resemble disomic homozygotes (a dosage effect), there will be 12·7 per cent high excretors among mongoloids if the frequency of disomic high excretors is 5 per cent and 23·7 per cent if the frequency of disomic high excretors is 10 per cent. The expected excess of high excretors among mongoloids under these assumptions is considerably less than the 4–6-fold excesses previously reported[7,8]. Further, if these assumptions are not met, the excess will generally be smaller.

Taurine is considered to be derived from cysteine via cysteine sulphinic acid[15]. Its urinary excretion is highly variable and reflects primarily protein intake as evidenced by its lowered excretion under fasting conditions. Gartler, Dobzhansky and Berry[16] and others have found little evidence of heritability in the excretion rates of taurine, though the difference between mono- and di-zygotic twins was in the direction expected if genetic factors were involved. In the independent urine samples from the same individuals mentioned above, taurine excretion levels were 0·64, 1·00 and 0·87 mg/l. for the one mongoloid and 32·7 and 35·2 mg/l. for the other.

Table 1 provides the results obtained on taurine excretion from the present subjects. Because several values were under 1 mg, taurine is expressed in terms of the logarithm (taurine in mg/day or mg/l. + 1). Three subjects with exceedingly low excretion rates were excluded from the control group because they were on a taurine-deficient but nutritionally complete synthetic diet and one because he was fasting. No sex differences in taurine excretion were detected. The mean log for mongoloids was significantly lower than that for controls ($t = 5{\cdot}33$, $P < 0{\cdot}001$). As might be expected, taurine excretion was lower for younger subjects, but the difference between mongoloids and controls persisted when subjects less than 10 years of age were excluded ($t = 4{\cdot}90$, $P < 0{\cdot}001$), and when subjects less than 18 years of age were excluded ($t = 4{\cdot}77$, $P < 0{\cdot}001$). Though protein intake for these subjects was not rigorously established, the diet available to the mongoloids, based on an 1,800 calorie intake, includes 80 g protein, 80 g fat and 180 g carbohydrate. Subjects are served milk three times a day, meat twice a day and eggs once each day. Hence, it does not appear that the protein intake is low. The low heritability previously shown for taurine excretion and the large proportion of mongoloids with low excretion rates discourage the inference that a gene or genes regulating taurine metabolism is on chromosome 21. On the other hand, the absence of a theoretical genetic basis for calculating the expected proportion of low taurine excretors among mongoloids prevents testing such a hypothesis.

Other differences in amino-acid excretion patterns of some mongoloid subjects were observed and will be reported elsewhere[11], together with further details of the experiments reported here.

Addendum. Work under way at the time the foregoing paper was submitted has been completed[17] which establishes a theoretical genetic basis for the high frequency of low taurine excretors as well as for the high galactose-1-phosphate uridyl transferase activity[18], and high neutrophil alkaline phosphatase activity[19] among mongoloids. These striking differences are probably due to overdose effects, that is, to genes which when present in triple dose give rise to phenotypes different in quantity or kind from the effects of the same gene in double dose. With assumptions that crossing over and timing of non-disjunction are independent of parental genotype, it can be shown that the various trisomic genotypes occur in the proportions:

Genotype	Frequency
AAA	$(p^3 + 1/2\ p^2qK)\ (1-m) + mp^2$
AAA'	$(3p^2q + 1/2\ pqK(1-3p))\ (1-m) + mpq$
$AA'A'$	$(3pq^2 + 1/2\ pqK(1-3q))\ (1-m) + mpq$
$A'A'A'$	$(q^3 + 1/2\ pq^2K)\ (1-m) + mq^2$

where p = frequency of A, q = frequency of A', m = proportion of post-fertilization non-disjunction, and $K = c + 2t - 3ct$, where c = frequency of crossing over, and t = proportion of second division meiotic non-disjunction. The excess of high excretors of BAIB among mongoloids previously reported[7,8] is not compatible with any assumptions regarding dominance relationships in the proposed model and is also dubious for the reasons stated. In contrast, the low taurine-levels and reported enzyme differences[18,19] are compatible with overdose effects in the model described, though the few data presently available and the lack of knowledge of gene frequencies preclude rigorous tests.

