Heavy Metals and the Fertilization of Rainbow Trout Eggs

THE effects of poisons on the development and survival of fertilized fish eggs have often been described but little information is available on the effects of such substances on either the gametes or fertilization. Mann, for example, has reported[1] that a dodecylbenzenesulphonate detergent caused a 26% loss of mobility of trout spermatozoa at a concentration of 5 mg $l.^{-1}$, and the anaesthetic drug tricaine methanesulphonate was found[2] not to affect fertilization provided that the concentration did not exceed 50 mg $l.^{-1}$. The effects of heavy metals on these life stages and processes do not, however, seem to be known, even though these poisons are common pollutants of river waters in industrial areas. We report here results of preliminary tests made at this laboratory into the effects of copper and nickel on the fertilization of eggs of the rainbow trout, *Salmo gairdneri* Richardson.

The eggs were obtained by section of the ovaries of a freshly killed female fish weighing 555 g. Spermatozoa were obtained from a 400 l. volume of water (of total hardness 260 mg $l.^{-1}$ as $CaCO_3$, *p*H 7.8, and temperature 8° C) into which a single male fish had been induced to discharge milt.

Three samples (2 l.) of this water were taken in borosilicate glass beakers and a solution of copper sulphate or of nickel sulphate was added immediately to two of the samples to give metal ion concentrations of 1.0 mg Cu^{++} $l.^{-1}$ in one and 1.0 mg Ni^{++} $l.^{-1}$ in the other; the third samples served as a control. The eggs, after removal from the ovary, were distributed in sub-equal lots between the three treatments. These operations were completed within a period of 5 min. The beakers were left undisturbed for the next 30 min, after which time each batch of eggs was removed and placed in a separate aquarium (from which light was excluded) in a fast, continuous flow of clean, well aerated, hard water (total hardness 260–280 mg $l.^{-1}$ as $CaCO_3$, copper 0.5 μg $l.^{-1}$, nickel nil) at a temperature of about 9° C. The number of eggs used and the percentages fertilized and hatching in each treatment are given in Table 1. The values of χ^2 obtained from comparison of the numbers fertilized in the control with those fertilized in the two metals are also given.

Table 1 Fertilization of Rainbow Trout Eggs in Solutions of Copper and Nickel Salts

Treatment	No. of eggs	% fertilized	χ^2	Fertilized eggs hatching (%)
1 mg Cu^{++} $l.^{-1}$	389	22.4	1.78	97
1 mg Ni^{++} $l.^{-1}$	419	23.4	2.88	99
Nil (control)	332	18.0	—	100

The hatching rates of eggs from the three treatments are shown in Fig. 1. Observations were made daily at 0900 h and 2000 h up to the start of hatching. The time origin in the figure is the time of the last observation, 35 days after fertilization, at which all the eggs were still intact. The periods to median hatch (on the same time scale) are given in Table 2. The data relating time to percentage hatch were analysed by the methods of Litchfield[3].

The proportion of eggs fertilized was low but it compares reasonably with those obtained elsewhere by methods involving ovarian section[4]. The $\chi^2_{(1)}$ values indicated that the differences observed between each treatment and the control were not statistically significant ($P > 0.05$). Fertilization in the metal solutions did, however, increase the rate of development, particularly in the case of copper, and the eggs from this treatment all hatched before any had done so in the controls. The rate of hatching of the eggs fertilized in the copper solution was also significantly different ($P < 0.05$); it was slower than that of the control eggs or of those fertilized in the nickel solution—the slopes of the curves of these last two were similar.

Table 2 Period of Time from 0900 h on Day 35 to Median Hatch

Treatment	Period to median hatch (min)	95% confidence limits (min)	Slope function
Nil (control)	11,500	11,320–11,680	1.07
Nickel	9,800	8,850–9,150	1.09
Copper	6,400	6,170–6,640	1.19

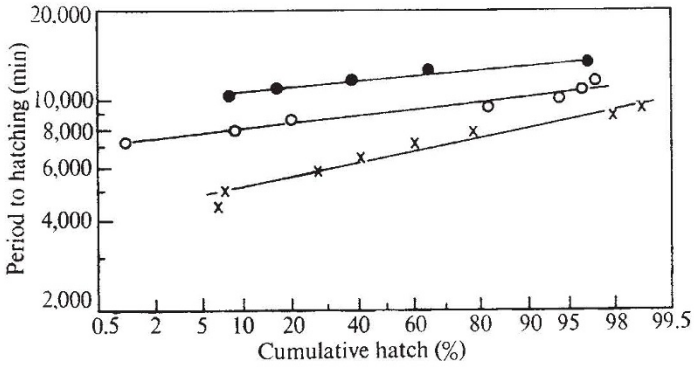

Fig. 1 Hatching of rainbow trout eggs following fertilization in solutions of copper and nickel. The period of hatching was measured from the time, 0900 h 35 days after fertilization, when all eggs were last observed to be intact. ●, Control; ○, nickel; ×, copper.

Because the concentrations of copper and nickel used here exceed those typically found in British rivers, even those badly polluted, it seems that, at least in hard waters, neither of these two poisons is likely to be responsible for any impairment of fertilization among trout. This does not, however, imply that successful development would occur under conditions of continuous exposure to these poisons. It is not known whether the differences observed in the development have a biological significance.

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Cyanide Insensitive Culture Form of *Trypanosoma brucei*

ON the basis of ultrastructural and cytochemical observations, Vickerman has suggested[1–3] that the single mitochondrion of sleeping sickness trypanosomes undergoes a cyclical activation and repression during the life cycle of the parasite. Detailed studies of the respiratory metabolism of this group of trypanosomes have been confined to bloodstream stages and forms from established *in vitro* cultures[4–7]. The culture forms are believed to correspond to the midgut stage of the tsetse fly vector[8]. The bloodstream forms lack cytochromes and a functional Krebs cycle[7]; terminal respiration is cyanide insensitive and is mediated largely by extramitochondrial L-α-glycerophosphate oxidase[6]. Established culture forms have cyanide sensitive terminal respiration and a full functional Krebs cycle[7].

We describe here a culture form of *Trypanosoma brucei* which develops during the transformation of bloodstream forms into established culture forms and differs from either of these forms in its respiratory metabolism.