These initial studies have shown that cells of the inner ear differ in their vulnerability to viral infections. Each virus caused a different pattern of infection: influenza virus infection was limited to perilymphatic structures, mumps virus to endolymphatic cells, and measles virus involved both perilymphatic cells and the organ of Corti and the crista. All three viruses infected ganglion cells of the eighth cranial nerve. It is significant that both measles and mumps viruses infected endolymphatic structures or cells in the organ of Corti, since an endolymphatic labyrinthitis has been implicated as the original cause of damage in patients with deafness associated with these two virus infections[1-3].

This work was supported by grants from the United Cerebral Palsy Research and Educational Foundation and the National Institutes of Health.

L. E. Davis
Susan Shurin
R. T. Johnson

Department of Neurology,
Johns Hopkins University,
School of Medicine,
Baltimore, Maryland 21205

Received November 12, 1974.

1 Lindsay, J. R., Davey, P. R., and Ward, P. H., *Ann. Otol.*, 30, 918–935 (1960).
2 Lindsay, J. R., and Hemenway, W. G., *Ann. Otol.*, 63, 754–771 (1954).
3 Lindsay, J. R., Caruthers, D. G., Hemenway, W. G., and Harrison, M. S., *Ann. Otol.*, 62, 1201–1218 (1953).
4 Myers, E. N., and Stool, S., *Laryngoscope*, 78, 1904–1914 (1968).
5 Davis, G. L., and Strauss, M., *Ann. Otol.*, 82, 584–593 (1973).
6 Sando, I., Masuda, Y., Wood, R. P., and Hemenway, W. G., *Ann. Otol.*, 80, 826–834 (1971).
7 Stuart-Harris, C. H., *Lancet*, i, 497–499 (1939).
8 Kilham, L., *J. Am. med. Ass.*, 146, 1231–1232 (1951).
9 Burnstein, T., Jensen, J. H., and Waksman, B. H., *J. infect. Dis.*, 114, 265–272 (1964).
10 Stephens, C. B., *Acta Oto-laryng. Suppl.*, 296, (1972).
11 Sainte-Marie, G., *J. Histochem. Cytochem.*, 10, 250–256 (1962).

Effect of electric fields on growth rate of embryonic chick tibiae *in vitro*

Numerous investigators have reported work on the stimulation of bone growth by electric currents in the microampere range, with varying degrees of success. Such work, along with the various tentative theories of the underlying mechanisms, have been summarised by Bassett[1], and some quantification of the effect has been attempted[2,3]. Hambury *et al.*[3] concluded that the necessary crudity of the surgical techniques involved, and the overriding effects of the trauma militated too severely against accurate quantification of the electrically-stimulated bone growth, and proposed[4] that *in vitro* experiments might yield more definitive results. They also concurred with other workers that if electric currents do influence bone growth, then so should electric and magnetic fields, and Norton[5] has already performed work in this area *in vivo*, using chickens. We report that the gross development of embryonic chick tibiae grown *in vitro* is affected by a pulsed, transverse electric field, but that no significant changes were observed when a static, non-varying field was used.

Between one and three bones were placed in each of eight Petri dishes, four of the dishes being subjected to a 1,000 V cm^{-1} electric field, and four being placed between the inactive plates of a second control apparatus (see Table 1 for detailed method). Each bone was measured before and after a 9 d incubation period. The ratio of the final to the initial bone length was calculated for each bone, giving the fractional increase in length in each case. Also, the experiments were systematised so that the two ratios obtained for the bones from each embryo could be related and compared. Where bones distorted somewhat, the lengths of the longitudinal median lines were measured incrementally. Table 1 gives the results in terms of the fractional increase in length

Table 1 Effect of an invariant electric field on embryonic chick tibiae growth *in vitro*

Batch	Fractional length increase			'One-tail' t test		
	Test	Control	Test/Control	t	d.f.	Confidence limit
A	1.50	1.50	1.00			
	1.42	1.57	0.90			
	1.64	1.92	0.85			
	1.29	1.43	0.90	1.52	6	>5% (less growth)
	1.67	1.67	1.00			
	1.58	1.67	0.86			
B	1.46	1.26	1.16			
	1.27	1.24	1.02			
	1.31	1.21	1.08			
	1.18	1.25	0.94	1.35	7	>10%
	1.34	1.28	1.05			
	1.34	1.25	1.07			
	1.58	1.44	1.04			
	1.43	1.38	1.04			
C	1.46	1.60	0.91			
	1.43	1.48	0.97			
	1.42	1.37	1.04			
	1.43	1.50	0.95	0.68	7	Not significant
	1.57	1.48	1.06			
	1.49	1.44	1.03			
	1.44	1.41	1.02			
	1.30	1.47	0.88			
D	1.59	1.34	1.48			
	1.41	1.48	0.95			
	1.48	1.44	1.03			
	1.43	1.45	0.99	1.67	7	>5%
	1.64	1.21	1.36			
	1.50	1.51	0.99			
	1.39	1.35	1.03			
	1.46	1.46	1.00			
E	1.30	1.16	1.12			
	1.19	1.16	1.03			
	1.19	1.15	1.03			
	1.20	1.19	1.01	0.65	6	Not significant
	1.14	1.09	1.05			
	1.20	1.21	0.99			
	1.14	1.26	0.90			
Overall	1.40	1.39	1.01	0.21	37	Not significant

