## Maintenance *in vitro* of *Haplometra cylindracea*

During the past two years, attempts have been made in this laboratory to keep alive *in vitro* sterile trematodes of the species *Haplometra cylindracea* (Zeder, 1800) obtained by aseptic dissection from the lungs of frogs (*Rana temporaria*). Of the 338 frogs dissected to date, 194 were uninfected with this trematode and the remaining 144 frogs provided a total of 576 living flukes. One remarkable frog had 23 flukes in the left lung and 17 in the right lung; but the majority of infected frogs had either two or three flukes in each lung.

In previous work of this kind with *Fasciola hepatica* it was shown[1,2] that Hédon–Fleig is a valuable saline to use. In the present work it was used once more; but sodium dihydrogen phosphate was substituted for disodium hydrogen phosphate, with consequent alteration of the *p*H range from 8·4–8·8 to 8·2–8·4. In addition, a frog Ringer's solution with the following composition and *p*H range was used: sodium chloride 6·5 gm., potassium chloride 0·25 gm., calcium chloride 0·3 gm., sodium bicarbonate 1·2 gm. and distilled water 1,000 c.c.; *p*H 8·1–8·3. Both solutions were filtered as in previous work and both were used either with or without additions of D-glucose (0·5 per cent). *Haplometra cylindracea* continued to live in a fairly active condition in these solutions when kept at 20° C. for the periods specified as follows:

| | Duration of culture period (days) | |
|---|---|---|
| | Ranges | Means |
| Hédon–Fleig – glucose | 48–105 | 69 |
| Hédon–Fleig + glucose | 60–82 | 71 |
| Ringer – glucose | 50–88 | 68 |
| Ringer + glucose | 54–84 | 64 |

Thus, *H. cylindracea* remains alive under these simple conditions of culture for much longer periods than does *F. hepatica*, partly no doubt because of the lower temperature of incubation but also because of its smaller size. There seems to be no significant difference in periods of survival in Hédon–Fleig and Ringer's solutions with or without additions of glucose, whereas *Fasciola* will not continue to live for more than about 48 hr. in the absence of glucose, while in the presence of glucose it has been found to live under these conditions for as long as 34 days. In a sterile medium somewhat enriched with nutrients ('Bacto' beef extract 0·3 per cent, sodium chloride 0·5 per cent, 'Bacto' tryptose 1·0 per cent and D-glucose 0·5 per cent) and having a *p*H range of 7·2–7·4, twelve separately treated *Haplometra* continued to live for 57–110 days, with a mean period of 88 days. In all these experiments, as in those with *Fasciola*, histological examination of the fluke revealed abnormalities of spermatogenesis, but in *Haplometra* these first appeared much later and only after about five days of culture.

After dissecting out the compact testes of *Haplometra* it was easy to make permanent fixed and stained smears showing stages of spermatogenesis, and examination of these revealed some variability in the rate of meiotic division in the primary spermatocyte stage. The mean rate is fairly constant *in vivo* at about 24 divisions per 1,000 spermatocytes examined at random, and this falls only slightly to about 14 per 1,000 after 24 hr. of culture in dextrose broth, but it falls to zero in the Ringer's solution without glucose and to 4 per 1,000 in this saline with added glucose. An initial fall to 14 per 1,000 is seen after 4 hr. of culture in all the solutions employed, so that in dextrose broth the meiotic division-rate does not fall appreciably after 4 hr. for a further 20 hr., and there is some foundation for the statement that it does not fall below this figure for periods up to five days.

Both flukes freshly removed from their hosts and flukes which had been maintained in the Ringer's solutions with or without glucose and in dextrose broth were examined for glycogen deposits after freeze-drying. In fresh flukes glycogen is stored in the muscles and in the cells of the parenchyma. Flukes cultured in Ringer's solution plus glucose and in dextrose broth showed heavy deposits of glycogen in the muscles and only slightly reduced deposits in the parenchyma, but flukes cultured in the Ringer's solution without glucose showed practically no glycogen in the parenchyma, though deposits were still present in the muscles. Quantitative studies based on the photometric estimation of glucose by Nelson's method[3] showed clearly that glucose is abstracted from the medium by the flukes and that in Ringer's solution plus glucose and in dextrose broth the glycogen deposits are maintained at the *in vivo* level of 1·5 per cent wet weight for periods up to three weeks, but that in Ringer's solution without glucose the gross deposits of glycogen are substantially reduced to a mean of 0·8 per cent wet weight after one week. However, Warburg determinations show that the rate of respiration of flukes maintained in Ringer's solution plus glucose falls from a mean of 4·8 μl./mgm. dry weight per hr. at 25° C. to about one-half of this value after about two weeks.

It appears that *H. cylindracea* depends on aerobic respiration to a greater extent than do any other helminths so far studied[4]. It dies within 4 hr. under anaerobic conditions, but after shorter periods of anaerobiosis the oxygen debt incurred is completely repaid when the medium is placed in equilibrium with atmospheric oxygen. Experiments are now proceeding to determine the amount of increase in glucose uptake from solutions and to estimate lactic acid production by worms placed under anaerobic conditions.

*Note added in proof:* Recent estimates indicate that lactic acid production during a short period of anaerobiosis (2 hr.) is increased threefold.

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[2] Clegg, J., Ph.D. thesis, University of London (1957).
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## Colonial Differentiation between *Escherichia coli* B and *Escherichia coli* B/r

A great deal of radiobiological research has been carried out on *Escherichia coli*, strain B, and its radiation resistant counterpart, *Escherichia coli*, strain B/r[1]. Aside from the fact that *E. coli* B/r demonstrates increased resistance to various physical and chemical agents[1–4] when it is compared to the more sensitive parental type, there is little else to distinguish one strain from the other.

It was recently noticed in our laboratory that *E. coli* B and *E. coli* B/r exhibited an unmistakable