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EFFECTS OF ELECTRIC CURRENTS ON BONE *IN VIVO*

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IT now seems evident that bone functions as a transducer, converting mechanical energy to electrical energy. This behaviour has been ascribed to solid state or semiconductor characteristics in bone by some observers[1–3], and to classic piezoelectric phenomena, such as those observed when quartz crystals are stressed, by others[4–6]. Regardless of the mechanism by which these electrical

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potentials are produced, the question of how they function remains obscure. While it has been recognized for many centuries that bone structure adapts to functional demands[7], the immediate controlling factors have not been identified. In 1962 it was suggested that the stress-generated electrical phenomena were capable of directing the activity of bone cells and were responsible for the orientation and/or aggregation pattern of macromolecules in the extracellular space[1]. Subsequent work in these laboratories demonstrated that collagen fibrils were oriented by weak, direct, electric currents (1 μamp) as they were reconstituted from solution[2]. The pattern of fibril orientation which occurred near the cathode was field-dependent, and at right-angles to the field. The present investigation was initiated to determine the effects of similar low-level, direct electric currents on bone, *in vivo*.

Electric currents were developed in bone by implanting units consisting of 2 iridium–platinum electrodes, a battery and a resistor. Within the units a 1·4 V mercury cell battery was connected in series to a resistor with a value of $1{\cdot}5 \times 10^6$, $1{\cdot}5 \times 10^5$ or $1{\cdot}5 \times 10^4$ ohms, so that the approximate current yield would be 1 μamp (microampere), 10 μamp or 100 μamp respectively. All connexions between battery, resistor and platinum electrodes were made with silver-epoxy cement. The entire unit was finally 'potted' in epoxy and coated with RTV silicone prior to implantation (Fig. 1*a*). Two holes, 13 mm apart, were drilled in the lateral, middle portion of the femora of 18 adult dogs, through an otherwise intact periosteum. Battery packs were inserted so that the platinum electrodes projected through the cortex, and 3–4 mm into the medullary canal. Muscle, fascia and skin were closed over the packs. Non-active controls were implanted in the contralateral femora. Current was monitored *in situ* continuously for 30 min, with a Hewlet-Packard d.c. meter connected to a strip recorder.

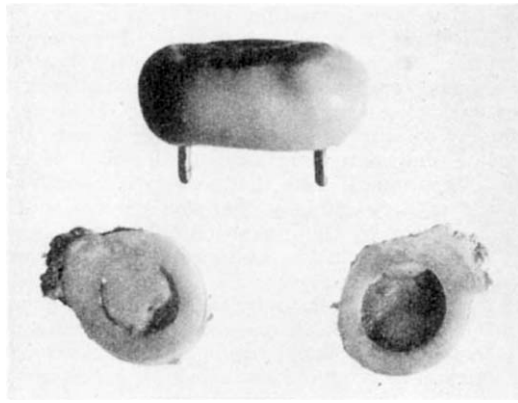

Fig. 1. (*a*) Silicone-encapsulated battery-resistor pack, with iridium–platinum electrodes which are 'plugged in' to holes drilled in femur so that tips of electrodes project 3–4 mm into medullary canal. (*b*) Cross-section of femur with 100-μamp pack in place for 14 days. Cathode left, anode right. Note the mass of intramedullary bone, nearly filling marrow cavity in specimen on left. The reaction about the anode, on the right, is approximately equal to that of the non-active control specimens

The results, depicted in Fig. 2, indicate that, most likely, as electrode polarization increases, resistance external to the pack increases and current falls. It seems reasonable to conclude that stability may be reached when the rate of gas production at the electrodes equals the rate of gas solution in the extracellular fluid. After 21 days in the animal, the 100-μamp packs were still delivering 3 μamp. When these animals were killed by the intravenous administration of an overdose of pentobarbital, the current fell rapidly to 2 μamp. This also suggests that current flow is dependent on the amount of gas being liberated and dissolved at the electrodes.

