but their free energy was within 5 kcalorie of that of the clover leaf. Several explanations are available for the anomalies: the thermodynamic data on which the energy assessments are based may be faulty in some way or the native clover leaf form may be trapped in a metastable state; but the authors incline to the notion that tertiary interactions may tip the balance in favour of the clover leaf, and in the worst case only 5 kcalorie would be required.

The experimental elucidation of such tertiary interactions by NMR techniques is the subject of an interesting paper by Reid *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 2049; 1975). They determined the NMR spectrum of several highly purified species of tRNA in the −9 to −15 p.p.m. region. The −11 to −15 p.p.m. region gives a signal believed to emanate from the hydrogen-bonded ring NH protons and proportional to the number of Watson–Crick base pairs. Nevertheless it has been suspected that this low field region should contain resonances due to other sorts of base pair and to tertiary interactions. Their first interesting observation was that $tRNA_1^{Val}$, $tRNA^{Arg}$ and $tRNA^{Phe}$ all from *E. coli* gave a resonance coresponding to two protons at about −10.5 p.p.m., whereas $tRNA^{Asp}$ from yeast gave a signal corresponding to about 10 protons in this region. The three *E. coli* sequences all indicate one GU base pair somewhere in their clover leaf structures while the yeast sequence indicates no less than three GU pairs and a GΨ pair as well. Since a GU pair involves two ring NH protons hydrogen bonded to oxygen atoms, the authors tentatively assign the −10.5 p.p.m. resonance to GU pairs.

The next point concerns a single proton resonance at −14.9 p.p.m. observed with these same three *E. coli* species but absent in several yeast tRNA spectra. For these *E. coli* species ring current shifts were not expected to result in any resonance below −14.5 p.p.m. Now the *E. coli* sequences all have s^4U in position 8, whereas yeast $tRNA^{Phe}$ has an unmodified U in this position which the X-ray diffraction data suggest is hydrogen bonded to A14. This suggested the attractive hypothesis that the −14.9 p.p.m. resonance corresponded to a S^4U8–A14 base pair. This was neatly confirmed in the case of *E. coli* $tRNA_1^{Val}$ by removing the sulphur by the action of cyanogen bromide. When this had been done, the tRNA was still active in aminoacylation (and hence presumably still had the native tertiary structure) but the −14.9 p.p.m. resonance had gone.

Other resonances in the −11 to −15 p.p.m. region probably remain to be defined and assigned, because in the case of *E. coli* $tRNA_1^{Val}$, the integrated intensity from this region corresponds to 26±3 protons. One of these is, no doubt, the s^4U–A tertiary interaction and 20 more no doubt correspond to the 20 Watson–Crick base pairs in the structure, but this leaves several more unaccounted for. The authors also suggest it likely that resonances corresponding to four protons in the −9 to −10 p.p.m. region in the case of *E. coli* $tRNA_1^{Val}$ should be assigned to amino protons hydrogen bonded to ring nitrogens. Several such hydrogen bonds appear in the tertiary interactions discerned in yeast $tRNA^{Phe}$ and *E. coli* $tRNA_1^{Val}$ has corresponding bases in corresponding positions of the sequence.

Clearly more work needs to be done to confirm some of these tentative assignments and to elucidate others but this technique is reaching the point where it can provide information concerning the three dimensional structure of nucleic acids which is almost on a par with that obtained from X-ray crystallography. □

Ectoparasite provides cercarial model

from F. E. G. Cox

DIGENETIC flukes typically use two hosts during their life cycle, a mollusc and a vertebrate. The larval stage infective to the vertebrate is the cercaria which passes out from the snail and often has a short free-swimming existence before it gains access to its final host. Cercarial activity and infectivity have been studied in economically important species such as *Schistosoma mansoni* and *Fasciola hepatica* but it has been difficult to obtain much quantitative information from these studies because of problems such as having to dissect the final host to find out how many cercariae have actually established themselves. Because these problems are inherent in dealing with any internal parasite there has been no particular interest in developing alternative laboratory models and the study of the digeneans has therefore tended to suffer.

A convenient model is now available and it is an ectoparasite so easy to maintain that it will obviously become very important in the future. This fluke is *Transversotrema patialensis* and it lives under the scales of many freshwater fish including *Brachydanio rerio*, the Zebra Danio, which is sold by many shops stocking tropical fish. The snail host is *Melanoides tuberculata* which is also available from aquarists. When infected fish are put in a tank containing uninfected snails the life cycle maintains itself indefinitely and the tank provides a convenient source of every stage required for experimental investigations. It is also easy to see how many adult flukes have become established simply by looking at the fish alive.

Transversotrema patialensis is therefore an ideal subject for the study of cercarial survival, activity and infectivity. Anderson and Whitfield (*Parasitology*, **70**, 295; 1975) have found that cercariae survive for a maximum of 44 h and that infectivity is restricted to half this time. As these cercariae do not feed they can live only as long as their energy reserves persist; Anderson and Whitfield have produced theoretical models relating infectivity to activity and activity to the amount of glycogen available and have found good correlations between their models and their experimental observations. They used electron microscopy as well as the more usual histochemical techniques in order to observe the depletion of glycogen in the cercariae.

Because the period of infectivity is so short the cercariae must locate, identify and attach to an appropriate host as quickly as possible. In the logical follow-up to the first study Whitfield, Anderson and Moloney (*Parasitology*, **70**, 311; 1975) were able to conclude that the location of the fish is a random process but that essential information regarding the suitability of the host is probably obtained by the use of receptors on the tail of the cercaria. Scanning and transmission electron microscope studies show that these receptors are unique but similar to types found in monogeneans, also ectoparasites, and which are thought to be concerned with chemoreception. After recognition of the host, attachment is accomplished first by means of adhesive pads, also on the tail, and then by the ventral sucker on the body after which the tail is lost and the fluke attains its final position under a scale.

These experiments open up a whole new field of investigation into the biology of flukes because the number of flukes that have actually established themselves can be counted without the need for killing and dissecting the host. These two papers are only a beginning but the standard set should ensure that further studies will also be based on critical experimental observations coupled with the use of techniques such as electron microscopy and mathematical analysis when necessary.