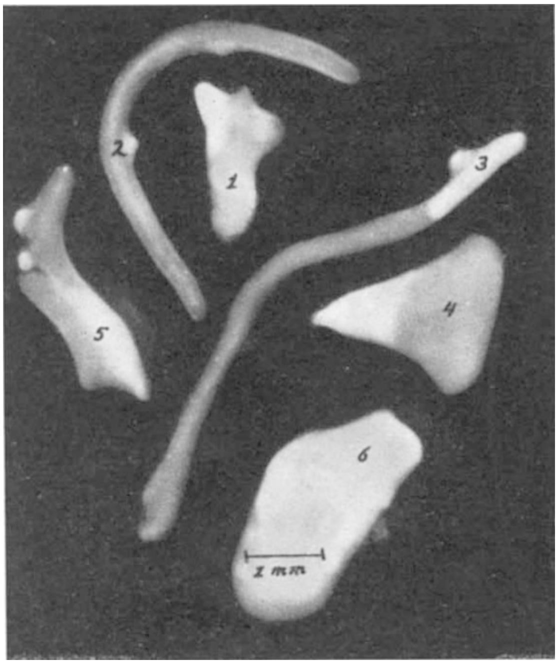
Differentiation of Skeletal Structures from Mouse Embryo Mince in the Peritoneum of Adult Mice

IN the course of attempts, as yet unsuccessful, to produce a non-malignant ascites growth of undifferentiated cells in peritoneal exudate, virgin female mice of two genotypes were injected intraperitoneally with homologous embryonic tissue mince, dissociated in a Waring micro-blendor and capable of passage through a 22-gauge hypodermic needle. Embryos ranged from the 4½-mm. 13-day stage, at which condensations of mesenchyme appear at the location of the future skeletal axes, to 8 mm. in length. Each of ten hosts received an inoculum equivalent to three embryos.



Skeletal structures recovered from the peritoneum of mice three weeks after injection with mouse embryo mince.
(1) Talus; (2) abnormally regenerating rib; (3) distal portion of 9th or 10th rib; (4) head of humerus; (5) ascending ramus of pubis; (6) bone teratoma

The treated mice failed to produce ascitic fluid, such as obtained by Klein[1] with a representative variety of dissociated neoplasms. Three to five weeks after injection, all the hosts were found to contain various teratomata, islands of bone and cartilage attached to the surfaces of spleen, liver, diaphragm and mesentery, and numerous fully differentiated, anatomically defined bones, including whole ribs, phalanges, sesamoids and vertebræ, corresponding in size to the skeletal proportions of mice about one week after birth. The bony structures (see photograph) are of especial morphogenetic interest, in that they provide further evidence (similar to the *in vitro* results of Fell and Robinson[2] with chick femoral rudiments) that skeletal primordia surpass other tissues in their capacity to attain not only advanced histological differentiation, but also to continue their normal anatomical development in foreign sites. In a relevant experiment, Ephrussi[3] cultured portions of 10-day mouse embryos homozygous for the lethal brachyury gene and their normal sibs and obtained differentiation of cartilage, but no definitive bone. It remains to determine the earliest developmental stage from which anatomically recognizable parts of the skeleton may be grown *in vitro* or *in vivo* and the minimal size of fragment still permitting organized bone growth.

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[1] Klein, G., *J. Exp. Cell Res.*, **11**, 291 (1951).
[2] Fell, H. B., and Robinson, R., *Biochem. J.*, **23**, 767 (1929).
[3] Ephrussi, B., *J. Exp. Zool.*, **70**, 197 (1935).

Toxicity of Copper in Solution to the Stone-loach

IN June 1951, stone-loach (*Nemacheilus barbatulus* L.), which had been kept alive in an aquarium since March 1951, died when it became necessary to replace the water supply, which came from a nearby reservoir with lake-water from Windermere. Chemical analysis showed that the original water contained 0·15 p.p.m. copper (Cu^{++}) whereas the lake-water had 0·28 p.p.m. copper, presumably derived from the inflow pipes. Stone-loach, when placed in water containing copper in solution from nil in steps of 0·05 to 0·30 p.p.m. copper, lived in concentrations less than 0·20 p.p.m. copper, but all except one fish died within twenty-four hours at concentrations of 0·20 p.p.m. and above. It seems reasonably certain, therefore, that the fish died of copper poisoning. This note is published because it may be of practical interest.

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An Improved Block for Spore-Formation in Yeast

V. Hartelius and E. Ditlevsen[1] recently directed attention to the fact that gypsum blocks used for spore-formation in yeast will not tolerate the strong heating necessary for adequate sterilization. After trials with various materials, they finally chose a block cast of Portland cement and kieselguhr, which after hardening in carbon dioxide is sufficiently porous.

Nearly sixty years ago, H. Elion[2] referred to the disadvantages of the use of gypsum blocks for the study of spore-formation in yeast. He stated that he had been using for several years small cubes, size 2 cm. × 2 cm. × 2 cm., made to his specifications of unglazed porcelain, and that these blocks, which could be obtained from laboratory supply houses, can easily be cleaned, sterilized and used repeatedly. Elion described the excellent results which he had obtained by using these porcelain blocks in the study of spore-formation in yeast, and during many years thereafter he continued to use these blocks to his great satisfaction.

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[1] Hartelius, V., and Ditlevsen, E., *Nature*, **168**, 385 (1951).
[2] Elion, H., *Cent. Bakt. und Parasit.*, **13**, 749 (1893).