

$$N = \frac{fIT}{0.693} \left[1 - \exp\left(-\frac{0.693t}{T}\right) \right]$$

where f is the fraction of inhaled material which is not exhaled or quickly swept out of the respiratory tract and swallowed. Both f and T may vary considerably with the character of the aerosol.

Considering the zirconium-95 + niobium-95 results and taking an average burden per person as 245 pc., the fraction f may be estimated using the foregoing expression as follows. The average zirconium-95 + niobium-95 activity in the air at Sutton, Surrey, during the period January–May 1962 was approximately 1 pc./kg air⁹. If the biological half-life (T_b) is much greater than the physical half-life (T_r) of a nuclide in the lungs, the effective half-life (T) is only slightly less than T_r . In the case of zirconium-95 + niobium-95 ($T_r = 65$ days), if T_b is not less than the 120 days suggested by the I.C.R.P.⁸ for insoluble particles, the effective half-life will be between 40 and 65 days. Supposing that the daily inhalation is the standard 26 kg air, the value of f , calculated with these parameters in the above equation, lies in the range 14–19 per cent. The I.C.R.P. suggests 12.5 per cent for f .

Assuming that the particle size distribution of plutonium-239 was similar to that of the zirconium-95 + niobium-95, so that the same value of f was effective, the lung burden of plutonium-239, at the end of June 1962, may then be predicted from the above equation. With $f = 14$ –19 per cent, $T = 120$ days and taking the average activity of plutonium-239 in the air as 20×10^{-17} c./kg air for the three months prior to this time and 7×10^{-17} c./kg for the period 1955 until March 1962 from the foregoing results and others¹⁶, the predicted burden is 0.10–0.13 pc., which is of the same order as that measured. Thus, the foregoing results and the 'standard man' parameters appear to be reasonably consistent.

Possible levels of plutonium-239 in other tissues. From ref. 16 and the results given here, the total plutonium-239 inhaled up to June 1962 by a 'standard man' in England can be calculated to have been of the order of 5 pc. Of the 14–19 per cent suggested here as being retained in the lung with an effective half-life of 120 days or longer, approximately 0.16 pc. was still there in June 1962. Absorption of plutonium via the gastrointestinal tract is

extremely small⁸, so, assuming only 19 per cent of the inhaled material was available for intake to the body fluids, not more than 0.8 pc. would have been absorbed and transferred to other tissues from the lungs. If it is assumed that this activity of plutonium-239 is redistributed in the proportions suggested by the I.C.R.P., then the activities in bone, liver and kidney would be less than 0.6 pc., 0.12 pc. and 0.016 pc. respectively. (The natural α -activity of these tissues has been given⁷ as 400 pc., 9 pc. and 1 pc. respectively.) The only reported value which can be compared with the foregoing is a measurement of plutonium-239 in human kidney¹⁸. This gave ten times the activity suggested here as an upper limit.

I thank Prof. W. V. Mayneord for his advice and encouragement. I also thank all those members of the Department who have assisted in some way or other, in particular, Mr. J. O. Crookall, who designed and carried out the chemical separation procedure, Dr. C. R. Hill and Dr. R. P. Parker. I also thank Dr. W. O'Bank (of the Department of Forensic Medicine) and Dr. P. J. Roylance for providing the post-mortem material. This work was performed under tenure of a Department of Scientific and Industrial Research research studentship.

¹ Diamond, H., *et al.*, *Phys. Rev.*, **119**, 2000 (1960).

² Burton, W. M., and Stewart, N. G., *Nature*, **186**, 584 (1960).

³ Cottini, C., Gatti, E., Giannelli, G., and Rozzi, G., *Nuovo Cimento*, **3**, 473 (1956).

⁴ Anderson, W., Bentley, R. E., Parker, R. P., Crookall, J. O., and Burton, L. K., *Nature*, **187**, 550 (1960).

⁵ Turner, R. C., Radley, J. M., and Mayneord, W. V., *Brit. J. Radiol.*, **31**, 397 (1958).

⁶ *Health and Safety Lab. Quart. Rep.*, HASL, 131 (1962).

⁷ Hill, C. R., *Health Phys.*, **8**, 17 (1962).

