PHYSICAL FACTORS IN MOSQUITO ECOLOGY.

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Whilst it has been the common experience of all field workers on Malaria from the original investigations of Stephens and Christophers (1902) that the various species of *Anopheles* have different breeding places, and that in one single breeding place a succession of species may be found at various seasons, the chemical and physical factors that cause such phenomena were not investigated until the pioneer work of MacGregor (1921) on the influence of hydrogen-ion concentration. His preliminary conclusions were that *A. maculipennis* became diseased in acid waters. The same author (MacGregor, 1924) continued his English observations in Mauritius, and reached the generalization that Anophelines are alkaliphiles, and Culicines acidiphiles. In the same year Buxton (1924), on a third Anopheline Fauna, that of Palestine, found six species, all in alkaline water. The present author (Senior-White, 1926) published the first really long series of larval-pH findings, made in Ceylon, finding that, though for each species there appeared to be an optimum value, the range found for the majority was very wide, almost, in fact, that of the whole series of waters examined. The conclusion reached was that only extremes, both of acidity and alkalinity, are inhibitory to breeding. But, from an investigation of the 'residual pH', that is the value after expelling CO_2 by shaking or boiling, it was found that, for Anophelines at least, acidity other than that due to CO_2 is definitely inhibitory. The first conclusion, that only extremes of 'actual' pH have any inhibitory effect, was confirmed by experimental work by Buxton (1927). Later, in East Central India and at Delhi, it was shown (Senior-White, 1928) that nine species common to Ceylon, East Central India and Delhi exhibited very different toleration limits and even optima in these three areas, and that therefore pH *per se* was not the controlling factor in making water suitable or otherwise to various species of *Anopheles.*

MacGregor (1929) summed up all work on this factor to early 1928, and made careful laboratory experiments. His conclusions are '(*a*) if the pH of the normal environment is changed the development of the larvæ is adversely affected: (*b*) this statement is not true under bacteriologically sterile conditions: (*c*) consequently the acid or alkaline reaction of the medium, within ordinary limits, has no direct effect on the development of the larvæ'. He concludes that 'pH is of unquestionable importance..........in.......... that.........it indicates the favourable or unfavourable association of chemical and biological factors in the breeding places.'

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Thus pH is no more than an 'indicator', and other factors have to be evaluated.

The study of other chemical water factors had been commenced in 1922 by Lamborn (1922) in Malaya, using a few analyses performed for him on nine measurable factors, from which he was unable to deduce any certain conclusions. Purdy (1925) failed to obtain larval correlations with pH, dissolved oxygen or mineral solutes. Hacker (1923) was able to correlate the abundance of two species, one a vector and the other harmless, with the albuminoid content of the waters, the carrier being most common in the presence of low values, the non-carrier of the opposite.

The present author took up the study of various chemical factors in addition to pH (Senior-White, 1926), measuring dissolved oxygen, mineral solutes (by conductivity) and saline ammonia. It was shown that high dissolved oxygen content appeared to favour the use of natural waters by Anophelines, and that this was especially important in rice fields, but the principal discovery announced was that *saline ammonia in amounts of less than* 1 *p.p.m. was inhibitory to natural water breeders*, especially Anophelines. In a later communication (Senior-White, 1928), in addition to pH, the following factors were studied and shown to be of no value in controlling breeding :—Conductivity, carbonates and albuminoid ammonia. Residual pH and dissolved oxygen after further work may still be studied in the hope that they will yield results of value. Phosphates in fresh water appear to be much less important than they are in sea water, but are worth further study. The conclusions formerly arrived at with regard to saline ammonia were confirmed for three vector species studied but not for the non-carrier *subpictus*. This value has since been confirmed by Beattie (1932)* for a Neotropical species, which shows this criterion is the only one of importance so far discovered. Whether it is applicable to all the 170 known species of the genus *Anopheles* is, however, quite another matter, still uninvestigated. The factor is not, however, a real one. It cannot be repeated in the laboratory, where larvæ can withstand far higher concentrations (up to 100 times) of various ammonium salts than the natural limit. An old paper by Waddell (1902) was found, in which 250 p.p.m. of *liquor ammoniæ* were found to be fatal to mature larvæ. But such concentrations are quite unlikely to occur in Nature. The factor, therefore, that we measure as saline ammonia must be only one in some way correlated with NH4, and not that ion itself.