Cross and longitudinal sections of both femora were obtained following a perfusion of the hind quarters with 10 per cent neutral formalin, 14 and 21 days after operation. In the gross specimens, a mass of bone trabeculæ surrounded all platinum electrodes where they projected into the medullary canal. There was no difference between the amount of newly formed bone around cathode or anode of the active, 1-μamp packs and the inactive controls. Marked differences were observed, however, between controls and the specimens bearing the 10-μamp and 100-μamp packs. In both of these groups with active packs, massive production of new bone was noted around the cathode, while the reaction about the anode resembled that of the 2 inactive platinum electrodes in the controls (Fig. 1*b*). The result was the same, whether the cathode was proximal or distal to the anode. Frequently with the 100-μamp packs a dense, cathodal bone mass nearly filled the medullary canal. Bone production in the region of the negative electrodes had apparently reached a peak at about 2 weeks, since continuation of the stimulation for 21 days did not increase the trabecular masses. Although the differences were small, it appeared that animals with 100-μamp packs formed more bone at the cathode than did animals with 10 μamp packs.

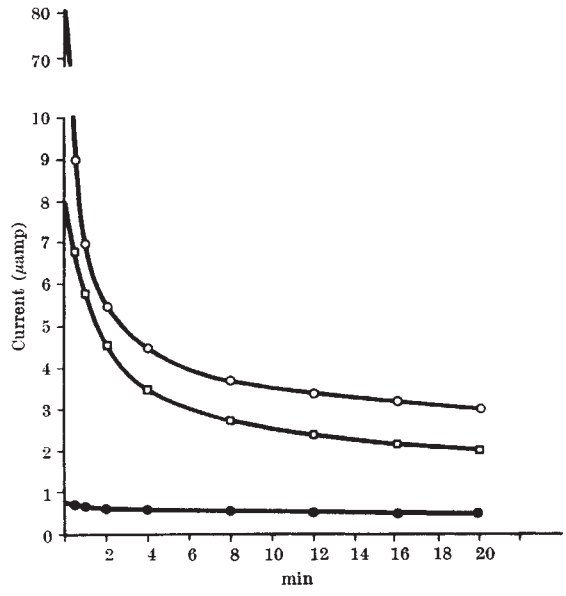

Fig. 2. Graph of current flowing in living bone after various intervals. Initial current values were 0·8–8·0 and 80 μamp. ○, 100-μamp pack; □, 10-μamp pack; ●, 1-μamp pack

Microscopic examination of sections, prepared from specimens decalcified in formic acid, and embedded in paraffin, and stained with hæmatoxylin and eosin, paralleled the results listed here. There was bone formation about all active and control electrodes. While the reaction about control electrodes, cathodes and anodes was qualitatively similar, with minor exceptions, there was a greatly increased quantity of non-oriented, young, trabecular bone about the cathodes of all 10- and 100-μamp packs.

Osteogenic activity was judged by the number of osteoblasts on trabecular surfaces, the number of large lacunar spaces, and by the general cellularity. On the basis of these criteria, more bone was being formed about all control and active electrodes on the 14th than on the 21st post-operative day. At the cathodes of the 10- and 100-μamp packs, however, the picture at 14 days was one of florid osteogenesis. Many of these specimens contained microscopic fields which were extremely cellular

and in which the newly formed osseous matrix was poorly organized or non-existent (Fig. 3). In some areas, the tissue might have been mistaken for osteogenic sarcoma, had the cells appeared slightly more pleomorphic. Few of these histological characteristics were present in the cathode region of the 10 and 100 μamp packs at 21 days, and these were uniform fields of maturing fibre bone (Fig. 4). Osteoclasis was not observed at the anode. Multinucleated cells were frequently found on the trabeculæ and at some distance from them, but they were distributed uniformly throughout the areas of reaction. A sheet of brownish, homogeneous material, 30–40μ thick, possibly denatured protein, encased the active anode, but not the other electrodes. In the femoral cortex, between the cathode and anode of the 10 and 100 μamp packs, remodelling of osteones occurred and seemed to increase in magnitude between the 14th and 21st days. Bone resorption and deposition were evident along the course of the Haversian and Volkmann's canals in these specimens. Osteoblasts and osteoclasts did not appear to accumulate selectively in the region of the anode or cathode.

