Table 2 Hypotheses for the Function of Cornicle Factor

I Slow release—intermittent, partial opening of cornicle valve to release small amounts of volatile factor. No droplet visible.
- (*A*) Territorial— (1) intra-specific spacing of aphids. (2) prevent influx of other species.
- (*B*) Dispersal— Induce migration in aphids to the new growth of the plant.

II Fast release—visible droplet released.
- (*C*) Defence— (1) Self-individual aphid repels a predator. (2) Social—individual aphid's release of factor when attacked by a predator causes other aphids to disperse or drop from the leaf.
- (*D*) Excretory— Release of metabolic matter or toxic plant compounds.

addition contaminate its mouthparts when eating an aphid. Hypothesis *B* is attractive, also, because emerging radish leaves will have a high density of fairly mature *M. persicae*, which migrated from other regions of the plant. Aphids on older leaves may sense the emergence of new leaves and signal for dispersal.

A-1 is attractive even though the intermittent, partial opening of the cornicle valve has not been observed. *M. persicae* are grouped near the base of the cotyledons on radish plants in a fraction of the space available to them, but are dispersed over much of the new leaf surfaces (Fig. 1*A*). Initial attempts to extract the active factor indicate that some difficulty may be expected with a bioassay as ether, one of the solvents employed, repels aphids.

The presence of a repellent or alarm odour in aphids is not unexpected, as many other species in the closely related Hemiptera possess alarm substances[5]. The significance of this odour in the natural biology of the aphid is unknown; it is not known either whether the spread of plant viruses for which aphids act as vectors would be enhanced or diminished by using the odour to control aphid infestations on economic plants.

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Stimulation of Partial Limb Regeneration in Rats

INJURY[1,2], nerves[3] and hormones[4] have been identified as essential for limb regeneration in amphibia. There seem to be differences in the "current of injury" between regenerating and non-regenerating types of amphibians[5]; partial limb regeneration can be induced in the latter type by simulating the "current of injury" of the regenerating form[6]. The cellular process of fracture healing in the amphibian is directly related to the electrical phenomena produced by the fractured bone[7] and maximally effective ranges for current density can be determined at the cellular level. This led to the concept of a control system the key element of which was the induction of

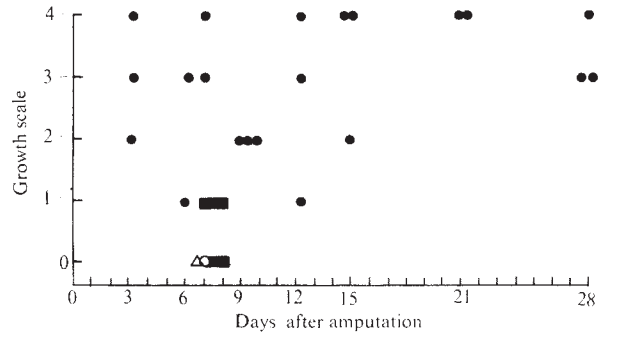

Fig. 1 Graphic representation of the results of all animals, control and experimental. The growth scale is as described in the text. ●, experimental series (twenty-two animals); ○, control series A (eight animals); ■, control series B (seven animals); △, control series C (two animals).

blastema formation in response to appropriate electrical factors[8]. The absence of regeneration in the mammal may therefore be due to the absence of adequate electrical factors. We report here the consequences of restoring factors which seem to be of practical as well as theoretical interest.

Measurements of the current produced by Smith's simple silver–platinum bimetallic couplings in Ringer solution indicated levels (5–15 nA) in excess of the optimal range determined for cellular stimulation in fracture healing. The insertion of a miniature 10 M ohm resistor at the junction of the silver and platinum wires resulted in a measured current closer to the desired level (3–6 nA). Several simple modifications of the basic device were manufactured to produce currents ranging from below to above the desired level and tested in a series of 21 day old, male, Sprague–Dawley rats. The experiment included three control series (A, B, C) and one experimental series with thirty-nine animals. All surgery was done under ether anaesthesia with aseptic precautions, each animal receiving 0.2 ml. of a long acting antibiotic (procaine penicillin, Pfizer) subcutaneously at the end of the surgical procedure. No hormonal supplement was used. Control series A consisted of eight rats with a guillotine type amputation through the right foreleg at the junction of the mid and lower thirds of the humerus (proximal to the distal epiphyseal plate) without the implantation of any device. Series B consisted of seven animals similarly amputated with the immediate implantation of a low current (0.001–0.1 nA) metallic device consisting of either two short lengths of No. 28 AWG silver soldered together (two animals), two similar sections of platinum wire (two animals) or a single length of silver wire (three animals). The solder joints and wires were encapsulated in medical grade silicon (Silastic-Dow Corning) except for 2–3 mm exposed wire at both ends. These (and all subsequent devices) were inserted beneath the remaining limb musculature, adjacent to the residual humeral shaft, with the distal end bent and inserted 2–3 mm into the medullary cavity. The proximal end was brought out through the shoulder muscles in the vicinity of the greater tuberosity and sutured in place to the superficial muscle fascia. Control series C consisted of two animals similarly amputated with the immediate implantation of a high current (5–15 nA) silver–platinum bimetallic coupling with no interposed resistor, similarly encapsulated with silicone. Twenty-two animals were used for the experimental series in which a device consisting of a silver–platinum coupling joined through a 10 M/ohm resistor and similarly encapsulated was inserted in the same fashion at the same amputation site. In all animals of this series, the platinum electrode was distal. All animals were maintained on standard laboratory rat diet with water *ad lib.* until they were killed by etherization. All control series were killed 7 days after amputation, while the experimental series were killed at intervals ranging from 3 to 28 days. No infections were noted in any animal of any series and no animals were discarded from any series. In each case, the implanted device was removed,

