system were also unsuitable, because they do not have space for writing on them, they have more positions than we needed, and they require special equipment for storage and retrieval. We could have used pre-scored, manually punched, smaller cards (I.B.M. Port-a-Punch) but these also have no space for writing on them (*5*). Therefore, we decided to use conventional edge-notched cards (*6*) in an inverted indexing system. The cards are stock items and no elaborate equipment is needed, so that the cost of the material required was low (less than $60).

Since the study was a descriptive ecology of the teaching of preventive medicine, it involved a large amount of data which had to be categorized topic by topic. As with any punch card system, once the data were organized and coded, it was a simple matter to store this information by punching the cards. The only equipment needed was a hand punch. In descriptive studies such as this one, a good deal of effort is spent in arriving at realistic categories. The method used here allowed one to modify categories, add or delete characteristics, without having to "start from scratch" each time. In working through the data, storage of satisfactorily classified information was not disturbed by alteration of other categories. Also, once each category was satisfactorily classified, coded, and stored on the cards, cross-comparisons among different categories was easy.

In utilizing this system, we assigned each medical school and school of osteopathy one of the 92 positions on the card. Each card was assigned a particular characteristic. Characteristics included such information as size of the city of location, nature of the academic complex, organizational pattern, components of curriculum, and responses of chairmen to opinion questionnaires. In arriving at categories or characteristics, we found it helpful to make notations on the face of cards as we proceeded, so that we could modify categories without going back to the "raw data" again. Characteristics were tabulated by counting notches. Several characteristics could be compared by superimposing the appropriate cards and counting the notches in common. For example, it was relatively simple to compare the age group of the departmental chairmen with the responses given on the opinion questionnaire.

For those engaged in short-term, small-scale research, where a small series of individuals are included, together with a large number of widely variable characteristics which do not lend themselves easily to ready-made categories, the use of edge-notched cards in an inverted indexing system is satisfactory for handling the data analysis.

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Venom Collection from Honey Bees

Abstract. *A device that provides an electric shock makes it possible to collect pure venom from several thousand honey bees* (Apis mellifera). *The collection apparatus fits underneath the brood chamber of a colony of bees and may be moved from hive to hive. Each colony is "milked" for 5 minutes. An average of 20 hives must be "milked" to obtain 1 gram of venom. Under optimum conditions this quantity of venom is produced by 10,000 worker bees.*

Bee venom has been used medicinally in Europe for several decades, especially for the treatment of rheumatic diseases. Both live bees and extracted venom have been used. The material has not been widely used in this country because it has never been available in sufficient quantity for analysis or clinical testing. In fact, because of a lack of knowledge of the chemistry of the substance, its use has been frowned upon by some, except for purposes of desensitization. The subject has been reviewed by Haydak (*1*) and Beard (*2*). The desensitization of some persons adversely affected by stinging insects has been accomplished through the use of extracts of whole insects. These extracts are used in part because venom has not been available in quantity. Some of the antigens responsible for allergic reactions in hypersensitive people seem to be common to bees and wasps (*3*).

Markovič and Molnar (*4*) were probably the first to subject bees to electric shock to obtain venom. Bees returning to the hive were caught between two revolving cylinders, where they were shocked and squeezed to make them sting. Plastic, rubber dam, and filter paper were tested as materials for the bee to sting. Since the bees were crushed, the filter paper was contaminated. The bees left their stings in the plastic and in the rubber dam (they died as a result). Moreover, the protruding stings made it difficult to scrape off the venom. As a final solution, Markovič and Molnar used a rubber dam with filter paper underneath.

In 1958 Weide (*5*) found that bees would sting moist filter paper when they were subjected to shock at low voltage. The treatment apparently did not harm the bees. He presumed that a method of obtaining venom, presumably for commercial use, by subjecting bees to electric shock was then in use in Germany.

Palmer (*6*) developed an apparatus for administering electric shock to obtain bee venom. His device consists of a magazine which holds as many as 200 bees; the bees sting through a sheet of silicone when shocked. The venom remains on the underside of the sheet when the current is turned off, and the stings are withdrawn. The magazines must be loaded before and after each shock. At Ohio State University bees were placed in "electric chairs" and shocked to obtain venom (*7*). O'Connor *et al.* (*8*) reported a similar method.