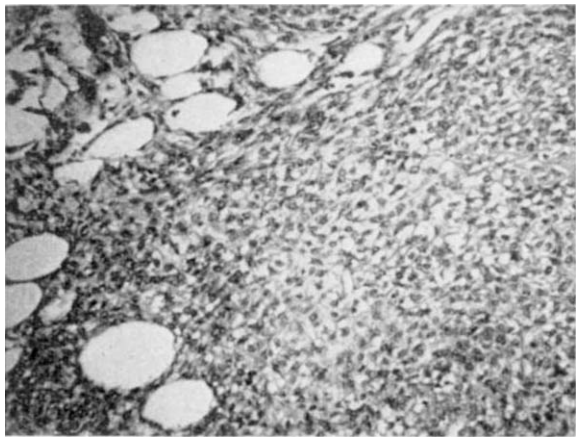

Fig. 3. Specimen 1(*b*), near cathode. Note marked cellularity, large vascular spaces and narrow 'seam' of osteoid at right of photomicrograph. (Hæmatoxylin and eosin, × 80)

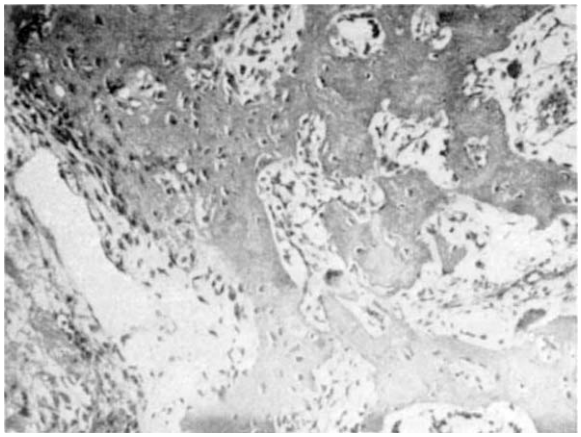

Fig. 4. Specimen 21 days after operation. Note fewer cells and more abundant extracellular matrix than are seen in Fig. 3. This picture was characteristic of the newly formed bone in most specimens with either active or non-active control packs. (Hæmatoxylin and eosin, × 80)

This work was begun without knowledge that investigators in Japan[9,10] had attempted similar experiments in 1956 and 1957, with results similar, in part, to those presented here. This present work, however, was undertaken as a test of the validity of a theoretical control system regulating bone structure and regeneration. The control system was proposed[2] as follows: mechanical deformation applied to the multiple apatite-collagen *pn* junctions in the bone matrix produces an electrical signal, which in turn stimulates differential cellular responses and causes the oriented deposition of newly formed collagen fibres to produce a structural change in the bone, enabling it better to resist the applied, deforming force. When the proposal was advanced in 1963, data were available to support each of its facets, with the exception of the relationship between electric currents and bone cell activity. Our results seem to indicate that such a relationship does, in fact, exist.

The massive osteogenesis occurring at the cathodes was probably not the result of electrophoretic action on collagen or mineral constituents. Had electrophoresis played a dominant part in the observations, a gradient effect might have been expected, with a greater or less amount of bone formed nearer the electrodes. Furthermore, on the basis of other studies (to be published), a preferred orientation of collagen fibres might also have been expected. Certainly, the mass of new bone was associated with an absolute increase in the number of young mesenchymal cells and osteoblasts. It is impossible to account for this degree of cellularity, unless more cells migrated to the region about the cathode, or the cells already present divided more frequently. Of the two possibilities, an increase in the mitotic rate seems more feasible, and the histological picture supports this. Furthermore, most cell membranes are negatively charged, and move towards the anode in electrophoretic chambers, under the influence of current densities vastly greater than the ones employed in this investigation[11,12]. In this connexion, it should be emphasized that while the current rating of packs before implantation ranged between 1 and 100 μamp, only 0·7 to slightly more than 3 μamp were actually delivered.

It was tempting to predict, on the basis of earlier studies[1,2], that bone formation on the concave surface of a deformed bone was related to its electro-negativity, while bone resorption on the convex surface was related to the relative positivity of that area. This work does not corroborate this hypothesis entirely. However, the experimental conditions, that is, direct continuous current, electrodes in line with long axis of bone and projecting into medullary canal, were deliberately chosen for simplicity. It is very unlikely that the current and field patterns produced by the implanted battery packs would ever be encountered in the living system in the natural course of events.

However, it seems that, under the influence of low level, direct electrical currents, bone formation mechanisms are affected in some way that depends on the polarity of the applied current, to produce new bone growth preferentially in regions of relative electro-negativity.

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