the leptolepid teleosts and their presence in all of the more generalized teleosts (*2, 3*). Among the pholidophoroids, intermuscular bones have been reported previously only in *Oligopleurus* (*4*) and have been little noted.

A remarkably well-preserved specimen of *Pholidophorus bechei* (the type species of the genus) shows a well-developed series of epineural intermuscular bones extending from the anterior end of the trunk to the last abdominal vertebra (Fig. 1) (*5*). The soft tissues of the head and body were either encased or replaced by calcite before much postmortem compression could take place. As a result, the elements of the axial skeleton retain practically their original spatial relationships. After removal of the scale covering from both sides of the body with an Air-Brasive machine (*6*), the specimen was submerged in xylene to render the calcite virtually transparent.

The epineuralia are very slender, delicate bones, about 5 mm in length in a fish of 140 mm standard length (*7*). Anteriorly, the elements attach near the bases of the neural arches and extend outward, backward, and downward for about three segments. Posteriorly, the epineurals attach higher on the arches and are slightly shorter. A neural spine, neural arch, and epineural form an osseous unit which is paired in each segment and free from the dorsal hemicentrum in each segment of the abdominal region (Fig. 2). In view of possible damage to the specimen, I deemed it unwise to uncover the most anterior vertebrae. No traces have been observed of either epicentralia or epipleuralia.

The Leptolepidae have been excluded from the Pholidophoriformes chiefly on the basis of the intermuscular bones (*3, 4*). In the light of the evidence presented above, it may be assumed that intermuscular bones were common among the Pholidophoriformes, but that few specimens have been preserved or prepared in a manner suitable for revealing them. In view of the presence of intermuscular bones among the pholidophoroids, the closely similar Leptolepidae can no longer be reasonably excluded from the Pholidophoriformes.

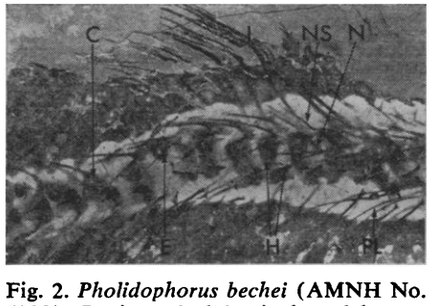

Fig. 2. *Pholidophorus bechei* (AMNH No. 6300). Region of abdominal-caudal transition. Abbreviations: *C*, first caudal vertebra; *E*, epineuralia; *H*, hemicentra; *I*, interneural; *NS*, neural spine; *N*, neural arch; *PL*, pleural rib.

RICHARD LUND

Department of Vertebrate Paleontology, American Museum of Natural History, New York 10024

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5. This specimen (AMNH No. 6300) was presented to the American Museum of Natural History by Dr. Colin Patterson of the British Museum (Natural History) in connection with my research on the Pholidophoroids and the origin of the teleosts.
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24 January 1966

Chimeric Mice with Donor-Type Liver Cells

Abstract. *C_3H mice, made chimeric by lethal x-irradiation followed by injection of $(C_3H \times T_6)F_1$ spleen cells, were later stimulated by CCl_4 to produce a vigorous burst of hepatic parenchymal cell mitoses. Cytogenetic studies of the regenerating livers of 11 chimeras identified 89 percent of the cells as donor type by the presence of the distinctive T_6 marker.*

Reports are increasing that mammalian cell systems may take up nuclear material such as nucleosides (*1*), DNA (*2*), and even chromosomes (*3*). Work in our laboratory and in others has suggested that this process in vivo requires circulating trephocytes (nursing cells) to transport the essential nuclear material to the needy cells (*4*), as opposed to total dependence on the extraction of building blocks from noncellular, circulating sources. To test these two concepts we selected as our experimental model the chimeras formed by the injections of massive numbers of $(C_3H \times T_6)F_1$ spleen cells into newborn and weanling, lethally irradiated, C_3H mice. This treatment schedule is known to allow replacement of host hematopoietic tissues, giving rise to the persistent circulation of donor red and white blood cells (*5*). Determinations of whether the liver cells were of host or donor type were based on the results of chromosome preparations made during the peak of regenerative activity of the parenchymal cells, which followed carbon tetrachloride (CCl_4) necrosis. This is a preliminary report of identification of the distinctive T_6 marker in these preparations which indicates donor-type cells.