⁸ *Intern. Comm. Radiol. Protect.*, Committee II Rep. (Pergamon Press, London, 1959).

⁹ Parker, R. P. (private communication).

¹⁰ Peirson, D. H., Crooks, R. N., and Fisher, E. M. R., *Harwell, A.E.R.E. M-620* (1960).

¹¹ *Defense Atomic Support Agency Rep.*, DASA-539B, Washington (1961).

¹² Rundo, J., and Newton, D., *Nature*, **195**, 851 (1962).

¹³ *U.S. Congressional Hearings on Fallout from Nuclear Weapons Tests*, **3**, 2197 (1959). Krey, P. W., Bogen, D., and French, E., *Nature*, **195**, 263 (1962).

¹⁴ Osborne, R. V., *Nature* (in the press).

¹⁵ Turner, R. C., Radley, J. M., and Mayneord, W. V., *Nature*, **181**, 518 (1958).

¹⁶ *The Hazards to Man of Nuclear and Allied Radiations*, 89, M.R.C. (H.M.S.O., 1960).

RETINAL PHOTOCOAGULATION BY LASERS

By N. S. KAPANY and N. A. PEPPERS

Palo Alto Medical Research Foundation, Palo Alto, California, and Optics Technology, Inc., Belmont, California

AND

H. C. ZWENG and M. FLOCKS

Palo Alto Medical Research Foundation and Division of Ophthalmology, Stanford University School of Medicine, Palo Alto

WHEREAS coagulation of detached retinae with light has been performed for many years, the present-day techniques suffer from long exposure time, large size of the lesions, and awkward and expensive apparatus. With the advent of lasers the use of a high-energy light source which can deliver the required amount of energy over a short time and cause lesions of small size has become possible. Furthermore, the overall instrumentation can be compacted to the size of conventional ophthalmoscopes.

Photocoagulation is used largely for the treatment of retinal detachment. This common eye disorder occurs in thousands of people each year and, if allowed to develop fully, ultimately results in blindness. The technique offers advantages over previous methods of retinal coagulation. For example, retinal coagulation by diathermy

needle takes several hours and general anaesthesia is required. Furthermore, this is often a 'blind' procedure in the sense that the portion of the retina which is to be coagulated is not viewed directly during the use of the needle. Also, the recuperation period is prolonged. In contrast to this method, photocoagulation is a very simple procedure which can be accomplished in a few minutes. Moreover, a general anaesthetic is never required, the spot which is to be coagulated is always directly in view, the affected area is relatively small and well defined, and the recuperation period is not painful and rarely exceeds a few days.

Investigations of damage by light to the retina¹ were carried out as early as 1867 when an attempt was made to burn the retina of rabbits with sunlight. However, it was not until 1945, when intense artificial light sources

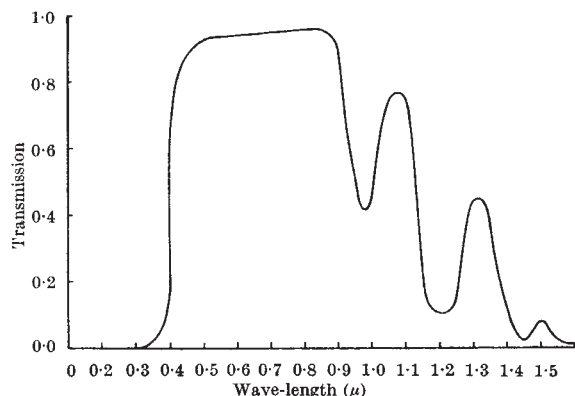


Fig. 1. Spectral transmission curve of the refractive media of the human eye

were available, that therapeutic light coagulation appeared promising. By 1949 Meyer-Schwickerath³ and Littman had developed a technique and an instrument for practical therapeutic light coagulation. In this instrument, light from a continuously operated high-pressure xenon lamp is transmitted along a train of optical components located in a long, movable arm which extends from the instrument. At the end of the drum is a mirror with a small hole through which viewing and aiming are done. The lens of the patient's eye causes the light to be focused on the retina where a lesion is produced. The light intensity can be adjusted to obtain the desired effect in an exposure of about 0.25–1.5 sec.