The stylet (shaft) of the sting of a honey bee is about 2.0 mm long. From its sharp tip, it widens until it is about 0.1 mm in diameter where it joins the shaft bulb. There are several barbs on the stylet, some of them as long as 0.03 mm. It is these barbs which hold the sting in the body of an object that has been stung, and which usually cause the honey bee to lose its sting. In the process of stinging, the shaft normally becomes imbedded for about half its length.

The best material that we have found to date is nylon parchment taffeta. The bees do not pierce the individual nylon strands; rather, the stings are inserted into the holes between strands. The strands of the taffeta filling are 0.18 to

0.22 mm wide, while the strands of the weft (which run perpendicular to those of the filling) are 0.3 to 0.32 mm wide. The holes between the strands are rectangular, often square. The average length of the side is about 0.035 mm, with the range from 0.02 to 0.05 mm. A small percentage (up to 5 percent) of the venom is deposited on the top of the sheet, but most of the venom is deposited as clear crystals on the underside. Remarkably few stings are left in the sheet of nylon taffeta—seldom more than a dozen stings as a result of "milking" one hive. The nylon is so slippery that the barbs on the sting fail to catch and hold the sting in place, and thus few bees lose their stings and die. Rarely is a bee electrocuted by the venom-collecting apparatus as described here; more than 99 percent of the bees seem to survive. The long-range effects of "milking" colonies have not yet been studied. Bees are able to replenish some but not all of their supply of venom after losing a portion as a result of stinging or attempting to sting (*1*).

Bees in colonies from which venom is being collected become irritable; the process of stinging includes the release of an alarm odor (*9*), which excites bees in the vicinity and causes them to sting the nearest susceptible object. Thus, clothing normally worn in the apiary does not provide sufficient protection, and special clothing is needed as protection against excessive stinging. People walking within several hundred yards of an unprotected apiary where collections are being made are likely to be stung.

The device used to collect the venom consists of a wooden frame having a flat area 42.54 by 36.20 cm (16¾ by 14¼ inches) over which copper or steel wires are stretched at 3.18-mm (⅛ inch) intervals (Fig. 1). Alternate wires carry an electric charge. The uncharged wires are grounded, and all the wires are open on one end; the circuit is completed when a bee comes into contact with any two consecutive wires. A 15.88 mm (⅝ inch) plywood board, 32.07 by 40.32 cm (12⅝ by 15⅞ inches), rimmed by 6.35 mm (¼ inch) wooden strips 3.96 mm (5/32 inch) high, fits under the wires, and a glass plate, 40.00 by 30.80 cm by 1.57 mm (15¾ by 12⅛ by 1/16 inches), fits inside the rim of the plywood board. A piece of nylon parchment taffeta is stretched tightly over the glass surface and held in place by thumbtacks. The board is raised so that the nylon taffeta touches the under surface of the wires. A variable transformer with a capacity of 135 volts is used. It is powered by line voltage or by a 12-volt d-c wet-cell battery in conjunction with a converter (which converts 12 volts of direct current to 115 volts of alternating current). An electrical timer is used to break the circuit to the collecting plate for 4 seconds at 3-second intervals.