A total of 54 male and female C_3H mice (*6*) were made chimeric by x-irradiation and injection with F_1 spleen cells (Table 1); F_1 animals were used as donors to minimize graft-versus-host reactions and to take advantage of the close genetic relation to parent strains. Of the 24 animals that survived these treatments, 18 had metaphases numerically and technically suitable for study; the results of these studies of liver chromosomes also appear in Table 1.

The x-irradiation was a single, total-body dose in a compartmented lucite container, with full back scatter (*7*). The air dosages of 606 and 680 r were contrived to give calculated tissue dosages of 900 r to group I and 950 r to groups II and III, respectively. Puramycin (*8*), in a dosage of 15 ml per 4000 ml of drinking water, was given to groups II and III 5 days before and 14 days after irradiation.

The cells for injection were prepared, from pooled spleens from male and female donors, in Ringer's lactate solution and a few drops of heparin (Lipo-Heparin, Riker) with a ground-glass homogenizer. The intravenous injections were made immediately after irradiation; the intraperitoneal, in one or two doses within 24 hours. The booster doses of donor spleen cells given to the animals of group IIa, were injected intraperitoneally immediately after 2-minute exposure of the mice to CCl_4 fumes in a closed container. It was postulated that any uptake of nuclear material could be facilitated during the intense mitotic activity that followed such treatment.

Approximately 4 weeks after irradiation and injection of $(C_3H \times A)F_1$ spleen cells, the control animals of group III were grafted with skin from $(C_3H \times A)F_1$ donors. The grafts all appeared healthy when the mice were killed, showing that reversion of the chimeric state had not occurred, despite the greater age of these animals and their

lower doses of donor cells (than the ages and doses of groups I and II).

In preparation for the terminal chromosome studies all mice were injected subcutaneously, with a 40-percent solution of CCl_4 in sesame oil, at a dosage of 0.1 ml per 20 grams of body weight. The animals of groups I and III were injected intraperitoneally with 0.002 mg of colchicine (Lilly) per gram of body weight approximately 48 hours after the administration of CCl_4. Because of a delay in the peak of hepatic regeneration, it was necessary to delay the colchicine treatment of group II mice until 54 hours after the injection of CCl_4. All animals were decapitated approximately 6 hours after the colchicine treatment, and samples of liver and spleen were taken for histologic study.

The technique for chromosome preparations of regenerating liver is essentially the colchicine–hypotonic citrate technique of Ford and Hamerton (*9*), combined with the air-drying technique of Rothfels and Siminovitch (*10*): the entire process is fully described elsewhere (*11*); here we summarize the essential points.

Preparation required the 20-minute incubation of 1-mm cubes of liver in 0.250 g percent trypsin (Difco, 1:250) in phosphate-buffered saline (*p*H 6) at 37°C. A cell suspension was prepared in a magnetic stirrer and exposed to a 1-percent solution of sodium citrate for 30 minutes at room temperature. The swollen cells were centrifuged for 5 minutes at 500 rev/min, the citrate solution was removed, and methanol–glacial acetic acid (3:1) fixative was added without disturbance of the pellet at the bottom of the centrifuge tube. After 2-hour fixation, the cells were suspended, centrifuged, and resuspended in fresh fixative. Slides of the air-dried preparations were stained with Giesma and coverslipped.

Spleen-cell suspensions for chromosome studies were obtained by treating small fragments of the tissue in a 1-percent solution of sodium citrate in a ground-glass homogenizer. After 20-minute exposure to the citrate at room temperature, the cells were centrifuged for 10 minutes at 2000 rev/min and fixed, stained, and similarly coverslipped.