Coagulation Using Lasers

A new class of unique light source, namely, optical masers and lasers³⁻⁵, is now available for photocoagulation work. These sources are monochromatic and emit light at various wave-lengths both in the visible and infra-red. A common solid-state laser configuration consists of an active material (polished flat and parallel at the ends) which is placed on the axis of a helical xenon flash lamp. For a period of time (typically $\approx 300 \mu\text{sec}$) an intense flash from the xenon lamp causes more active material ions to exist in an upper quantum state than are in the ground state. During this period, light which travels parallel to the cylinder axis is amplified and an intense, highly collimated, monochromatic, coherent light beam is emitted.

(a) *Selection of Wave-length.* In selecting a wave-length for retinal photocoagulation one must consider the optical properties of the eye as well as the difficulties of obtaining required amounts of light at a given wave-length. Fig. 1 shows the transmission of the refractive medium of the human eye². It appears that for photocoagulation work the wave-length of light should be confined to the portion of the spectrum between 0.4 and 0.9 μ or possibly to the infra-red region around 1.08 μ . At these wave-lengths the light will pass through the vitreous and aqueous humours and the retina, and will be absorbed in the pigment epithelium and the choroid. At the present time there are only two laser materials which conveniently give practical amounts of energy in these regions. These materials are chromium in alumina (ruby), which emits at 6943 Å, and the neodymium in glass, which emits at 1.06 μ .

The fraction of the incident light which is absorbed in the fundus varies several hundred per cent⁶ from one eye to another and also varies at different regions in the retina. However, the absorption does not vary rapidly with wave-length in the visible and slowly falls towards the infra-red. Because of this and because the transmission of the refractive media of the eye is higher in the visible than in the infra-red, ruby appears to be favoured over neodymium. However, there is a possibility that

instrumental considerations associated with neodymium may make its selection advantageous.

(b) *Photocoagulation Process.* All the biophysical details of the absorption-coagulation process are not fully understood. Certainly the effect is dependent on power although the extent is not known. Soesterberg⁷ has advanced a theory which appears to be applicable to light sources less powerful than lasers. A mathematical description of the process is reported by Rose *et al.*⁸ Qualitatively, the process is quite simple. During the time ($\approx 300 \mu\text{sec}$) when light energy is delivered to the fundus, very little energy can escape to the surrounding tissue either by convection within the capillary system or by conduction to surrounding tissue. Because of this, the temperature of the small amount of tissue which absorbs the energy becomes highly elevated. If a large amount of energy is delivered rapidly, tissue fluid is vaporized and the vapour erupts through the retina into the vitreous humour and a bubble is formed. A haemorrhage or a retinal hole can also be caused under these conditions. If more moderate amounts of energy are delivered, the absorbing tissue is not vaporized and some of the stored energy is passed on to the retina causing it to be coagulated and 'welded' to the choroid.

Experimental Laser Photocoagulator

An experimental laser photocoagulator has been built to determine laser parameters pertinent to photocoagulation and also to provide an instrument for clinical testing on rabbits, cats and human beings. Fig. 2 illustrates, diagrammatically, the optical features of this instrument. Here a collimated beam of light is emitted from the active laser material and is focused by the lens of the eye on to the retina where a lesion occurs. Provision is made so that the active medium can either be a cylindrical ruby crystal or a cylinder of neodymium-doped glass. The instrument is about the size and weight of a normal ophthalmoscope so that it can be controlled easily with one hand. Direct ophthalmoscopic viewing is provided with an auxiliary light source and several examining lenses, both negative and positive. The laser configuration consists of a 0.25 in. \times 3 in. rod placed between two linear xenon flash lamps surrounded by a specular reflector. For safety and reliability, the lamps are connected to independent storage capacitors although they are triggered from the same source. The capacitors are capable of delivering up to 800 joules of energy to the pumping source, that is, xenon lamps.

The energy output of the laser can be altered by varying the voltage to which the capacitor banks are charged. The result is that energy output and average power output are not independent variables, but rather they are related in some complex manner. Moreover, the instantaneous power output is a rapidly varying function of time and no attempt is made to control it⁹. In this instrument the total energy output of the laser was varied continuously from zero to 0.25 joule.

