histologically on random sections, although single tumour cells could be identified in the pulmonary capillaries.
It is possible that the flow of tumour cells was somewhat increased by the prior increase in tissue pressure induced by the introduction of 20 µl of Evans blue. However 1 serially sectioned lymphatic from the animal which did not receive Evans blue did not contain an evidently different number of cells from the similarly sectioned lymphatics from animals given Evans blue. It is unlikely that this is an important factor.
These findings establish for the first time the approximate number of tumour cells in lymph efferent from a transplanted tumour and show that lymphatic metastasis under the conditions studied is a continuous phenomenon.

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Copper-induced heart malformations in hamsters[1]

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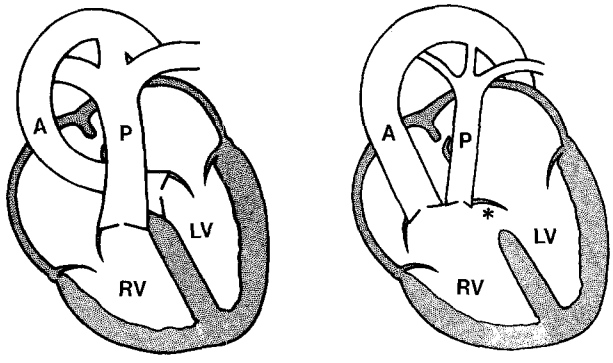
Summary. The injection of copper citrate into pregnant golden hamsters induces a specific pattern of cardiovascular malformations in their embroys. The syndrome consists of double-outlet right ventricle, pulmonary hypoplasia and a ventricular spetal defect.

Although the release of copper from an IUD during the early stages of pregnancy inhibits implantation of the blastocyst[2,3], the release of copper from wires placed in the uteri of several experimental animals was not found to have toxic effects on their post-implantation embryos[4]. On the other hand, mouse blastocysts were killed rapidly when cultured in association with copper[5] and there is evidence that the administration of copper to hamsters induces congenital malformations in their offspring. The embryos of pregnant hamsters injected i.v. with copper salts showed, amongst other abnormalities, thoracic wall defects with or without an associated case of ectopia cordis[6]. It is this latter finding that led to this study on the effects of copper on cardiac development.
Materials and methods. Pregnant golden hamsters were i.p. injected on the morning of the 8th day of gestation with a final treatment concentration of 2.7 mg copper citrate solution per kg b.wt. The day following the evening of mating is considered the 1st day of gestation. The mothers were individually caged, fed ad libitum and sacrificed at gestational days 12 and 13. A group of control hamsters were treated in the same manner except that the solution injected contained solely deionized water. Embryos were removed from gestation sacs, placed in 0.9% saline solution, checked for viability and live embryos were examined for gross pathologic and gross teratogenic effects. Embryos showing gross malformations (e.g. mostly tail and limb defects) or edema were embedded in paraffin, sectioned with a microtome through the thoracic cavity and examined for cardiac anomalies with a light microscope. Whenever a cardiac malformation was found the littermates of this embryo were then sectioned in a similar manner and examined for defects. In addition, several litters of copper-treated, normally-appearing embryos were also processed for light microscopy. Embryos from control group litters were selected at random and, in several cases, the entire litter was processed for light microscopic observation.



Normal day-12 or day-13 hamster embryo heart (left) and a hamster embryo heart of the same age affected by copper citrate (right). Note the narrowing of the pulmonary trunk (P), the malpositioning of the aorta (A) and the ventricular septal defect (*) between the left and right ventricles (LV and RV, respectively).