the residual upper extremity (humerus with attached musculature) was removed *en bloc*, fixed in buffered formalin, prepared in the usual manner, serially sectioned longitudinally and stained with haematoxylin and eosin. Growth was assessed on a scale of 0–4+, 0 being that consistently seen following simple amputation, 1+ any growth in excess of control but not as a recognizable blastema, 2+ presence of a recognizable blastema, 3+ a blastema with the differentiation of a specific tissue type such as cartilage, bone or muscle within the blastema and 4+ the appearance of an organized multi-tissue structure with anatomical characteristics appropriate to the morphological area.

Fig. 1 summarizes the results. Simple amputations without implantation of any device resulted in some subperiosteal osteogenesis and bridging of the cut surface with new bone and formation of a simple fibrous cap (Fig. 2*A*). No control animals with very low current devices (control series B) demonstrated growth in excess of 1+, while the animals with high current devices (control series C) demonstrated bone destruction and cyst formation (Fig. 2*F*). In the experimental group, only two animals were as low as 1+ with the remainder higher than this, including eight instances of 4+ growth. Seven of the experimental animals were killed at or before the seventh post-amputation day and may thus be directly compared with all

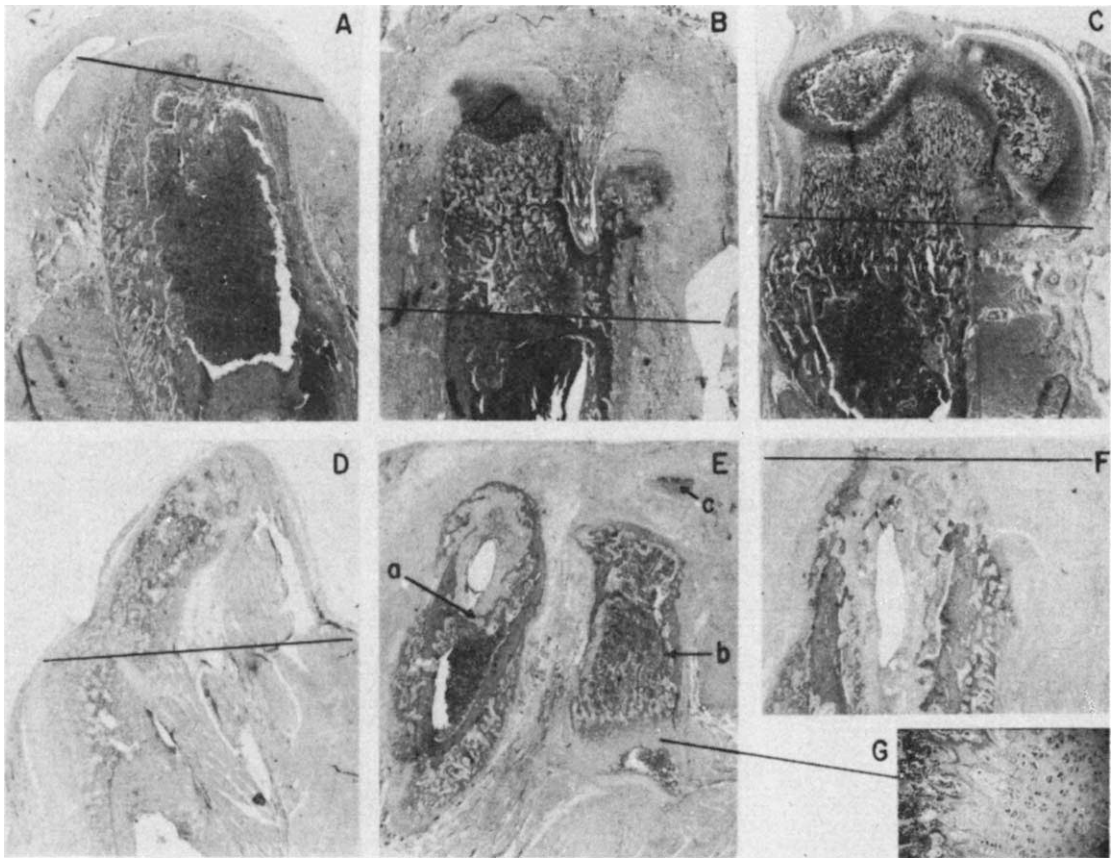

Fig. 2 Low power (× 10, unless otherwise noted) photomicrographs of longitudinal sections through the amputation site. All sections were stained with haematoxylin and eosin and the horizontal dark line indicates the approximate original level of amputation. All sections, except *G*, are oriented with the distal portion uppermost. The level of the original amputation was determined either by new bone which was clearly distinguishable from the old bone or by angulated or eccentric new growth on the original shaft. *A*, Control rat (series A, no implanted device) 7 days after amputation. The subperiosteal new bone growth and the tendency to bridge across the cut surface of the humerus are evident. *B*, Experimental rat (implanted device with 10 M ohm resistor) 14 days after amputation. Considerable growth in length has occurred with a cancellous bony shaft and two cartilaginous growth centres distally. New muscle formation was noted in this specimen distal to the cartilaginous "epiphyses". *C*, Experimental rat (implanted device with 10 M ohm resistor) 3 days after amputation. Again, considerable new bone growth has occurred, though less than in *B*. The distal portions have formed two well developed epiphyses with a well organized epiphyseal plate proximally and normal appearing joint type cartilage distally. The epiphyseal centres contain borders of cancellous bone and centres with apparently normal haematopoietic marrow. The 50% offset of the new growth on the shaft is apparent, the deviation occurring towards the side of the humerus to which the device was attached. *D*, Experimental rat (implanted device with 10 M ohm resistor), 7 days after amputation. A long "finger" of cancellous bone has grown from the original resection