indeed indigenous to the tumor cells and relatively independent of the host organism.

The GW-39 and GW-77 tumors growing in the cheek pouches of golden hamsters were excised, and 1 g of each was homogenized in 19 (GW-39) or 9 (GW-77) volumes of distilled water. The homogenate was centrifuged at 12,000*g* for 30 minutes. The supernatant was assayed for antigen content. Three other heterotransplantable tumors derived from human surgical tumor specimens, but which were found to be highly malignant in hamsters, were used as controls. These three tumors had species-specific properties more consistent with hamster than with human tissue (GW-176, originally a gastric carcinoma; GW-365, originally an adenocarcinoma of the lung; and GW-478, originally a gastric lymphoma) (*6*). The radioimmunoassay developed by Hansen *et al.* (*3, 7*) was used to detect CEA. Briefly summarized, known quantities (0 to 25 ng) of CEA diluted in 10 ml of 0.01*M* ammonium acetate (*p*H 6.8) were incubated with 0.1 ml of a 1 : 2500 dilution of goat antiserum to CEA (*8*) for 30 minutes at 45°C. A portion (2.4 ng) of [^{125}I]CEA, prepared and labeled according to the method of Thomson *et al.* (*9*), was then added to complex (with the antiserum in excess) after the first incubation with unlabeled CEA, and again incubated as before. The reaction was stopped by addition of 5 ml of zirconyl phosphate gel (*10*), which adsorbs the [^{125}I]CEA-antigen-antibody complex at *p*H 6.25, but not the free [^{125}I]CEA. The antigen-antibody complex was then sedimented by centrifugation, washed in 0.1*M* ammonium acetate buffer (*p*H 6.25), and centrifuged again. The pellet was used for counting radioactivity in a Packard gamma scintillation counter. The lower the counts, the greater is the amount of antiserum to CEA that is complexed by the original unlabeled CEA in the mixture, and is thus unavailable for precipitation with [^{125}I]CEA. Hence radioactivity (counts per minute) and quantity of CEA (nanograms) are inversely proportional. A standard curve was then established with known quantities of CEA (Fig. 1); the amount of antigen present in our tumor samples is deducible from the ^{125}I (counts per minute) values obtained in a repetition of the assay.

Table 1 provides the final average values of CEA per gram of each of the five tumors examined. The high amount of CEA in the GW-39 and GW-77 neoplasms not only agrees with other evidence that they retain properties characteritsic of human cells despite their long-term propagation in hamsters (*4, 11*), but supports their high degree of functional differentiation in this animal model. Our repitition of this assay for CEA in GW-39 and GW-77 tumors of other transplant generations has confirmed these results. Conversely, the lack of CEA in the other three neoplasms does not permit us to decide whether they are human, hamster, or human-hamster hybrid tumors.

Table 1. Average CEA content of five tumors of human origin continuously propagated in hamster cheek pouches.

Tumor	Passage No.	CEA content (μg/g)
GW-39	94	162
GW-77	102	41
GW-176	234	0
GW-365	155	0
GW-478	118	0

Our radioimmunoassay for CEA is not identical to that used by Thomson *et al.* (*9*), since we are only measuring an ion-sensitive site on CEA (*7*). This antigenic site has been found *not* to be restricted to gastrointestinal cancers (*3*). In colonic tumors, however, it can be considered as identical with Gold and Freedman's CEA. It thus appears that our GW-39 and GW-77 tumors contain CEA and an antigenic site on CEA similar to that found in many different human cancers (*3*). The capacity of these tumors to continue to produce such antigens when serially grafted in xenogeneic hosts constitutes strong evidence that these antigens are indeed specific to the tumors themselves and not products of any peculiar interaction between tumor and host. Accordingly, our results do not support the view of Apffel and Peters (*12*) that such tumor-specific antigens as CEA are "neither original constituents nor products of tumor cells," but glycoproteins produced by the liver (so-called "symbodies") in response to the tumor and coating the tumor cells. In order for us to accept this thesis, we would be obliged to prove the production of human glycoproteins in hamster hosts. The presence of a human tumor-associated antigen in our tumor xenografts introduces a new aspect to heterotransplantation of human tumors, and perhaps also a unique approach to studying the biogenesis of such human tumor-associated antigens in an animal model.

