the mobilization period. The control spongiosal bones were collected from identical places of normal human femora of cadavers in 11 cases – regadless of sex, the average age being 35 years. The necropsy revealed no kind of skeletal disorder in the control cases.

Results and discussion. The results of the radiomicrometric values measured are presented in the Figures. The scheduled data indicate a remarkable decrease of the thicknesses of the spongiosal trabecules of the osteoatrophic bones originating from operated patients (Figure 1). The intertrabeculare spaces were widened by this process. The differences in the spongiosal trabeculare thicknesses between the operated osteoatrophic and normal cadaver bones represent a rarification of the trabecules and explain the increased radiotransparency that may be observed on the radiograms (Figure 2, a and b). The radiomicrometrically measured data resembling the histomorphometric results of FROST and SCHENK[4,5] are in a good agreement.

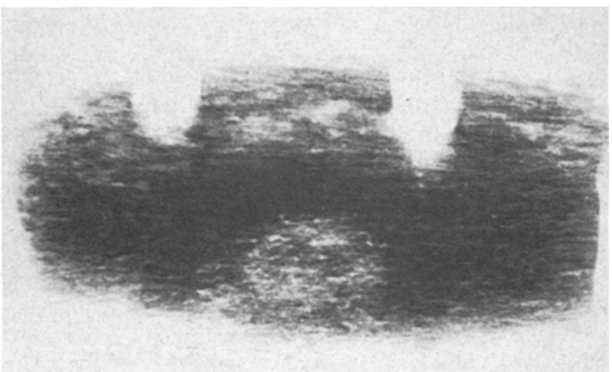

Fig. 3. Slab radiogram of a removed osteoid osteoma. × 5.

In one case of the localized cortical bone atrophy surrounded with sclerosis, after its operative removal the rarification of the cortical trabecules may be seen on slab radiogram as the nidus (Figure 3). The trabeculare thicknesses measured in the nidus were found to be 180 μ thick (Figures 1 and 3). On histological examination this alteration was characteristic of an osteoid osteoma. This cortical atrophy was in a close relationship with the increased vascularity[6].

This manner of evaluation and the results indicate that the trabeculare radiomicrometry on slab radiogram is a suitable and much more exact method for the estimation of the rarification of the spongiosal trabecules by the decreases of their thicknesses, than the visual means. By these radiomicrometric measurements, the trabeculare changes could be demonstrated convincingly in a quantitative way.

Zusammenfassung. Bericht über eine radiomikrometrische Messung der Trabekel des spongiösen Knochens welche genauere Werte ergibt als die gebräuchlichen Methoden.

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[1] Kl. J. THIEMANN, Internist 7, 564 (1966).
[2] W. BESSLER, Radiologe 9, 154 (1969).
[3] L. BEREK, Experientia 25, 633 (1969).
[4] H. M. FROST, Henry Ford Hosp. med. Bull. 9, 145 (1961).
[5] R. SCHENK and W. A. MERZ, Dt. med. Wschr. 94, 206 (1969).
[6] Å. LINDBOM, N. LINDVALL, G. SÖDEBERG and H. SPJUT, Acta Radiol. 54, 327 (1960).
[7] Permanent address: Dr. med. L. Berek, Sallai Imre u. 37.II.1., Budapest, XIII (Hungary).

Preparation of Large Volumes of Linear Gradient Solutions for Zonal Ultracentrifugation

Zonal ultracentrifugation techniques in sector-shaped rotors require preparation of large volumes of gradient solutions of sucrose, cesium chloride or other solutes[1]. The gradient solutions used are either linear or exponential with respect to rotor volume. These solutions are prepared in a suitable gradient former and immediately delivered to the rotating zonal head. The volume of the solution used ranges between 45 and 1,675 ml, depending on the type of the rotor employed. It is desirable to perform the whole loading operation within a period of not more than 10–20 min. Therefore, a gradient-forming device capable of delivering a constant liquid output of up to 150 ml/min may be required.

The preparation of large volumes of linear gradient solutions used in zonal ultracentrifugation presents particular difficulties which will be discussed briefly. A linear gradient solution can be prepared by filling 2 identical reservoirs with the heavy and the light end of the gradient, respectively. The 2 reservoirs must be connected so as to keep their contents at the same level at all times. The content of the chamber corresponding to the light end of the gradient is continuously stirred as it receives solution from the reservoir containing the heavy solution. As the gradient is formed, the solution is withdrawn and fed into either centrifuge tubes or the zonal rotor.