The 8–9-d-old embryos were aseptically decapitated, and extracted and the tibiae carefully excised. Each bone was then placed on a thin siliconised cellulose acetate mat supported by a 3 mm thick sponge of similar material, both items being chemically clean and sterile. Each assembly, consisting of a sponge, mat and two or three bones was placed in a 35 mm disposable polystyrene Petri dish containing sufficient medium to thoroughly soak the sponge without immersing the bone. The bones must be allowed free exposure to the incubator atmosphere of CO_2–air (5:95). Chemically-defined BGJ-b nutrient medium[6] was used, as described previously. In particular, the medium was changed every 2 d by gently removing the cellulose mat and placing it on a new sponge in a new Petri dish. This enabled most of the metabolic by-products of the preceding 2 d to be removed in the interstices of the sponge, the mat to which the bones adhered being too thin to retain more than a very small portion of these products. The bones were incubated for 9 d at 37 °C, after which they reached their maximum length. This period was defined by trials in which the length of each bone was measured and plotted against time until the graph became horizontally asymptotic. These measurements were made outside the Petri dishes using a micrometer scale mounted on a dissecting microscope eyepiece. After excision, one bone from each embryo was placed in a Petri dish inserted between the plates of the apparatus applying the electrical field. The other bone was placed in a dish within a similar, but inactive, apparatus as control. Both sets were placed in the same incubator. The apparatus for applying the field consisted essentially of two circular stainless steel plates, 12.7 cm in diameter, and held 1.4 cm apart by a Perspex framework. A Keithley model 244 high voltage power supply was used to apply 1,400 V across the plates, so that a uniform invariant field of 1,000 V cm^{-1} existed between them, the lower plate being grounded. A Plexiglass disk, 3 mm thick, drilled with four holes 38 mm in diameter, was used to slide the four 35 mm Petri dishes into the space between the stainless steel plates. In the fully operating system, an interlock microswitch was incorporated into the incubator so that the opening of the door switched off the power supply. The stainless steel plates of the second apparatus, containing the controls, were short-circuited and grounded.

of each bone subjected to the field, compared with that for each corresponding control bone from the same embryo. The ratio of each pair of fractional increases is also included. Further, a 'one-tail' t test was applied to each batch, and also to the complete series. This shows that there is no significant difference between the test and control bone fractional increases.

A second series of experiments was carried out using a 1,000 V cm^{-1} field pulsed at approximately 1 pulse s^{-1} using a high voltage relay driven at this pulse repetition rate by a unijunction transistor circuit. The live (upper) plate was alternately connected to the −1,400 V supply and to ground by this means, so that a good square wave of approximately 1 : 10 mark–space ratio was obtained. Table 2 shows that for batch A, the chances that the pulsed electric field had no effect were no more than 0.5%; and for batches C and E, were no more than 2.5%. These results can therefore be considered significant, whereas those for batches B and D could not, being more than 10 and 25%, respectively. The 'one-tail' t test was also applied to the entire series taken as a whole (Table 2). The overall confidence limit was less than 0.5%, clearly very significant.

Bones were photographed in their Petri dishes at the end of each test to provide a record of their macroscopic appearance, then fixed in neutral formalin for 3 d. After manual processing, they were embedded in paraffin wax and sectioned longitudinally. The sections were then fixed to glass slides by means of a glycerin–albumin mix and dewaxed in a hot air oven. They were then regressively stained with haemotoxylin, counterstained with eosin, and examined with an optical microscope. This did not reveal any differences between test and control slides; nor did a macroscopic inspection of the photographs reveal any differences in growth patterns.