area. The distal portion of the growth is a reasonably well organized single epiphysis with a small centre of ossification and a proximal epiphyseal plate. This growth also was eccentric on the shaft and the residual shaft of the humerus is represented here by the tangential section visible at the lower border of the photomicrograph. The cancellous bone between this and the resection line is actually the normal subperiosteal bone growth sectioned tangentially. *E*, The same animal as in *D*, showing the supernumerary limb forming adjacent to the humeral shaft. This growth was also eccentric to the midline of the original shaft which is here represented by the tangential section (*a*). The supernumerary humerus (*b*) is well formed with a proximal head epiphysis, a well organized epiphyseal plate (*G*), a bony diaphysis containing normal appearing haematopoietic marrow and a distal cartilaginous plate. Distal to this structure is a zone of transversely organized fibrous tissue followed by a small osteo-cartilaginous structure (*c*) which is taken to represent the anlage for either radius or ulna. *F*, Control rat (series C, implanted device with no resistor). The bony resorption back from the original amputation level is particularly evident on the right side of the shaft in the photomicrograph as is the reactive cyst that formed near the site of the electrode implanted in the medullary cavity. *G*, Higher power (× 100) view of the proximal epiphyseal plate observed in the supernumerary humerus (*E*). This figure is rotated 90% from its corresponding placement in *E* to show the longitudinal organization of the epiphyseal plate. In this figure the right hand portion represents the proximal portion of the epiphyseal plate and the left hand portion is distal, closest to the bony diaphysis.

control groups. In this portion of the experimental group only one demonstrated 1+ growth, one 2+ growth, three 3+ and two 4+. In the remainder of the experimental group that went for periods longer than 7 days, a higher percentage of 4+ growth was noted. The commonest form of 4+ growth consisted of regrowth of the distal humerus complete with two epiphyseal centres. The majority were organized as shown in Fig. 2*B*, one was structurally indistinguishable from the normal except that it was laterally displaced by approximately half the diameter of the humeral shaft (Fig. 2*C*). In one case the longitudinal growth was strongly curved and contained only one epiphyseal centre (Fig. 2*D*). A larger than normal blastema, however, had formed in this case with a large mass extending proximally along the humeral shaft. Developing within this mass was a complete supernumerary humerus (Fig. 2*E*) with appropriate morphological appearance including a proximal epiphyseal plate (Fig. 2*G*), head epiphysis, bony diaphysis with a medullary cavity containing apparently normal haematopoietic marrow, a distal cartilaginous surface and an osteo-cartilaginous anlage of another bony structure distal to this. In all animals exhibiting 3+ or 4+ growth, there was formation of new, organized skeletal muscle distal to the transection line accompanied by ingrowth of nerves and blood vessels.

