character of the germinations is quite conspicuous. From the first germinations on September 2 to the last in the following April, they are spread over a period of no less than 215 days. If we ignore the fluctuating numbers and only regard periods when there were uninterrupted daily germinations, these number no less than thirty, forming about ten groups of active germination. Germinations occurred on 96 of the 215 days over which they were spread. It should, however, be stressed that biologically a succession of flushes of germinations probably have a similar biological significance whether interrupted or not unless the actual discontinuity be so marked, as in periodic types of germination, as to preclude the build-up of pests or predators.

Incidentally, it may be noted that during the very severe weather of January the seedlings were as brittle as glass, but some that had been transplanted when removed showed no sign of injury after they had thawed.

We could reasonably expect that the factor, or more probably factors, responsible for the differing natural dormancy of contemporaneous seeds would exhibit degrees of intensity from being very marked to perhaps only slight and that this variation would be normal and continuous. The discontinuity that we actually observe might be imparted by the erratic occurrence of the meteorological conditions that bring about the marked temperature changes which observations have shown stimulate germination of these seeds that have attained a condition to respond. For *Capsella*, such an interpretation conforms to the observed facts that while all the germination flushes occur in relation to abrupt changes of temperature such changes may nevertheless occur without any accompanying germination response, presumably because no seeds were contemporaneously in an appropriate physiological state.

E. J. SALISBURY

Croindene,
Strandway,
Felpham,
Bognor Regis.

[1] Masters Memorial Lectures, *J. Roy. Hort. Soc.*, **87** (1962).

## Electron Paramagnetic Resonance in Non-irradiated Bone

THE generation of electrical potentials by bone subjected to mechanical stress has been recently reported by two groups of investigators[1,2]. Bassett and Becker suggested a semiconduction mechanism as being the source of the phenomenon, while Shamos *et al.* favoured a classical piezo-electric mechanism similar to that earlier described by Fukada and Yasuda[3]. The semiconduction theory has recently been reported on in more detail[4] and evidence presented for the formation of multiple *pn* junctions from the apatite crystal (*p* type) collagen fibre (*n* type) in the bone matrix. Similar inorganic *pn* junctions have been shown to be exceedingly stress sensitive[5,6].

Since the semiconduction theory would require the presence of free charge carriers while the piezo-electric theory would have no such requirement, the application of electron paramagnetic resonance techniques to this problem seemed to be of interest. If semiconducting materials with adequate concentration of donors are present in bone, resonance signals should be observable without previous irradiation, and should be increased by light and thermal activation and possibly by mechanical stress. Electron paramagnetic resonance signals have previously been reported in electron irradiated bone[7] and in X-ray irradiated teeth[8] and bone[9]; no signals have been reported in non-irradiated material.

Cortical bone samples, human tibia and bull-frog tibia, were obtained by surgical dissection from extremities rendered bloodless by tourniquet or vessel ligation. The periosteum and inner cancellous bone were removed and

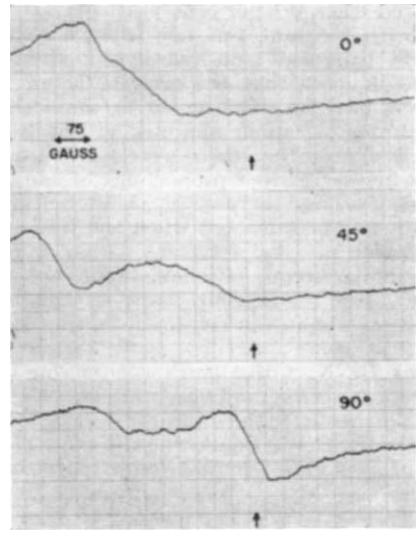


Fig. 1. Electron paramagnetic resonance spectra obtained from amphibian whole cortical bone. The orientation of the sample long axis relative to the *H* field is indicated in the upper right on each tracing. The arrow indicates *g* 2·0 value

cortical fragments approximating 3 mm × 3 mm × 5 mm were obtained by osteotome. Further dissection was done with quartz knife and probe under 20 times magnification. All surfaces were shaved free of any possible metallic contamination and sections with visible vascular capillaries were discarded. Final specimens were approximately 0·5 mm wide and 3 mm long, the original long axis being represented by the larger dimension. Only dense mature cortical bone was utilized. Samples were mounted on quartz filaments with a small amount of 'Cenco' red wax and suspended in 5-mm diameter quartz tubes, in such a manner that the angular relationship between the long axis of the bone sample and the main magnetic field (*H*) could be altered. Control tracings were made in each case by removing the sample, retaining the original mounting wax, quartz filament and tube. All electron paramagnetic resonance spectra were obtained at room temperature, the first derivative curve being recorded. A Varian *X* band spectrometer with 100-kc/s field modulation was used in conjunction with a 6-in diameter Varian electromagnet with ring shim pole caps and gap diameter of 2·58 in.