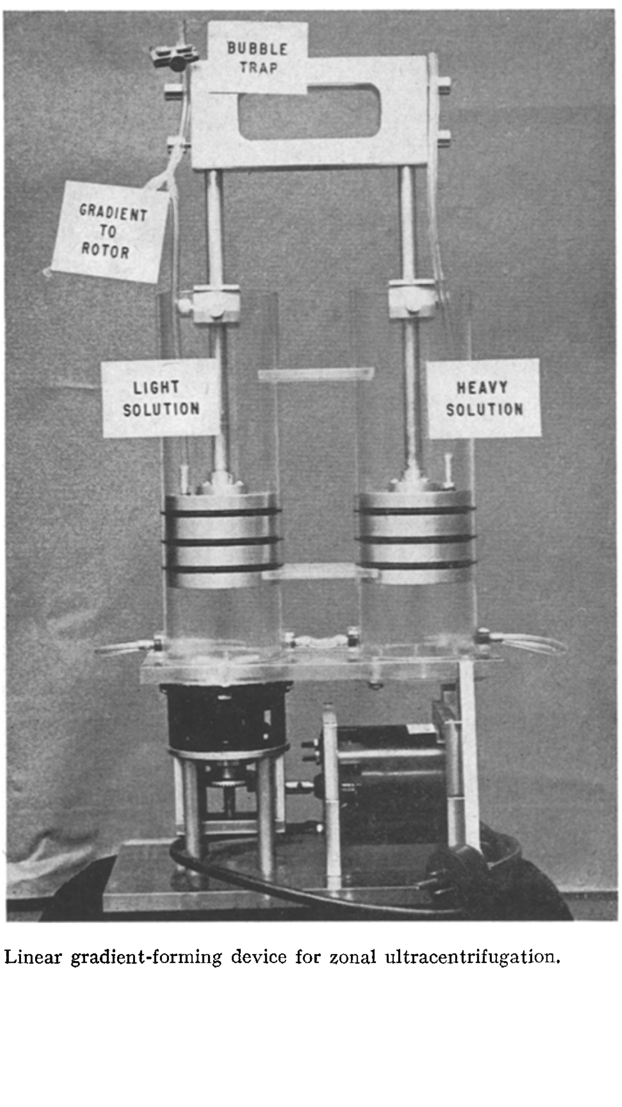
In the gradient device described originally by PARR[2], the linear gradient was obtained by the simultaneous emptying of the 2 chambers under the influence of gravity. Modifications of this open chamber-device have been described by others[3,4]. These simple gradient-forming devices are not suitable for zonal ultracentrifugation work. In the first place, in the gradient formers described above, solution mixing is achieved by means of magnetic bars or

[1] N. G. ANDERSON and E. RUTENBERG, Analyt. Biochem. 21, 259 (1967).
[2] C. W. PARR, Biochem. J. 56, 27P (1954).
[3] R. M. BOCK and N. S. LING, Analyt. Chem. 26, 1543 (1954).
[4] G. A. DAVIS, R. J. SANTEN and B. W. AGRANOFF, Analyt. Biochem. 11, 153 (1965).

stirring paddles, which are inadequate for the rapid mixing of large amounts of dense sucrose solutions. Secondly, the turbulence caused by the operation of a powerful stirring device within an open mixing chamber would result in the introduction of an excess of bubbles into the gradient stream, making the procedure impractical. In fact, gradient formers of the open chamber type cannot be used for the accurate preparation of linear gradients of very viscous sucrose solution at rates faster than 2–5 ml/min.

The problem of introducing air bubbles in the gradient is eliminated by using closed-chamber devices. These machines must be provided with variable volume chambers, capable of a simultaneous decrease in volume. Several gradient formers which operate on this principle have been described[5]. In all these devices, the twin pistons used to close the chambers are motor driven. These carefully engineered machines provide an excellent gradient delivery rate and ensure reproducibility of the characteristics of the gradients, but are in general very expensive.

Material and methods. In order to simplify the construction of a closed chamber, linear gradient-forming device, it was thought that the piston-driving motors and their complicated gear systems could be eliminated if the force of gravity were used for this purpose. A machine assembled by us which operates on these principles is shown in the Figure.



Linear gradient-forming device for zonal ultracentrifugation.

The construction of this device is greatly simplified by the fact that the basic stirring mechanism and the propeller are parts from a commercially available pump with magnetic drive (Cole Parmer No. 7004-3) used in the manner described in a previous paper[6]. The solution reservoirs are 2 identical plastic cylindrical chambers of 8 cm diameter. Extra-long aluminium pistons were found necessary in order to keep the whole system uniformly centered. In addition, the weight of the pistons helps to ensure smooth displacement during operation. Three loosely fitting 'O'-rings per piston were found to be more satisfactory than a single, tight one, in order to keep the chambers hermetically sealed without interfering with the vertical movement of the pistons. The apparatus is completed with suitable outlets for easy chamber loading and unloading, a chamber interconnection, a bubble trap, and a rigid piston-to-piston clamp.

Results and discussion. For operation, the chambers are filled with equal amounts of the light and the heavy solutions, and stirring initiated. The mixed gradient is gradually withdrawn from the chamber corresponding to the light end of the gradient by means of a peristaltic pump. Simultaneously, an equivalent volume of heavy solution is fed into the light solution chamber due to the parallel displacement of the aluminium pistons.

The described device was used for the preparation of up to 1,500 ml of a linear sucrose gradient, ranging from 10–65% w/w. A maximum of reproducibility was found for volume rates of up to 100 ml/min at an impeller rotation speed of 750 rev/min. This volume rate does not constitute a theoretical limit, and higher delivery rates may be achieved by using a more powerful stirring mechanism.

Despite its appearance, the described gradient device can be constructed in a few hours at any moderately equipped machine shop and without much expense. The apparatus provides a fast, convenient way of preparing the large amounts of linear gradient solutions required for zonal ultracentrifugal work. In addition, the same device can be used for the preparation of exponential gradient solutions, by removing the piston-to-piston clamp and adjusting the height of the piston corresponding to the light end of the gradient. After use, the device is easily disassembled for cleaning, and no lubricated moving parts are in contact with the solution used[7,8].

Résumé. On décrit la construction d'un appareil à chambre close pour la préparation de gros volumes de solutions à gradients de densité linéaire. Ces solutions de sucre ou d'autres substances sont généralement utilisées pour l'ultracentrifugation zonale en gradient de densité.

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[5] C. DE DUVE, J. BERTHET and H. BEAUFAY, Prog. Biophys. biophys. Chem. *9*, 325 (1959).
[6] J. E. PARIS, Biochem. biophys. Acta *165*, 286 (1968).
[7] The assistance of Dr. N. I. KRINSKY and Mr. R. SCHROEDER is acknowledged.
[8] This work was performed in the Department of Radiology of the University of Maryland Hospital, Baltimore (Maryland, USA), under Grant No. CA-06518 of the National Cancer Institute, National Institutes of Health, U.S. Public Health Service.