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4 October 1971

Electric Enhancement of Bone Healing

Abstract. *A human congenital pseudarthrosis of the tibia, unresponsive to conventional treatment, was stimulated to healing by direct electric current. The method was modeled after prior experimental work in vivo in rabbits. X-ray photographs, histological techniques, and electron microscopy confirmed the presence of newly formed bone in the defect region.*

Recent experiments (*1–7*) dealing with electrical stimulation of bone tissue may be divided into two types. *Electrode-sensitive* experiments (*3, 8–10*), which tend to be equivocal, relate to remodeling effects at the electrode sites with accretion occurring at the negative electrode and resorption at the positive. Other investigators have performed the *current-sensitive* experiments (*1, 4, 7*) which evaluate the effects of current in promoting healing of lesions placed *between* the electrodes. The latter method prevents misinterpre-

tation of spurious results occurring at the electrode sites due to (i) the tissue–foreign body interactions, (ii) localized ionic imbalances, and (iii) possible heating effects. Our group (*5*) placed such experimental lesions between electrodes in rabbit femora and demonstrated that direct current in the range of 2 to 4 μa increases the normal healing process by a factor of 2 when compared to nonstimulated controls, in agreement with Yasuda *et al.* (*1*) and Iida (*2*).

We now describe the first case of human congenital pseudarthrosis of the tibia successfully treated with electric current; in this case we used the same experimental methods that produced bone consolidation in rabbits. Congenital pseudarthrosis is a rare, local dysplasia in which the possibility of effecting union by conventional methods is scant.

The pseudarthrosis in a 14-year-old male was followed since his birth. The child was braced until he was 4 years old, when he underwent a posterior bone graft which healed the defect. At the age of 11, the patient sustained trauma to the tibia, which fractured through the old pseudarthrosis site. He was treated with casting for 6 months. There was no evidence of healing. He then underwent an open reduction with the application of dual onlay bone grafts. The grafts resorbed and the nonunion persisted. One year later he was operated on again and underwent reversal of the diaphysis of the tibia which included the pseudarthrosis, with the concomitant insertion of an intramedullary rod. The defect did not heal despite rigid immobilization for 12 months.

As an alternative to amputation of the limb, in October of 1970, direct electric current was passed across the pseudarthrosis defect and beginning bone union was obtained within 4 months.

Prior to electrical treatment, the previously inserted intramedullary rod (Fig. 1A) was removed; there was an obvious nonunion, with motion at the fracture site. A biopsy of the pseudarthrosis was performed. Two drill holes (0.25 cm) in diameter were made 1.9 cm proximal to and distal to the area of nonunion; platinum electrodes were inserted into the medullary cavity of the tibia through the drill holes. The platinum was insulated with shrinkable tubing up to the periosteum, such that the current path traversed bony tissue on both sides of the defect. A Steinman pin was placed in the calcaneus and proximal tibia to maintain alignment, the skin was sutured around the electric leads, and a long, leg plaster cast was applied.