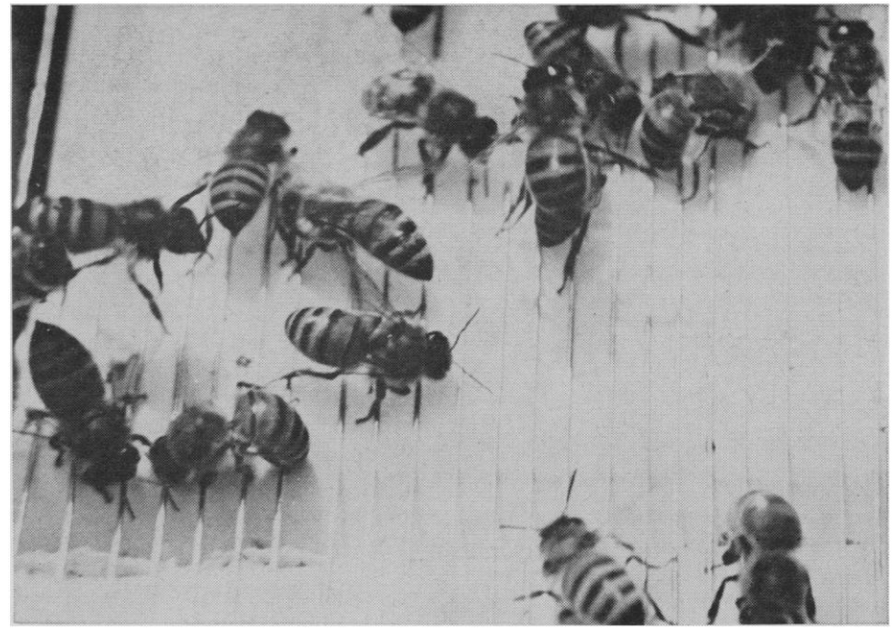

Fig. 1. Bees receiving an electrical shock bend the tip of their abdomens downward and sting. When the current is turned off the stings are easily withdrawn from the nylon material, and thus very few bees lose their stings.

The rim of a standard Langstroth bottom board (a flat board, 41.3 by 55.9 cm, [16¼ by 22 inches] upon which the colony rests) is raised 12.7 cm to make room for the collection apparatus under the brood chamber of the colony. Thus there is a space of 1.9 cm between the wires and the bottom bars of the frames. The apparatus is connected to the timer, and the transformer is adjusted to 33 volts. The power is turned on for 5 minutes; at the end of this time the collector is disconnected from the timer and slowly removed from the hive, and the bees are brushed off the surface of the collecting plate. At this point the operator must take extreme care not to be stung excessively. The device is transferred to the next hive. No smoke should be used in the apiary near the apparatus (smoke is commonly used to calm angry bees) because of the danger that the venom will be contaminated by tar.

The yield of venom is greater from some hives than from others, and the nylon taffeta may actually become wet from the venom. When this occurs, the taffeta should be allowed to air dry for a few minutes before it is inserted into the next hive. If this is not done, the bees become extremely irritable, and the operator may be severely stung.

The dried bee venom is easily scraped from the glass plate and the underside of the nylon sheet with a razor blade. It is necessary to wear an aspirator while removing the venom, as it is highly irritating to the mucous membranes. Persons in the room where the venom is being removed sneeze excessively. The venom is in clear, crystalline form, free from contaminants.

We expect that the device will prove satisfactory for collecting venom from other species of stinging, social bees, from wasps, and perhaps from ants.

A method of evaluating bee venom on the basis of a biological standard has been developed (*10*). Arrangements for chemical analysis and further testing of the venom have not yet been made (*11*).

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Measurement of Bone Mineral in vivo: An Improved Method

Abstract. *The mineral content of bone can be determined by measuring the absorption by bone of a monochromatic, low-energy photon beam which originates in a radioactive source (iodine-125 at 27.3 kev or americium-241 at 59.6 kev). The intensity of the beam transmitted by the bone is measured by counting with a scintillation detector. Since the photon source and detector are well collimated, errors resulting from scattered radiation are reduced. From measurements of the intensity of the transmitted beam, made at intervals across the bone, the total mineral content of the bone can be determined. The results are accurate and reproducible to within about 3 percent.*

An improved method for measuring the mineral content of bone in vivo by photon absorption techniques has been developed. The methods previously described in the literature (*1, 2*) are based on measurement of the transmission through bone of photon beams which are generated by standard x-ray tubes. The transmission is usually determined by densitometric measurements of x-ray films. The method described here differs from each of these earlier methods in one or more ways: in this method (i) the transmission of the photon beam is measured directly by counting techniques, by means of a scintillation detector system; (ii) the photon beam used is essentially monochromatic; (iii) the photon beam and detector are well collimated; and (iv) the effects of the tissue around the bone are taken into account. These factors eliminate errors resulting from the variability of x-ray films and film development techniques, reduce uncertainties in absorption coefficients, reduce the effects of scattered radiation, and reduce errors arising from the presence of tissue.

Figure 1 is a schematic diagram of the equipment used. The radioactive photon source used at present is iodine-125 (5 mc) contained in a thin-walled stainless steel tube 1 cm long, 3 mm in diameter. [An americium-241 source (1 mc) in tubing of similar dimensions is also used.] The tube is placed in a hole 3 mm in diameter in a small lead cube. The hole is drilled in such a way that the end of the tube is 5 mm below the surface of the lead, for purposes of collimating the photon beam. The tube is viewed end-on by the crystal detector system, which is also collimated, as shown schematically in Fig. 1. Two holes, each 3 mm in diameter, drilled in a pair of lead plates, each 5 mm thick, serve as collimating apertures. The plates are 4 cm apart, and the two holes are aligned on a common axis with the photon source.

