the entire proximal convoluted tubule of the kidneys was found with accumulation of eosinophilic material within the remaining basement membrane and in the tubules in the outer medulla, in which cellular debris including droplets of basophilic material was present. The glomeruli, distal convoluted tubules and remaining kidney structure appeared normal. The placenta appeared normal in structure. No dye was found in the unstained sections of any of the tissues examined.

Table 2 shows the stage of development attained by the embryos at the time of killing. All exhibited general developmental retardation of the order of one half to one stage[4], but no abnormalities were present. As the entire litter was either aborted or retarded in development, it seems probable that this was related to the degeneration of the renal proximal tubule in the mother, rather than to a direct effect of the dye on the embryos.

Chromatography of compound *A* showed that it was not identical with either of the commonly described red or purple contaminants of trypan blue.

Table 2. Developmental Stage of Embryos at Time of Killing in Injected Group as compared with Control Rats

Day of killing	Developmental stage	Normal stage reached
10·5 (1)	8 at stage 16 7 at stage 17	18–19
10·5 (2)	4 at stage 16 4 at stage 17	18–19
11·5 (1)	3 at stage 20 (early)	20 (late)–21
11·5 (2)	2 at stage 19 9 at stage 20 (early)	20 (late)–21
11·5 (3)	3 at stage 19 5 at stage 20 (early)	20 (late)–21
12·5	8 at stage 22 (early)	22 (late)

The chemical formulae of trypan blue and the other known teratogenic disazo dyes tested in Table 1 include two 8-naphthol rings (one in the case of compound 8) substituted in the 1 and 7 positions with amino groups, and with two sulphonic acid groups in the 3,6 positions, linked through di-*o*-toluidine, or di-*o*-anisidine. As *o*-toluidine has been found not to have teratogenic activity, it seemed likely that reduction of trypan blue *in vivo* over the disazo linkage could release the active part of the molecule, that is, compound *A*. However, this investigation has clearly shown that this is not so, and it would appear that it is to the whole molecule rather than parts of it that one must look for teratogenic activity.

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Biphasic Dose-response Relationship with Acetylcholine on the Heart of the Mollusc *Tapes turgida*

The heart of the mollusc *Tapes turgida* has been shown to be very sensitive to acetylcholine[1] and provides a satisfactory alternative to the heart of *Venus mercenaria*, a species not found in Australia, for the assay of acetylcholine.

In the experiments described here, only the ventricle was used, and this was suspended between threads tied at the anterior and posterior vessels, in an organ bath containing sea-water. In most experiments, oxygen containing 5 per cent carbon dioxide (carbogen) was bubbled through the bath, though in some, no aeration at all was provided. Under the conditions of assay, when the bath fluid was changed frequently, the heart continued to beat constantly without aeration.

Acetylcholine produces a negative inotropic effect on the hearts of both *Venus* and *Tapes* and the dose-response relationship is particularly steep in both species. A concentration of acetylcholine between 10^{-10} and 10^{-9} g/ml. is generally sufficient to produce complete block.

During an examination of the effect of concentrations of acetylcholine less than 10^{-10} g/ml. we have found that the heart showed another inhibitory phase, often proceeding to a complete block, but only when aeration with carbogen was used. The dose-response curve over the range of acetylcholine concentrations studied showed a biphasic effect (Fig. 1).

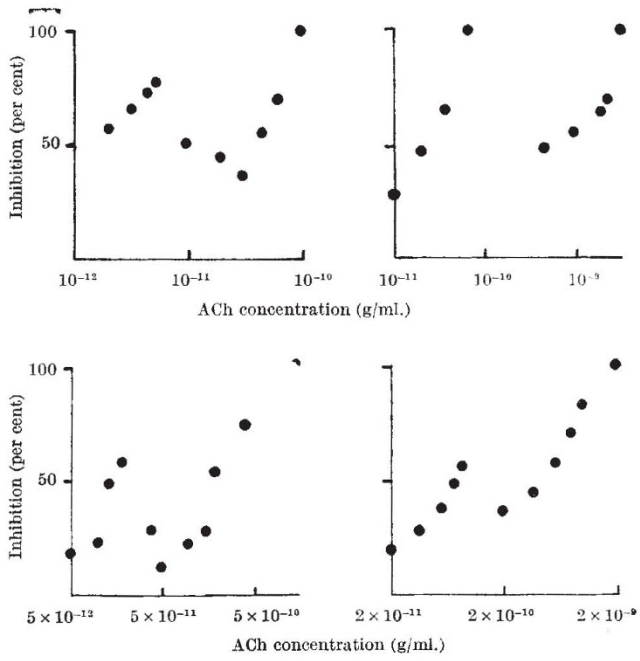

Fig. 1. Graphs showing the relationship between concentration of acetylcholine and the percentage reduction in the amplitude of the heart beat in four hearts of the mollusc, *Tapes turgida*

Preliminary experiments without aeration failed to reproduce inhibition at the lower concentrations of acetylcholine.

A similar biphasic dose–response relationship with acetylcholine has been described recently for the inhibitory effect on the perfused frog heart[2], and for the stimulation of the frog rectus abdominus muscle[3].

This biphasic relationship presents an inherent source of error when the *Tapes* heart, aerated with carbogen, is used as an assay preparation for acetylcholine. Particularly is this so if the 'matching' technique is used, where the concentration of the unknown solution is estimated by matching a response on the heart (generally about 50 per cent inhibition) with that produced by a standard solution of acetylcholine.

With this technique it is possible to match responses produced by the unknown and standard solutions on different slopes of the biphasic dose–response curve.

However, preliminary experiments suggest that this difficulty does not arise if the heart is allowed to beat in sea-water without aeration.

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