The hepatic chromosome preparations were surveyed until a minimum of ten metaphases appeared, comparable in quality to the one shown in Fig. 1. As an additional check, slides from the treated animals (Table 1) were pooled with slides from known C_3H, $(C_3H \times T_6)F_1$, and T_6T_6 chromosome preparations. The genetic background was diagnosed on each slide, without knowledge of the origin.

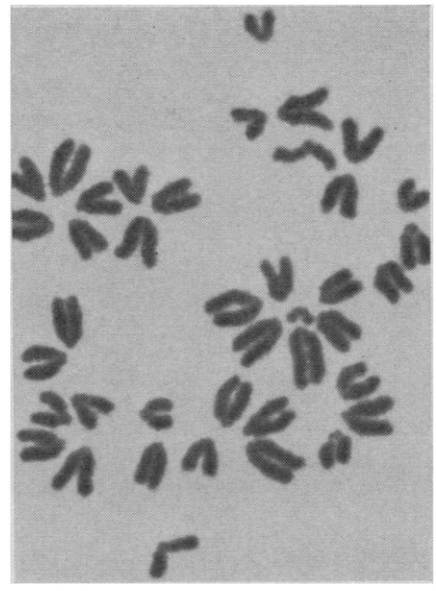

Fig. 1. Air-dried chromosome preparation from the liver of an irradiated $C_3H/(C_3H \times T_6)F_1$ mouse chimera. The small T_6 chromosome is readily seen. (Giemsa; × 1970)

The injection of 40-percent CCl_4 in doses of 0.1 ml per 20 grams of body weight or the 2-minute inhalation of the fumes produced a classic centrilobular necrosis affecting an estimated 40 percent of the liver (Fig. 2, top). This amount of necrosis was invariably followed by a period of intense regenerative activity that reached maximum 48 to 72 hours after the exposure to CCl_4. Especially pertinent was the specificity of the proliferative reaction. Approximately 93 percent (range, 83 to 98 percent) of the mitotic figures were found in the liver parenchymal cells (Fig. 2, bottom). In contrast with the results of surgical hepatectomy (*12*), proliferation of the Kupffer cells and infiltration by acute and chronic inflammatory cells were minimal when the mice were killed.

Study of the cytogenetic preparations disclosed that the T_6 marker was present in 98 of the 110 metaphases (89 percent; range, 70 to 100 percent) of good quality from the 11 liver-cell preparations of groups I and II; none was apparent in group III, a control group designed to check the effects of irradiation on liver chromosomes. During the study done without knowledge of the source of the cells, the T_6 marker was always identified where present.

No falsely positive diagnoses were made in animals where the T_6 chromosome was absent. All metaphases selected for study had approximately the diploid number or 40 chromosomes (range, 38 to 41).

Of special interest were two metaphase preparations of tetraploid cells from two group-I chimeras. Two T_6 markers were seen among the 80 chromosomes. The T_6 markers were consistently present in the spleen-cell preparations of groups I and II.

In summary, we report two key findings: (i) Recovery from CCl_4 treatment caused an intense mitotic activity limited almost exclusively to the hepatic parenchymal cells; and (ii) the T_6 marker was found in diploid and tetraploid cells from these rapidly regenerating livers. Our interpretation is that at least some of the liver parenchymal cells contained donor F_1 chromosomes; a corollary assumption is that these liver cells accepted large aggregates of genetically active material from donor cells.

It must be recognized, however, that there is a gap between the histologic and cytologic studies. It is impossible to identify the T_6-marker chromosome in tissue sections of liver; nor can one determine the type of cell in mitosis in the cytogenetic studies. Although there is a minimal degree of inflam-

Table 1. Experimental scheme and results. The host C_3H mice were decapitated after injection of CCl_4 and colchicine. IV, Intravenous; IP, intraperitoneal.