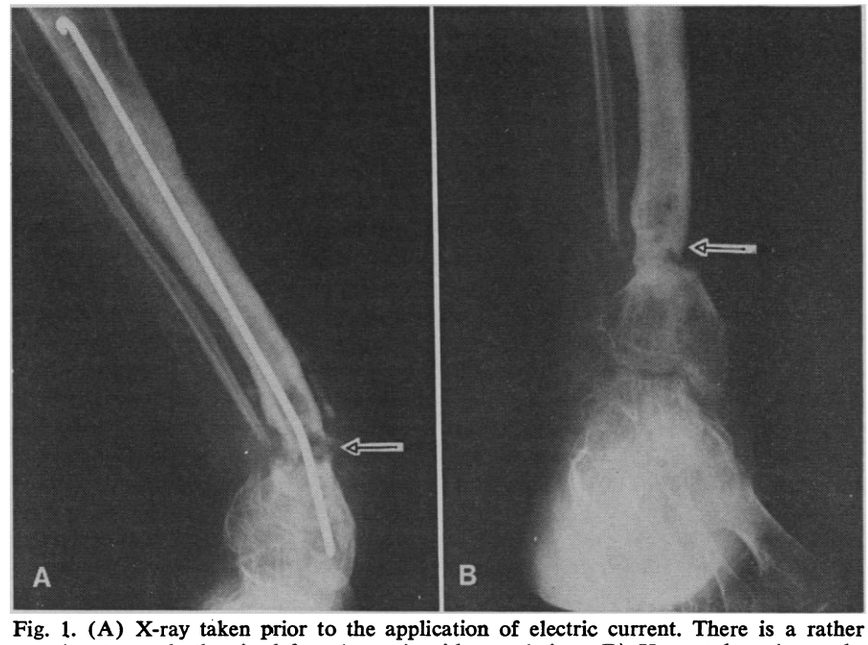

Fig. 1. (A) X-ray taken prior to the application of electric current. There is a rather prominent pseudarthrosis defect (arrow) with angulation. (B) X-ray taken 4 months after continuous application of 4 μa of current. The healing site (arrow) is being replaced by bone.

The external circuit for the power supply (Fig. 2) consisted of two D cells (3 volts) in series with a 0- to 15-μa meter and a resistance of 0.63 megohm. The effective tissue resistance between the implanted electrodes was 0.14 megohm. The current, monitored continuously with a strip-chart recorder, did not vary from the 3.9 μa reached after completion of the surgical procedure. Thus, the potential difference across the platinum electrodes was 0.55 volt. During the entire treatment period (125 days) the current was applied at least 92 percent of the time. A total of 39 coulombs of charge was supplied to the patient during this treatment. Except for extremely brief checkout procedures, the polarity (distal lead positive) was kept the same during the treatment period.

Two months after electrical treatment was begun, the Steinman pins were removed, and 2 months later the cast and platinum electrodes were removed. The area was surgically inspected and a biopsy was taken. There was no evidence of motion at the pseudarthrosis site upon manipulation. X-ray photographs, histological studies, and electron microscopic investigations revealed beginning bony union (Fig. 1B).

Electron microscope studies of the pseudarthrosis before treatment (Fig. 3) reveal marked differences between bone and defect area. The bone at this transition area stains more densely (histologically and in electron microscope studies), suggesting the possibility of a barrier that prevents the bone from growing into the defect. The inset illustrates collagen coming from bone and forming a major component of the defect. However, bone and collagen appear normal. Some cells found in this defect area are typical fibrogenic-like cells with rough endoplasmic reticulum and normal mitochondria.

Electron microscope pictures taken after the electrical treatment revealed that collagen formation and osteogenesis was occurring in the former defect. Figure 4 shows a fibrogenic cell whose prominent features are a large nucleus, numerous cytoplasmic fibrils, mitochon-

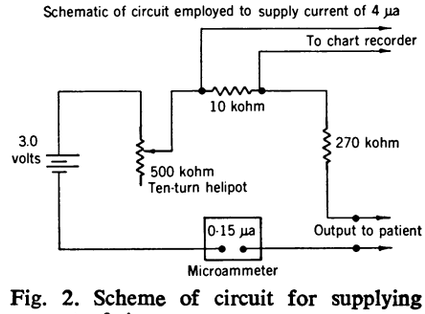

Fig. 2. Scheme of circuit for supplying current of 4 μa.