Results and discussion. Maternal survival was unaffected by the administration of copper. The effects of maternal copper treatment on the embryos, as well as the control data, are summarized in the table. Embryos collected from the water-injected mothers exhibited normal survival and were free of gross pathologic and teratogenic effects. Light microscopic sections of these embryos revealed normal cardiac development. In the copper-injected group, only embryos with edema had heart malformations but not all edematous embryos had cardiac defects. Nevertheless, the presence of edema served as a reliable clue for the detection of these copper-induced anomalies.
The unusual observation is the specificity of the heart malformations found in the day-12 and day-13 embryos of copper-treated hamsters. 4 of the 17 copper-treated mothers bore 1 or more embryos with a specific array of cardiac lesions. As shown in the figure, the pulmonary trunk of the normal day-12 and day-13 embryonic hamster heart has a greater diameter than the aorta. The cephalad portion of the aorta lies ventral to the pulmonary trunk. As these

Day-12 and day-13 hamster embryo response to maternal, i.p. injection of deionized, distilled water or copper citrate (2.7 mg/kg) administered on the morning of the 8th day of gestation

Group	Number of hamsters	Number of implantations	Number (%) of embryos live at sacrifice	live embryos with edema	live embryos with heart defects	Percent of edematous embryos with heart defects	Number of hearts examined microscopically
Deionized, distilled water	12	150	145 (97)	0 (0)	0 (0)	0	50
Copper-treated	17	215	144 (67)	21 (15)	7 (5)	33	34

vessels are traced into the heart however, the aorta comes to lie dorsal to the pulmonary trunk and connects with the left ventricular outflow tract. The ventricular septum is usually complete by day 12 and is well-developed by day 13. These features of cardiac development follow a different course in the affected embryos. The most striking abnormality is that the pulmonary trunk is remarkably narrow throughout its extent (figure). The pulmonary arteries are noticeably hypoplastic also. The aorta does not course behind the pulmonary trunk in the normal manner but comes to lie alongside this outflow tract. The result of this malpositioning is that the aorta and pulmonary trunk both arise from the right ventricle. In addition, the interventricular septum is deficient in these cases. This pattern of cardiac maldevelopment has been classified as pulmonary hypoplasia and double-outlet right ventricle with an associated ventricular septal defect.

The pathognomonic feature of this syndrome, which was present in the copper-induced cases, is that the aorta and pulmonary trunk both arise completely from the right ventricle[7]. A ventricular septal defect is an important adaptive component of the double-outlet right ventricle syndrome because it provides the only means of exit for the oxygenated blood in the left ventricle. A ventricular septal defect was found in all the experimentally-induced cases and is a consistent finding in human cases with this cardiac lesion[8]. Associated defects in the pulmonary trunk are also thought to have survival value. Pulmonary arterial stenosis is a common but non-essential component of this syndrome in humans[8], and it is of interest that pulmonary hypoplasia was present in all of the experimentally-induced cases in hamsters. Since the right ventricle becomes the systemic ventricular chamber in cases of double-outlet right ventricle, Taussig[9] suggested that a narrowing in the pulmonary trunk may protect the lungs from undue pressure.

The effects of copper on heart development are of considerable interest because animal models for the investigation of abnormal cardiogenesis are rare. A comprehensive study[10] in Keeshond dogs suggests that the occurrence of ventricular outflow tract lesions are under a genetic influence. The induction of a specific array of defects in the hamster heart by copper citrate renders this system a useful experimental model. Observation of embryonic hamster hearts following copper exposure at successively earlier stages, may reveal a sequence of events in the pathogenesis of double-outlet right ventricle.

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Octopus chromatophores accumulate nickel

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Summary. Microprobe analysis of the pigment granules in the superimposed chromatophore layers of *Octopus vulgaris* reveal rising amounts of calcium, nickel and sulfur as the size and electron-density of the granules increases. The colours, associated with these increases, shift from yellow/orange to red and finally black.

Octopuses and cuttlefish - like all other modern cephalopod molluscs - have a dense array of coloured spots (chromatophores) in the surface layers of their skin. The spot matrix is used by the nervous system to display a range of patterns and colour changes unmatched by any other kind of animal. As it is not known what particular pigments are responsible for the individual hues - yellow, orange, red, dark brown or black - that are revealed when the chromatophores are spread, we have looked at them under the electronmicroscope and used the opportunity of microprobe analysis to throw some light on their chemical nature.

The members of the chromatophore array lie in staggered positions relative to each other and in successive layers of