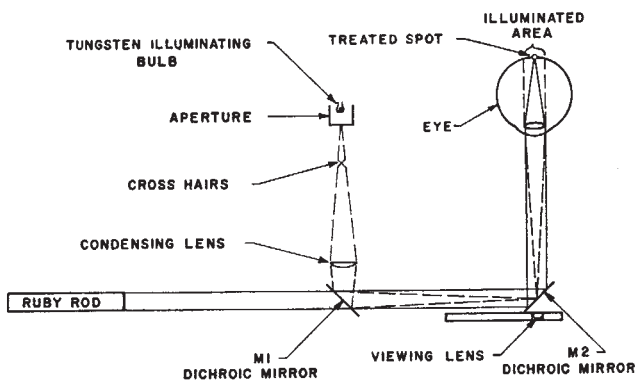


Fig. 2. Diagrammatic illustration of the optical features of the experimental laser coagulator

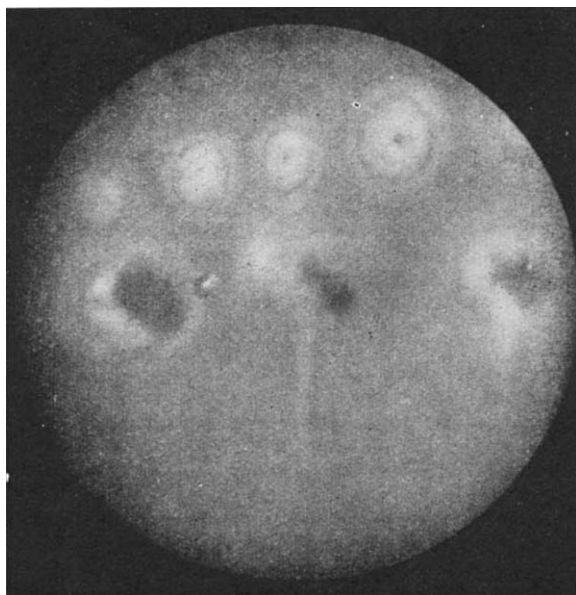


Fig. 3. Two rows of lesions showing effects of increasing laser energy on the size of lesions

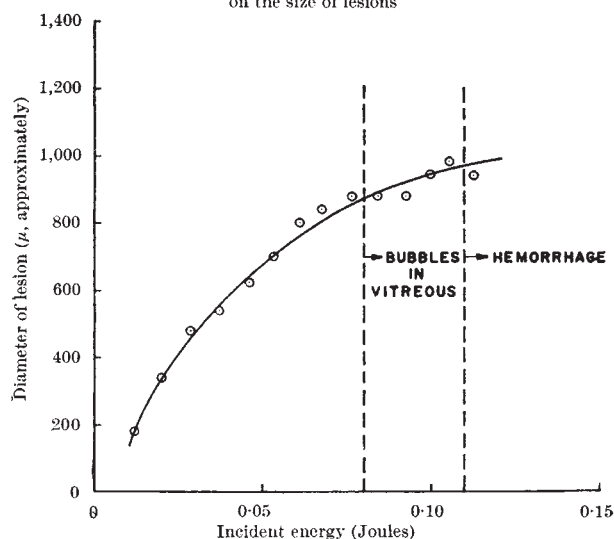


Fig. 4. Plot of the diameter of lesions versus incident laser energy. The bubbling and haemorrhage thresholds are indicated

Experimental Results

So far all experimental work has been performed on rabbits or cats. In the experiments the animal is anaesthetized with intravenous pentobarbital and the pupils dilated with 10 per cent phenylephrine. After treatment with a laser photocoagulator and/or a Zeiss photocoagulator, photographs of the lesions are taken. Histological sections of the lesions are also made.

Immediately after laser coagulation the structure of the lesions is more apparent than in those caused by the Zeiss photocoagulator and are smaller in size. In the centre of the lesion is a dark crater, indicating a high local density, and concentric with this is a dark ring with smaller and larger white rings on either side. During the next few days the lesion changes in appearance and begins to look more like a uniform disk of affected tissue. This disk of affected tissue apparently is firmly attached to the choroid.