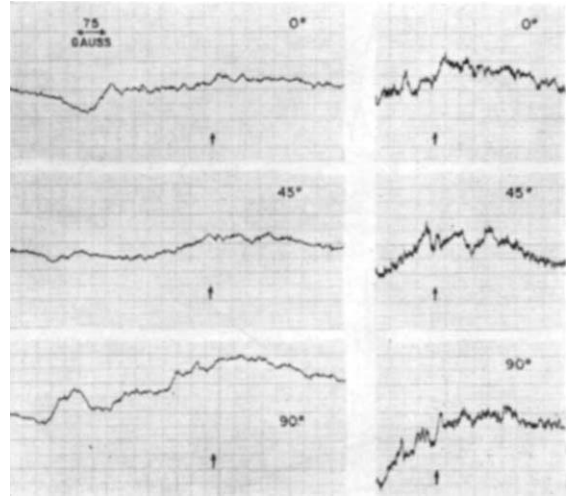


Fig. 2. Electron paramagnetic resonance spectra obtained from human whole cortical bone. Sample long axis orientation and *g* 2·0 value are indicated in the same fashion as in Fig. 1. The tracings at the left were obtained with similar amplification to those of Fig. 1. The recordings to the right were obtained with an amplification of three

Resonance signals with $g$ values dependent on the angular relationship of sample long axis to $H$ field were observed in all samples of bone from each species. In amphibian bone (Fig. 1) a large well-defined resonance about 200 gauss wide was noted in the vicinity of $g$ 2·3, with a shift in $g$ value with change in angular orientation. In the 90° position a well-defined, almost symmetrical resonance appeared at $g$ 2·0 which was much more anisotropic, appearing to merge with the $g$ 2·3 peak in the 0° position. In human bone (Fig. 2) a similar well-defined resonance was found in the $g$ 2·3 vicinity, being more prominent in the 0° and 90° orientation than in the 45° with again moderate $g$ value anisotropy. A very broad resonance signal appears, most prominently at 90° orientation, reaching a peak at approximately $g$ 1·8. Higher amplification scans of the $g$ 2·0 area demonstrated a small superimposed resonance, about 35 gauss wide with a $g$ value of 2·0 in the 90° position. This signal is light sensitive, increasing in amplitude with irradiation from a wide band (2000–10000 Å) source.

The similarity of the two major resonances obtained from non-irradiated bones of two different vertebrates as well as the evident anisotropic characteristics of the signal indicate that they are produced by actual components of the bone rather than surface contamination. At this time neither resonance can be definitely ascribed to a free charge carrier population such as is present in doped semiconductors[10]. Identification of the substances involved requires further separation and purification procedures.

ROBERT O. BECKER

State University of New York,
Upstate Medical Center,
Veterans Administration Hospital,
Syracuse, New York.

[1] Bassett, C. A. L., and Becker, R. O., *Science*, **137**, 1063 (1962).
[2] Shamos, M. H., Lavine, L. S., and Shamos, M. I., *Nature*, **197**, 81 (1963).
[3] Fukada, E., and Yasuda, I., *J. Phys. Soc. Japan*, **12**, 1158 (1957).
[4] Becker, R. O., Bassett, C. A. L., and Bachman, C. H., "The Bioelectric Control System Regulating Bone Growth", *Proc. Intern. Symp. Biodynamics of Bone* (Henry Ford Hospital, Detroit, Mich., March 1963) (in the press).
[5] Rindner, W., and Nelson, R., *Proc. Inst. Elect. Eng.*, **50**, 2106 (1962).
[6] Huber, F., *App. Phys. Letters*, **2**, 76 (1963).
[7] Slager, V. T., and Zucker, M. J., *Transplant. Bull.*, **30**, 146 (1962).
[8] Silver, A. (personal communication).
[9] Gordy, W., Ard, W. B., and Shields, H., *Proc. U.S. Nat. Acad. Sci.*, **41**, 983 (1955).
[10] Portis, A. M., Kip, A. F., and Kittel, C., *Phys. Rev.*, **90**, 988 (1953).