Williamson (1928) took this investigation a step further, and showed that the inhibitory effect was obtained when the relationship $\frac{\text{oxydised}}{\text{ammoniacal}} N < 1$. This suggests that the process at work is probably bacteriological, and so would explain how it is that the dissolved oxygen content appears to have

* Buxton's (1934) analysis of this author's findings should be studied *pari passu* with the original paper.

some connection with mosquito breeding, though Iyengar (1929) failed to trace much connection. On the other hand Bekhemlishev and Mitrophanova (1926) state that an optimal breeding place is characterized by oxygen saturation or supersaturation and a basic reaction, though they are without 'immediate' vital importance to the larvæ. I think the Russian authors, by 'immediate' mean 'direct'. The quotation is from their own English summary.

Beattie (1930) repeated in England the methods of earlier workers abroad. She failed to obtain any correlation with saline ammonia, but her findings for this factor were never in excess of 0·6 p.p.m. She considered that pH, H_2S, total organic nitrogen and dissolved oxygen had some bearing on the incidence of *maculipennis* and *bifurcatus*, but her results are not very convincing.

An entirely new line of work was opened up in 1932 by Hinman (1932) and (1932A), who showed that Anopheline larvæ could utilize material in solution and colloids in suspension in Seitz-Werke filtered water, but not in the dialysate from such water. He refers to amino-acids and monosaccharides particularly. This was independently worked at by Shipitsina (1930). Both authors seem to have been following the still unproved theory of Pütter (1911) regarding the alimentation of marine organisms. In this connection Hinman (1932B) has studied the enzymes of the digestive tract of larvæ, finding amylase, invertase, (sucrose) xylanase and a protease acting in alkaline medium. Negative results were obtained for maltase, lactase and an acid medium protease. This work was done with Culicines and not Anophelines. He points out that his protease finding is in accordance with my work on the pH of the intestinal tract, done with Anopheline larvæ (Senior-White, 1926) as well as Culicines.

Morin and Bader (1933) were the next in the field. They found that the ratio $\frac{\text{free}+\text{half bound}}{\text{total alkalinity}} CO_2 > 1$ in water that was breeding Anophelines. They developed this thesis further the following year (Morin and Bader, 1934), and pointed out that upward seepage through clay at certain seasons permits of the breeding of various species normally confined to springs and the upper reaches of rivulets, but they have so far failed to pursue the matter further. The authors have, however, definitely 'got something' out of a factor in which I (Senior-White, 1928) had failed to find anything of significance.

The present position has been summed up by Williamson (1936), in a publication which must be consulted by anyone interested in the subject. According to the later work of this author (Williamson, 1936A) it would now appear that it is not so much the water itself, as the underlying soil, which determines whether malarial vectors do or do not breed in the water. Excess albuminoid nitrogen in the presence of deficient oxidation characterizes non-malarial soil. Below a soil nitrogen content of 0·1%, malaria is to be expected from standing surface water: at levels much above 0·3%, stagnant, shallow waters are inimical to all Anophelines. A high degree of nitrification, giving a high ratio of nitrate to ammoniacal nitrogen, indicates adequate oxidation and is compatible with the breeding of malaria carrying Anophelines.

Thus the studies of single factors, which have yielded by no means conclusive results, as has been shown, have, through the genius of Williamson, been combined into a compound factor made of the interaction of ammonia and oxygen which has not only immensely clarified the problem, but opened up the way to 'naturalistic' methods of malaria control, the secret of which, in many instances, is seen to be better agriculture, raising the soil nitrogen content.

That author's 'herbage cover' method of stream control, recently shown by myself (Senior-White, 1936) to have great possibilities, is another method of producing excess albuminoid nitrogen in the presence of deficient oxygenation.

It has long been apparent to all workers that chemical larvicides have a most limited application owing to their excessive cost, and are, in fact, quite unsuited to the vast rural tracts of this and other Tropical Countries. Cheaper methods will have to be evolved if anything is to be done for rural malaria generally. We now see that by further work on the points so far discovered the method can be envisaged from afar, by improving the standard of agriculture by soil enrichment, a method which will doubly benefit the villager by raising his standard of living. We come to purely agricultural methods as our main weapon of attack, the raising of fodder crops, the stabulation of cattle, the conservation of cow-dung for manure instead of its wasteful expenditure as fuel. These are questions for the agriculturist and the economist rather than the malariologist, who can only strengthen the hands of these professions by pointing out that success in their immediate object will, in time, lead to the control of the 'King of Tropical Diseases'.

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