Group	Age at irradiation (wk)	Injection with F_1 spleen cells			Age at death (wk)	T_6 marker present	
		Dose after irradiation ($\times 10^6$)	Booster (IP)			Livers	Liver metaphases
			At age (wk)	Dose ($\times 10^6$)			
I	Newborn	IV 5*, IP 9*	1.5	100*	8	7/7	62/70
IIa	4	IV 200*, IP 800*	7	200*‡	13	3/3	27/30
			10	200*‡			
IIb	4	IV 300*, IP 300*			17	1/1	9/10
III	4	IV 100†			26	0/7	0/70

* $(C_3H \times T_6)F_1$ cells. † $(C_3H \times A)F_1$ cells. ‡ Followed 2-minute exposure to CCl_4 fumes.

matory-cell infiltration and littoral-cell proliferation, it is possible that these were the only cells to have survived the preparative procedures.

Evidence against immigrant, donor-type leucocytes as the cause of falsely positive results may be inferred from the absence of mitoses from the histologic and cytologic preparations of six mice in which the peak of hepatic regeneration was missed. Presence of numerous metaphases in the spleen preparations suggested that the mitotic activity of the leucocytes was independent of liver parenchymal-cell regeneration. Thus, if the leucocytes were the source of mitoses seen in the cytogenetic studies, they should have provided comparable numbers of metaphases regardless of the degree of hepatic cell proliferation; this was not the case. It is possible that the Kupffer cells are descendents of donor reticuloendothelial cells, from the spleen-cell injections, that lodged in the liver; thus they may cause erroneous interpretation. However, "transformation" of these cells cannot be ruled out and would be an important finding in its own right.

The finding of T_6 markers in two tetraploid cells reinforces the interpretation that the hepatic parenchymal cells of the host have taken up genetic material from the donor spleen cells. Binucleate and polyploid cells are characteristically found in the parenchymal component of mouse livers (*13*).

Current concepts of cytogenetics offer no clue to the mechanisms by which exchange of nuclear material may occur at the chromosomal level. However, these three factors may be important:

1) Ability of liver cells to take up necrotic cellular material and to undergo cellular fusion has been noted (*14*). Perhaps ingested nuclear material may retain its genetic identity and influence the subsequent genetic composition of the host cell.

2) The hepatic regeneration took place while the circulating leucocytes, or potential trephocytes, were of donor type (*5*). Such circumstances may favor the uptake of foreign nuclear material, especially when the mitotic surge creates such a tremendous demand.

3) Severe radiation damage to the chromosomes of the host liver cells may manifest itself by defective mitotic activity of long duration (see *14*). Such alteration of the local, native supply of genetic material may enhance the transfer of nuclear building blocks from the circulating trephocytes.

Our findings may broaden concepts of radiation chimeras; they seem to support and extend the concept of leucocytes as nucleoprotein trephocytes. This report is the first evidence known to us of uptake in vivo of functional nuclear material, larger than nucleosides, from nursing cells. The possibility of a circulatory transport system, capable of transferring genetically active material, opens exciting areas for investigation. Two questions are especially important: Can the entire complement