The source and the detector system are rigidly coupled by mechanical means and are driven simultaneously in 1-mm steps, in a direction transverse to the bone, by the motor-drive system. Measurements of the transmission of the photon beam through the bone are made for a 10-second interval after each step. The starting and stopping of the drive motor and scaler and the resetting of the scaler are automatically performed by the timer unit, and the operator need only record the scaler reading after each step. In the course of the scan, measurements of the transmission of the photon beam through tissue alone, on either side of the bone, are also made. From the data obtained in these measurements, an equivalent thickness of bone mineral can be computed, as follows.

Let I_o be the intensity of the unobstructed photon beam, (as measured at *a* of Fig. 1); I_o^* the intensity after passage of the beam through a thickness T of tissue, (as measured through *b* of Fig. 1); and I the intensity after passage of the beam through an equal thickness of bone mineral plus tissue $T_b + T_m$ (as measured through *c* of Fig. 1). If μ_b and μ_m are the mass absorption coefficients of bone mineral and of tissue, respectively, then:

$$I_o^* = I_o \exp(-\mu_m \rho_m T)$$

$$\begin{aligned} I &= I_o \exp(-\mu_m \rho_m T_m - \mu_b \rho_b T_b) \\ &= I_o \exp(-\mu_m \rho_m (T - T_b) - \mu_b \rho_b T_b) \\ &= I_o \exp(-\mu_m \rho_m T) \exp(-\mu_b \rho_b T_b + \mu_m \rho_m T_b) \end{aligned}$$

$$I = I_o^* \exp(-\mu_b \rho_b T_b + \mu_m \rho_m T_b)$$

$$T_b = [\log_e (I_o^*/I)] \,/\, (\mu_b \rho_b - \mu_m \rho_m) \qquad (1)$$

Equation 1 gives an equivalent thickness of compact bone mineral of density ρ_b for the point at which the intensity of the transmitted photon beam is I. Measuring I at closely spaced intervals across the bone gives a series of equivalent thicknesses which, when summed, give the equivalent cross-sectional area of compact bone mineral in the bone. A standard composition of bone mineral is assumed in these calculations (*3*). The absorption coefficient μ_b can be determined on the basis of this assumption from tabulated atomic absorption coefficients (*4*). It is also assumed that all non-bone-mineral substances absorb radiation to the degree that striated muscle tissue does. The absorption coefficient μ_m can be calculated in a similar manner, or it can be experimentally determined for the particular tissue under study.

As shown in Fig. 1, the bone and tissue under observation are placed between form-fitting pieces of tissue-equivalent material [for example, Mix-

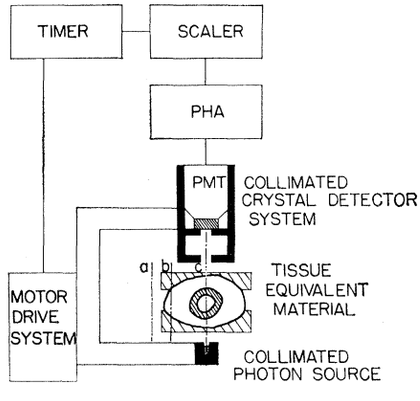

Fig. 1. Schematic diagram of equipment for measuring the mineral content of bone.

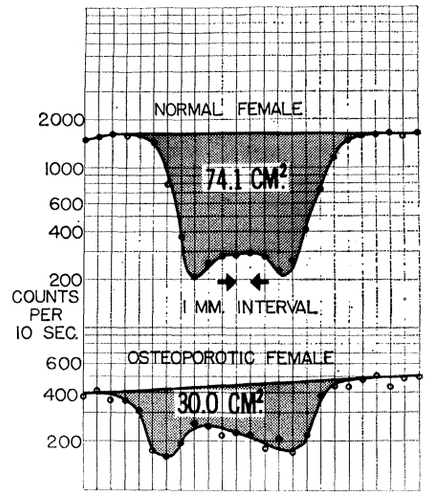

Fig. 2. Scanning records for a 28-year-old normal female and for a 75-year-old female with osteoporosis.