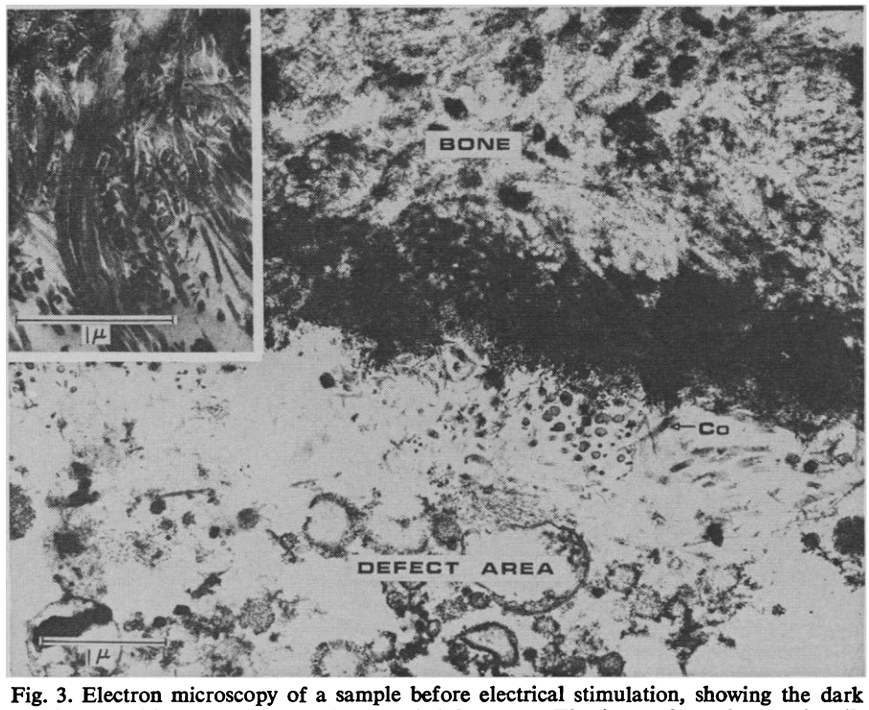

Fig. 3. Electron microscopy of a sample before electrical stimulation, showing the dark staining transition zone between bone and defect area. The inset of another section illustrates some of the prominent collagen fibers (*Co*) coming from the bone and entering the defect. This inset is a representative picture of the collagenous tissue lining the transition zone. The preparation was fixed with 10 percent formaldehyde buffered to *p*H 7.3 with phosphate. It was then postfixed with 1 percent osmium and stained with uranyl acetate and lead citrate.

