the test medium in sufficient amount, the growth of yeast greatly exceeds that when only hexose sugars are present.

Results of this nature have been found by Nickell and Burkholder[3] working with virus tumour tissue from the sorrel plant (*Rumex acetosa* L.)[4,5] grown *in vitro*. It was found that aspartic acid could serve, to a certain extent, as a carbon source for the growth of this tissue. Nitrate was found to be the best source of nitrogen; but aspartic acid,