Table 2 Effect of a pulsed electric field on embryonic chick tibiae growth *in vitro*

Batch	Fractional length increase			'One-tail' t test		
	Test	Control	Test/control	t	d.f.	Confidence limit
A	2.56	1.64	1.56			
	2.70	2.10	1.29			
	2.36	1.86	1.27			
	2.62	2.21	1.24	4.49	6	<0.5%
	2.32	2.25	1.03			
	2.40	2.17	1.11			
	2.50	1.87	1.34			
B	2.66	2.25	1.18			
	2.40	2.14	1.12			
	2.24	2.15	1.04			
	2.15	2.00	1.07	1.24	7	>10%
	2.00	1.91	1.05			
	2.30	2.43	0.95			
	2.43	2.09	1.16			
	2.05	2.24	0.92			
C	1.80	1.45	1.24			
	2.11	1.50	1.41			
	1.68	1.65	1.02			
	1.62	1.43	1.13	3.32	7	<2.5%
	2.06	1.37	1.50			
	1.83	1.62	1.13			
	1.74	1.65	1.05			
	1.83	1.78	1.03			
D	1.67	1.52	1.10			
	1.45	1.50	0.97			
	1.74	1.67	1.04			
	1.67	1.74	0.96			
	1.32	1.57	0.84	0.58	9	>25%
	1.39	1.61	0.86			
	1.56	1.05	1.49			
	1.58	1.72	0.92			
	1.67	1.25	1.34			
	1.64	1.60	1.02			
E	2.00	1.96	1.02			
	2.04	1.92	1.06			
	2.03	1.74	1.17			
	2.36	1.68	1.40	2.80	7	<2.5%
	2.00	2.03	0.99			
	1.90	1.87	1.02			
	2.22	1.82	1.22			
	1.90	1.76	1.08			
Overall	2.01	1.80	1.12	2.81	40	<0.5%

We propose to grow further batches and perform more comprehensive tests including cell counts, wet and dry weight measurements and DNA content analyses, and to determine the effect of variation in field strength, frequency, waveform, and the spatial relationship of the field to the bone.

If our trials are significant, it can be concluded that there is present in the embryonic bone a transducer mechanism which allows the electric field to interact directly and modify (at least) the growth rate. Since the bones are in a pre-osseous phase, this mechanism clearly cannot be related to any properties of hydroxyapatite, thus ruling out at least one mechanism, the collagen–hydroxyapatite semiconductor interface[1]. In addition, because only pulsed electric fields (as opposed to invariant fields) have an effect, it is likely that repeated cycles of charge separation are involved. This is equivalent to applying very small pulsed currents, and since such currents can also be induced by pulsed magnetic fields, a series of experiments using such fields has been initiated. Note that Bassett *et al.* have reported that pulsed magnetic fields do modify bone growth patterns *in vivo*[7].

We thank Mr H. J. Hambury of Swansea, who first suggested the use of bone cultures in the context of our work, and Professor C. A. L. Bassett of the Columbia University Orthopaedic Centre, for his encouragement and advice.

JOSEPH WATSON
WILLIAM G. DE HAAS*
SONIA S. HAUSER*

Department of Electrical Engineering,
University College, Swansea,
University of Wales,
Singleton Park, Swansea, UK

Received October 23, 1974; revised January 14, 1975.

*Present address: Division of Surgery, Faculty of Medicine, University of Calgary, Canada.

1 Bassett, C. A. L., *The Biochemistry and Physiology of Bone*, Part II ch. 2 (Academic, New York, 1971).
2 Lavine, L. S., Lustrin, T., and Shamos, M. H., *Nature*, 224, 1112-1113 (1969).
3 Hambury, H. J., Watson, J., Sivyer, A., and Ashley, D. J. B., *Nature*, 231, 5299 (1971).
4 Hambury, H. J., Watson, J., Toole, A., Sivyer, A., and Ashley, D. J. B., *Ann. N.Y. Acad. Sci.*, 238, 508-517 (1974).
5 Norton, L. A., *ibid.*, 466-476.
6 Biggers, J. D., *Expl Cell Res.*, 25, 41-58 (1961).
7 Bassett, C. A. L., Pawluk, R. J., and Pilla, A. A., *Science*, 184, 575–577 (1974).

Left-handed to right-handed helix conversion in *Salmonella* flagella

ALTHOUGH bacterial flagella can assume various helical configurations[1-3], the 'normal' and 'curly' configurations are encountered most frequently in nature. In *Salmonella*, normal and curly flagella have helical pitches of about 2.3 and 1.1 μm, respectively[4]. Macnab and Koshland[5] observed that normal flagella of living *Salmonella* were left-handed helical filaments, as shown for *Proteus* and *Bacillus*[6]. Flagella with this handedness and rotating counterclockwise (looking in the direction of travel), would cause helical waves to propagate distally and provide forward thrust[7-9]. Normal flagella can transform reversibly into the curly type when physiological conditions, such as pH, are varied[10,11]: this has been termed biplicity. Asakura *et al.*[12], using *Salmonella* strain SJ670 and others, found that reconstituted flagellar filaments also could be transformed reversibly, although they could not control this transformation completely. SJ670 is a motile strain that produces