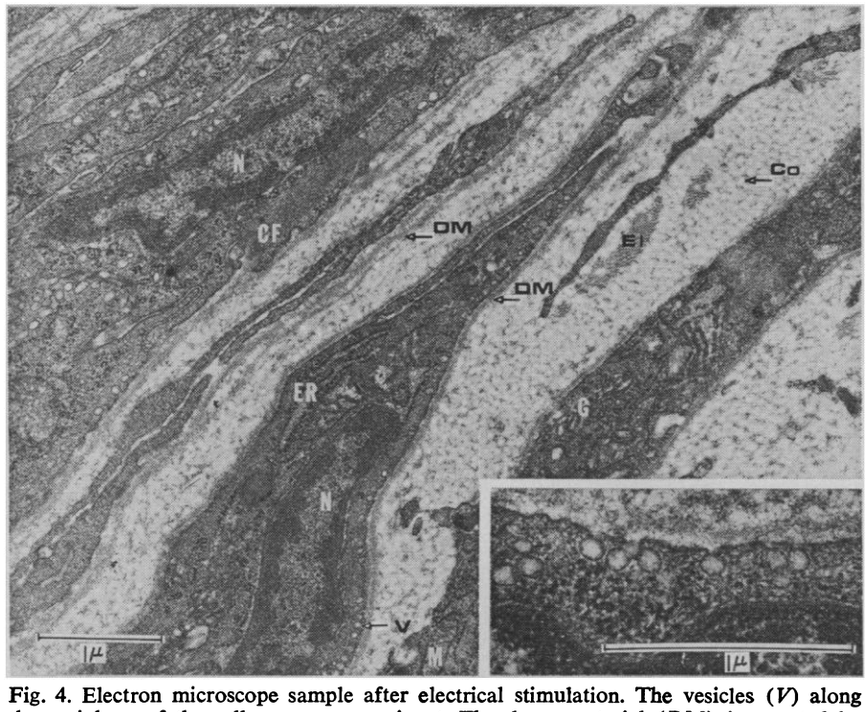

Fig. 4. Electron microscope sample after electrical stimulation. The vesicles (*V*) along the periphery of the cell are very prominent. The dense material (*DM*) is secreted by these cells. The inset is higher magnification of these vesicles. *N*, nucleus; *ER*, endoplasmic reticulum; *G*, golgi complex; *El*, elastin; *CF*, cellular fibrils; *Co*, collagen; *M*, mitochondria. The preparation was fixed with 3 percent glutaraldehyde and buffered at *p*H 7.4 with cacodylic acid. Postfixation and staining as in Fig. 3. The biopsy specimen was obtained 4 months after the one taken for Fig. 3. The area shown in this electron micrograph corresponds to the defect region shown in Fig. 3, and the magnification is exactly the same (except for the inset) as in Fig. 3.

dria, rough endoplasmic reticulum, and Golgi complexes. The presence of rough endoplasmic reticulum indicates that these cells are actively synthesizing protein (*11*). Vesicles near the periphery have been observed by others (*12, 13*). It should be emphasized, in the present case, that scores of such small vesicles line the cell membranes. These vesicles are secretory, as is indicated by the dense band of material of uniform width surrounding the cells. This dense band of material is layered, two or three bands being sequentially secreted near one cell interspersed with collagen. One must consider the possibility that the vesicles may secrete mucopolysaccharides, precollagen materials, and apatite precursors (*13, 14*). The inset photograph is another view of these membrane-bound vesicles, secreting into the extracellular environment.

It is clear that electrical potentials play an important role in directing the architectural and structural development of bone. The success of applied electrical current in treating this difficult orthopedic condition warrants, in our judgment, its application to other less rare problems, such as nonunion and delayed healing of fractures. Obviously, there are further ramifications. Understanding the coupling mechanisms linking electricity to basic cellular phenomena represents, in our opinion, a problem of primary biophysical importance.

We realize that this is only one case. However, the foundation for this report rests upon prior animal experimentation. In addition, the rarity of this disease precludes reporting more than one case at this time. The decision to experiment first with congenital pseudarthrosis in a human subject instead of delayed union was prompted by the treatment of choice, in this case amputation. In addition, the difficulties presented by this disease represent an extreme test of electrical stimulation. Fundamentally, we are motivated in presenting this report by its potential importance.

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15. We thank Adam Narbut for technical help in assembling the power unit and Dr. John Bruno for clinical assistance. This work was supported by an NIH grant.

19 August 1971; revised 18 October 1971 ■

Mercury Concentrations in Museum Specimens of Tuna and Swordfish

Abstract. *The mercury levels of museum specimens of seven tuna caught 62 to 93 years ago and a swordfish caught 25 years ago have been determined by instrumental neutron activation analysis. These levels are in the same range as those found in specimens caught recently.*

Although there is considerable evidence that man-made mercury pollution of freshwater rivers and lakes has in some instances definitely increased the mercury levels in freshwater fish (*1*), there is relatively little information as to whether man-made sources of mercury pollution of the oceans have significantly increased the mercury levels in wide-ranging ocean fish. The large-scale confiscation of swordfish and the lesser-scale confiscation of tuna, as a consequence of the establishment by the U.S. Food and Drug Administration of a maximum permissible level of 0.5 part per million (ppm) for mercury in fish, have been interpreted by many persons as a consequence of the discovery of man-made pollution. Goldwater (*2*) and Hammond (*3*), however, have questioned the validity of this assumption. For example, Hammond estimates that the total amount of mercury processed by man since 1900 would, if put into the world's oceans and well mixed, increase the average mercury concentration of seawater (approximately 0.1 part per billion) by at most 1 percent—an essentially negligible increase (*4*).