## A Graphical Method for comparing the Ratio of the Number of Cells to the Volume of Medium in Tissue Culture Containers of Various Sizes

IN the course of virus yield studies it is often necessary to convert a tissue culture system from one size container to another in such a way that the ratio of the number of cells to the volume of medium remains constant. An easy way of determining the various medium volumes which will give this constant ratio is to plot the relevant data graphically.

From previous work carried out in this Department, a value for the number of cells of a given tissue culture per unit area of confluent cell sheet was available. This value was obtained from six hundred counts of cells grown on cover-glasses. The confluent cell sheets were stained and a total of forty areas were counted on each cover-glass using stage and ocular micrometers, ten cover-glasses being counted for each batch of cells. The experiment was repeated six times using a different batch of cells each time. The particular tissue cultures used in this experiment were primary monolayers of embryonic chick fibroblasts and the mean value obtained was 1,150 cells/mm² of cell sheet.

Knowing this value, it was then necessary to measure the total area of the cell sheet in each type of flat bottle under consideration. As the contours of the bottles made

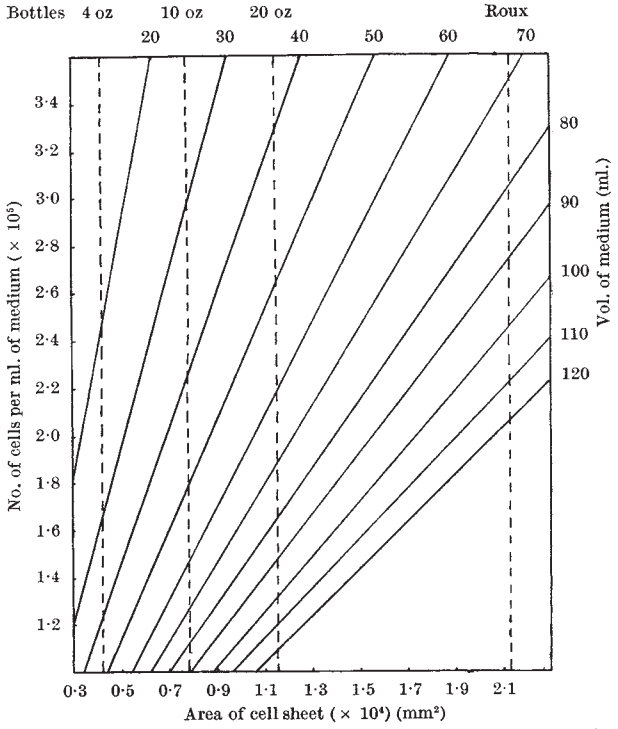


Fig. 1. No. of cells/ml. of medium plotted against area of cell sheet calculated for chick embryonic fibroblasts of density 1,150 cells/mm²

the exact shape of the cell sheets irregular it was found that the best way to measure the area was first to mark the edge of the cell sheet on the outside of the bottle. This outline was then traced on to thin paper and from there copied on to millimetre squared paper. A count of the squares enclosed by this outline gave the area of the cell sheet in square millimetres directly. Twelve experiments were made in this way to obtain a mean value.

The ratio of number of cells to volume of medium (expressed as cells/ml.) can be calculated from the following equation:

$$R = \frac{AN}{V}$$

where $R$ = cells/ml.; $A$ = area of cell sheet (mm²); $N$ = cells/mm² = 1,150 (as determined); $V$ = volume of medium (ml.).

Hence if $R$ is plotted against $A$ (for different sizes of bottles) then a straight line will be obtained with a slope of $N/V$. As $N$ is a constant for a given tissue, then the slope will be inversely proportional to $V$ and as the volume increases so the slope of the line decreases. Therefore, if graphs of $R$ against $A$ are plotted for various values of $V$ then a series of straight lines with different slopes will be obtained.

Fig. 1 gives the values for the cell sheet areas corresponding to four sizes of bottle (4 oz., 10 oz. and 20 oz. medical flats and Roux) which are marked by the dotted lines. The point where the dotted line intersects a continuous line marks the value of $R$ at that volume of medium represented by the continuous line.

This method can be used to translate a system from one type of bottle into the equivalent for another type. A 20-oz. bottle is represented by the dotted line on the graph at $1{\cdot}15 \times 10^4$ mm² on the $A$ axis. If this bottle contains 60 ml. of medium then the equivalent in other sizes of bottle is found by placing a ruler across this line parallel to the $A$ axis and where it intersects the 60 ml. line. Where the ruler crosses the 10 oz. line the nearest volume line is for 40 ml. ($A$ line for 41 ml. would be more accurate if drawn.) Similarly for a Roux bottle the nearest volume line is 110 ml.