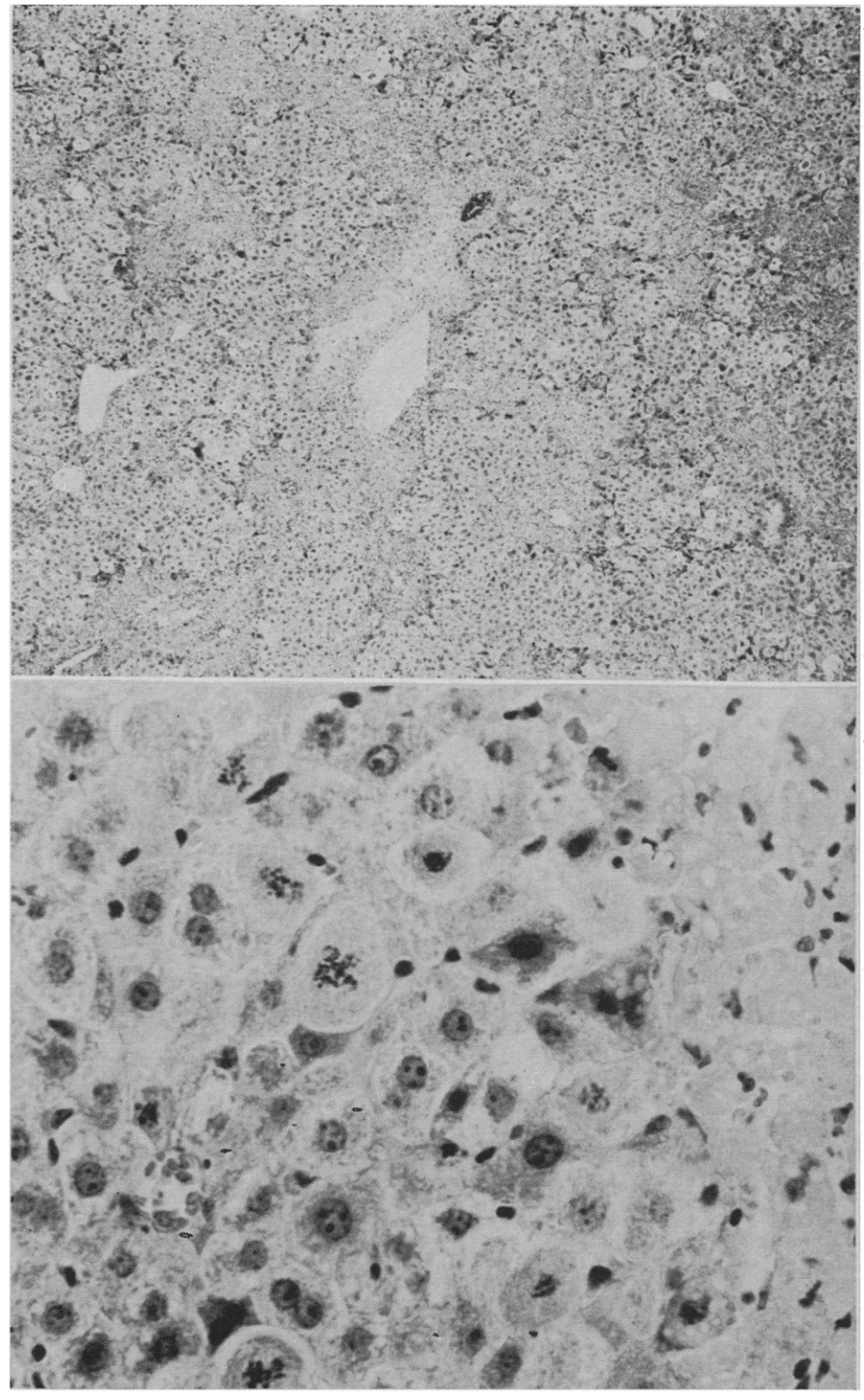

Fig. 2. Section of liver from a chimeric mouse killed 6 hours after injection of colchicine and 54 hours after injection of CCl_4. (Top) Typical centrilobular necrosis (hematoxylin and eosin; × 47). (Bottom) Same section, showing regeneration; all eight cells in mitosis are parenchymal in type; a small area of bland necrosis is on the right (hematoxylin and eosin; × 450).

of donor chromosomes be transferred to recipient cells? How many of the host cells develop donor characteristics? Both questions are fundamental to any hypothesis of genetic transformation of the host cells.

RICHARD C. HARD, JR.*
Pediatric Research Laboratories, Variety Club Heart Hospital, and *Department of Physiology, University of Minnesota, Minneapolis*
BIRGITTA KULLGREN
Department of Histology, Uppsala University, Uppsala, Sweden

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6. From the inbred C_3H_{He}/Bi stocks of Prof. C. Martinez, Univ. of Minnesota.
7. Factors: 220 kv; half-value layer, 0.89-mm copper; 15 ma; distance, 60 cm; 1.0-mm aluminum plus 0.25-mm copper filtration.
8. Ralston-Purina brand of oxytetracycline; 25 mg/ml.
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* Advanced research fellow, American Heart Association, 1964–5. Present address: Department of Pathology, University of Chicago, Chicago, Illinois.