To explore this subject further, we obtained samples of seven tuna and one swordfish that have been preserved in museums for varying lengths of time. The seven tuna (each slightly under 0.6 m in length) were caught between 1878 and 1909 and were preserved in toto, first in formaldehyde and then in alcohol, in the Smithsonian Institution. The single swordfish was caught in 1946, and its head was preserved in 40 percent isopropanol at the Museum of the California Academy of Sciences (*5*).

Samples of the flesh (*5*, *6*) of these eight fish were analyzed for mercury by means of purely instrumental thermal-neutron activation analysis (NAA). In each case, a weighed sample (about 1 g) was sealed in a quartz ampule, activated for 3 hours in the nuclear reactor of the University of California at Irvine at a thermal-neutron flux of 0.7×10^{12} n cm^{-2} sec^{-1}, and then counted (still sealed in quartz) with a gamma-ray spectrometer [36-cm^3 Ge (Li) detector, 4096 channel], after 3 to 7 days decay. Several freshly prepared aqueous mercury standard solutions, also sealed in quartz (*7*), were activated along with the fish samples and counted with the same gamma-ray spectrometer, under the same conditions. The mercury contents of the samples and standards were measured unambiguously by means of the

Table 1. Mercury levels in museum and recent specimens of tuna and swordfish, determined by instrumental neutron activation analysis.

Description	Date	Samples	^{197}Hg measurements	Hg, mean ppm and S.D. of mean	
				Wet-weight basis	Dry-weight basis
		Museum specimens			
Skipjack tuna, 21563*, Massachusetts	1878	2	4	0.27 ± 0.02	0.91 ± 0.06
Skipjack tuna, 21852*, Massachusetts	1878	1	2	0.64 ± 0.02	1.51 ± 0.04
Albacore tuna, 26873*, California	1880	2	8	0.27 ± 0.03	0.59 ± 0.06
Bluefin tuna, 37928*, Woods Hole	1886	1	4	0.38 ± 0.01	1.14 ± 0.04
Skipjack tuna, 41901*, San Diego	1890	2	4	0.45 ± 0.02	1.05 ± 0.04
Skipjack tuna, 52704*, Hawaii	1901	2	4	0.42 ± 0.02	0.92 ± 0.01
Skipjack tuna, 194901*, Philippines	1909	2	4	0.26 ± 0.01	0.53 ± 0.02
					0.95 ± 0.33‡
Swordfish, Baja California	1946	6	22	0.52 ± 0.10	1.36 ± 0.31
					1.36 ± 0.31‡
		Recent specimens			
Albacore tuna, fresh, California		1	2	0.13 ± 0.01	0.44 ± 0.05
Skipjack tuna, fresh, Pacific		1	2	0.18 ± 0.03	0.62 ± 0.10
Albacore tuna, canned (A), in water†		1	2	0.48 ± 0.04	1.53 ± 0.12
Albacore tuna, canned (A), in oil†		1	2	0.30 ± 0.02	0.66 ± 0.04
Albacore tuna, canned (B), in water†		1	2	0.38 ± 0.03	1.29 ± 0.11
					0.91 ± 0.47‡
Six swordfish, fresh, California		14	40	0.23 to 1.27	0.94 to 5.08
					3.1 ± 1.5‡

* Smithsonian reference number. † The letters (A) and (B) are used to distinguish two brands of tuna. ‡ Mean mercury concentration.