It was found that the coagulation threshold (minimum energy required for coagulation) varied grossly with colour of the fundus. This was expected and is related to the reflectivity and absorptivity of the fundus at 6943 Å, the wave-length of the radiation from the laser. In

general, the threshold energy for a lightly pigmented fundus is higher than a darkly pigmented fundus. Variations of more than a factor of five were observed. The amount of damage caused by a given incident energy is also a function of the colour of the fundus. It is possible for a given incident energy to cause a haemorrhage in a darkly pigmented fundus and yet not visibly damage an albino fundus. The energy-range over which good lesions can be caused without excessive damage varies markedly with the colour of the fundus. For example, for a particular Siamese cat with a pale fundus the ratio of energy required for a haemorrhage to coagulation threshold energy was about eleven to one. This same ratio for a cat with a darker fundus was three to one. Similar variations were found with rabbits.

Investigations were carried out to determine the area of affected tissue as a function of incident energy. In general, the diameter of the lesion increases with increasing incident energy but not indefinitely. Fig. 3 is a photomicrograph showing this effect on the retina of a rabbit, on which two rows of lesions were caused. It will be noted that the energy associated with the lesions increases from left to right in both rows, with the lesion in the upper left being caused by the least amount of energy. In experiments on pigmented rabbit's retina, as the energy is increased bubbles begin to form between 0.08 and 0.11 joule, and finally haemorrhaging occurs above 0.11 joule. Fig. 4 gives a plot of the diameter of lesions on a rabbit retina caused by varying the laser power output. The size of the lesions varies from 200 μ to 1 mm when the energy is varied from 0.01 to 0.15 joule.

Histological sections of many of the lesions were prepared to determine the general character of the lesions, as well as the depth of penetration. In the process it was found that the retina usually separates from the choroid except at the sites where lesions were caused. This is evidence that the retina is indeed 'welded down' in these regions. Fig. 5a is a section of a typical lesion caused

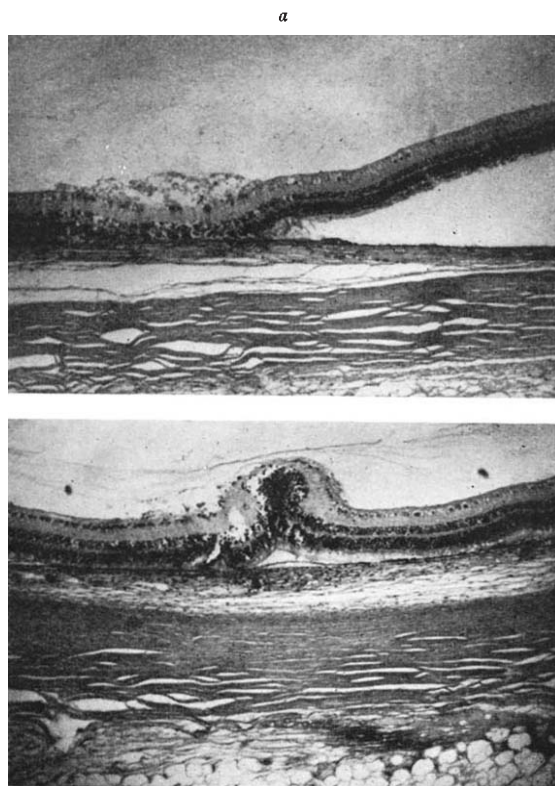


Fig. 5. a, Histological section of a normal lesion caused by laser photocoagulation; b, histological section of a lesion showing eruption through the retina due to high laser energy

in the retina of a rabbit. Scar tissue has formed above the retina and a general disruption of cells is apparent below this tissue. The retina has separated from the choroid except at the lesion. Fig. 5*b* is a section of a lesion through which a bubble erupted. It is obvious from the photomicrograph that the tissue which was vapourized was in the vicinity of the pigment epithelium and in fact probably was a portion of the pigment epithelium. The photomicrograph also shows that the retina has separated from the choroid except at the lesion. Other sections indicate that the pigment epithelium is affected at very low incident energies. Even at very high energies (0.25 joule) the damage does not appear to penetrate very deeply into the choroid.