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Glucose-6-Phosphatase in Tubular Endoplasmic Reticulum of Hepatocytes

Abstract. *The histochemical localization of glucose-6-phosphatase activity in neonatal mouse liver was studied under electron microscopy. The activity was demonstrated in the tubular endoplasmic reticulum, which pervades the glycogen areas of the cell during glycogenolysis. Activity was also demonstrated in the nuclear envelope and ergastoplasm.*

A number of electron microscopical studies have demonstrated a tubular form of endoplasmic reticulum in hepatocytes of mammals and other vertebrates (*1–5*). This tubular endoplasmic reticulum (TER) has been described as a branching or anastomosing latticework of agranular tubules in continuity with the ergastoplasm and, under physiological conditions, confined to cytoplasmic areas in which glycogen deposits are found. Peters, Kelly, and Dembitzer (*4*) have reported on the sequence of morphological events associated with the differentiation of TER during hepatic development in the mouse. TER is absent throughout the late gestational period when massive stores of glycogen accumulate. It becomes evident for the first time shortly after birth and proliferates extensively while glycogen rapidly declines on the first postnatal day. From these observations it was concluded that TER is related to glycogenolysis but not also to glycogen synthesis and storage as previous authors (*2*) had suggested. In view of biochemical information (*6*) that a rapid increase in glucose-6-phosphatase activity coincides with the early neonatal decline in glycogen, it was further postulated that the TER might function in glycogenolysis as a site of glucose-6-phosphatase activity. Histochemical studies on adult rat liver have shown the enzyme to be present in the ergastoplasm, nuclear envelope, and some smooth membrane (*7*). In order to determine whether TER is also a site of glucose-6-phosphatase activity the present histochemical study utilized the developing mouse liver at an age when hepatocytes are known to exhibit large aggregates of TER.

Samples of livers from 23- to 27-hour-old strain C3H mice were fixed for 1 hour in a 4-percent glutaraldehyde solution at *p*H 7.1. This fixative, as well as the subsequent wash, incubation medium, and postfixative, contained 0.05*M* sodium cacodylate as buffer. By the addition of 0.35*M* sucrose in the latter three solutions the osmolality throughout all the procedures was maintained at approximately 550 milliosmoles. The same *p*H was used in all solutions except the incubation medium, which was at *p*H 6.8. Low temperatures were maintained during fixation with an ice-water bath and during the wash period by refrigeration at 1°C; incubation, however, was at room temperature. After removal of excess glutaraldehyde by washing the samples for 2 hours, 35-μ frozen sections were obtained. The incubation media consisted of the cacodylate-sucrose solution and 3 m*M* lead nitrate, either with or without the addition of 4 m*M* substrate. The test substrate was glucose-6-phosphate, and the control substrates were fructose-6-phosphate; ribose-5-phosphate; fructose-1,6-diphosphate; adenosine monophosphate; β-glycerophosphate; and adenosine triphosphate. Incubation time for glucose-6-phosphate was 3 to 15 minutes, and for the control substrates, 15 minutes. After incubation, the sections were postfixed 1 hour in 1-percent osmic acid, dehydrated in graded concentrations of ethanol, and embedded in Maraglas resin prior to thin-sectioning for electron microscopy. Thin sections were studied either unstained or stained with lead citrate and uranyl acetate, used singly and in combination. For light microscopy 10-μ sections were handled similarly through the incubation, after which they were rinsed briefly in the cacodylate-sucrose wash solution and treated with dilute ammonium sulfide. An additional series of 10-μ sections was incubated in acetate buffer at *p*H 5.0 for 15 minutes prior to a 15-minute incubation in glucose-6-phosphate medium. This procedure is known to inhibit glucose-6-phosphatase activity while not affecting acid and alkaline phosphatases (*8*).

Under electron microscopy, the sections incubated in the glucose-6-phosphate medium showed lead reaction product in the ergastoplasm and TER (Fig. 1, top), and nuclear envelope. No reaction product appeared in the pericanalicular bodies, microbodies, the Golgi complex, mitochondria, or plasma membrane. With incubation periods of 10 to 15 minutes the reaction product appeared as large, very dense, closely spaced clumps often filling long stretches of the cisternae (Fig. 1, top). The ergastoplasm and aggregates of TER were easily distinguishable, even