Studitsky[9] reported that rats could regenerate a single minced skeletal muscle. Carlson[10,11] has confirmed the original report and extended the histological observations. Whether the process is truly regenerative is questionable, for no recognizable blastema appears at any stage. In a few cases, osteocartilaginous structures appeared within the muscle mass, arising apparently from fragments of the Achilles tendon (Carlson, personal communication). The rat otherwise is capable of only a small measure of muscle regeneration at the histological level[12]. Moreover, the regeneration of organized multi-tissue extremity structures is lost in the rat after the fourteenth day of gestation[13]. The only report of successful restoration of rudimentary limb regeneration in any mammal is that of Mizell[14] who implanted nerve tissue into the hind limb of the newborn opossum before amputation. The opossum. however, is born at a very early stage of development with particularly rudimentary hind limbs, and these results may be interpreted as enhancement of foetal type regeneration.

The results of the present study are interpreted as indicating support for the original thesis[8] that regenerative growth could be restored in mammals by the application of appropriate levels of electrical stimulation to produce the cellular changes necessary for blastema formation. The origin of the blastema formed in these experiments is unknown. Further experiments will be necessary to find the effect of simultaneous hormone (prolactin) administration, the most effective range and method of electrical stimulation, the efficiency of the method in other mammals including those in older age groups, and possible undesirable side effects.

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Lead Levels in Deciduous Teeth of Urban and Suburban American Children

LEAD poisoning is a serious threat to the health of American children living in urban ghettoes. Perlstein and Attala, studying 425 children treated for lead poisoning, found mental retardation in 22%, recurrent seizures in 20%, and cerebral palsy in 2%[1]. Many more children eat lead-containing substances and bear raised body levels of lead, but are not identified as being intoxicated[2]. If children with elevated tissue lead could be reliably identified, and their neurological and psychological function evaluated, the incidence of silent brain damage from lead ingestion could be established. Because elevations in blood lead are transitory, and decline once ingestion has stopped, blood lead levels are unsatisfactory indices of earlier exposure. Calcified tissues, however, store lead: bone biopsy procedures are impractical for large scale surveys, but it occurred to us that the exfoliated primary tooth might offer a way to examine the lead content of children who are at risk. Alshuler *et al.* reported a substantial increase in the lead content of deciduous teeth of children with fatal and treated plumbism[3].

Sixty-nine deciduous teeth were collected from dental clinics serving children who resided in the "lead belt" of urban Philadelphia, the area of the inner city from which most cases of lead poisoning are reported. Forty teeth were obtained from suburban dentists, where lead poisoning and, by inference, lead ingestion are largely unknown. The external surfaces of the individual teeth were cleansed with a detergent solution, then caries, filling materials, and adherent soft tissue manually removed. The teeth were rinsed, then crushed to a fine powder in a 'Freezermill' (Spex Industries) at $-196°$ C. The powder was mixed with 5 ml. of bromoform (specific gravity 2.85), exposed to ultrasonication for 15 s (30 W, maximum tuning) and centrifuged at 3,000*g* for 5 min. This procedure removed any residual particles of dental amalgam. A further density separation was performed at specific gravity 1.6 to remove carious tissue. Seventy per cent perchloric acid (2 ml.) was then added to the dried tooth powder to dissolve the inorganic components of the tooth and precipitate the dental proteins. The protein precipitate was washed twice with 1 ml. aliquots of 0.2 M perchloric acid and the washings were combined with the acid-soluble portion of the tooth. The perchloric acid was buffered with 1.0 ml. 0.2 M glycine-HCl (*p*H 2.6) and the *p*H adjusted to *p*H 2.2–2.8. The lead was chelated with 1 ml. 1% ammonium pyrolidine dithiocarbamate and the lead complex was extracted into 1 ml. of 2,4-dimethyl-6-heptanone. The diphasic solution was centrifuged at 3,000*g* for 5 min and the organic phase removed. The concentration of lead in the organic phase was determined on an EEL 140 atomic absorption spectrophotometer.

Fig. 1 shows the differential distribution of lead in children from the "lead belt" and from the suburbs. The mean tooth lead for suburban controls is 11.1 ± 14.8 p.p.m., and for children from the ghetto 51.1 ± 109.0 p.p.m. After the data were normalized by logarithmic transformation, because of the skewed distribution, a *t*-test was applied ($P < 0.01$). One child,