Conclusions

Because of the large variations in colour of the fundus it will be necessary to begin any treatment at low energies and gradually increase the energy until the proper effect is caused. This change in energy-level can be caused both by varying the condenser voltage and by using calibrated filters in the beam. This procedure, however, is not objectionable and is generally followed today with the existing coagulator. There are a number of advantages offered by laser photocoagulation. The fact that the lesion which is caused is small is important when

the macula of the eye is involved. The size of the lesion can be varied by varying the laser energy or by using an appropriate optical system. Also, since the lesion is caused in such a short time, the normal reaction of the eye to a bright light does not occur until after the process is over and thus the burn is not smeared across the retina. The ease of manoeuvring and aiming a hand-held instrument with an ophthalmic viewing head, the fact that the patient does not need to be anaesthetized, and the potential low cost of the instrument are all important practical considerations which make retinal photocoagulation by lasers appear quite promising.

We thank N. Silbertrust for his assistance.

This work was supported by Public Health Service Grants HE 07684 from the National Institutes of Health and NB 0215 from the National Institute of Neurological Diseases and Blindness.

¹ Czerny, *Ber. Wien. Acad. Wiss.*, **56**, 11 (1867).

² Meyer-Schwickerath, G., *Light Coagulation* (St. Louis, C. V. Mosby Company 1960.)

³ Schawlow, A. L., and Townes, C. H., *Phys. Rev.*, **112**, 1940 (1958).

⁴ Maiman, T. H., *Nature*, **187**, 493 (1960).

⁵ Javan, A., Bennett, jun., W. R., and Herriott, D. R., *Phys. Rev. Letters*, **6**, 106 (1961).

⁶ Rose, H. W., *Research Study of the Production of Retinal Burns*, DASA Tech. Rep. No. 1279, October 1961, Contract DA-49-146-XZ-046.

⁷ Soesterberg, *Bull. Math. Biophys.*, **24**, 115 (1962).

⁸ Hellwarth, R. W., in *Advances in Quantum Electronics*, edit. by Singer, J. R., 334 (New York, Columbia Univ. Press, 1961).

PARTITION OF DIVALENT CATIONS BETWEEN BACTERIAL WALL AND CELL CONTENTS

By PROF. J. M. VINCENT and MRS. BEVERLEY A. HUMPHREY

Microbiology Laboratory, School of Agriculture, University of Sydney

A DETAILED examination of the requirements of *Rhizobium trifolii* for magnesium and calcium¹ correlates well with the results we have since obtained on the disposition of these elements in the cell wall and within the cell itself, under contrasting conditions of calcium deprivation and sufficiency². The information so derived supports the indications we already have that calcium has a structural role in the wall of this organism. This role can be distinguished from the less specific part it shares with magnesium in meeting a much larger requirement by the cell for divalent cations.

Portion of the analytical data³ can be shown graphically (Fig. 1) in such a way as to indicate the concentration of both elements in walls and cells (height of histograms) and their total distribution between the two (area). For this purpose and subsequent calculations we have taken 20 per cent as a fair estimate of the proportion of total cell mass likely to be represented by wall³.

Three things call for comment: (a) Calcium is more concentrated in the wall than in the rest of the cell. (b) When calcium is severely limiting (histogram B) the wall retains a reduced but appreciable amount of this element, sufficient to account for all that is found in the whole cell analysis. (c) Magnesium only partly replaces calcium in the wall but fully compensates for deficiency of calcium within the cell (histograms A, B). In fact, the total divalent cation thus represented by magnesium on a molar basis is significantly greater than when calcium is present as well. On an actual weight basis, Fig. 1 is about the same whether calcium is present or absent. (Total divalent cation in cells receiving calcium, 4.11 mg/g dry cell substance; without supplied calcium, 4.38.)

The apparently more particular relationship between calcium and the cell wall is, we think, significant in relation to the spherical condition of calcium-deprived cells that is quite different from the appearance of cells limited by a

shortage of magnesium⁴. It is apparent that each element has a distinct role. Ultra-thin sections of the calcium-deprived cells viewed under the electron microscope show a well-defined double-layered cell wall⁴ which is quite different from what we have seen in penicillin-induced spheroplasts of the same organism. Moreover, although the proportion of viable cells grown under con-

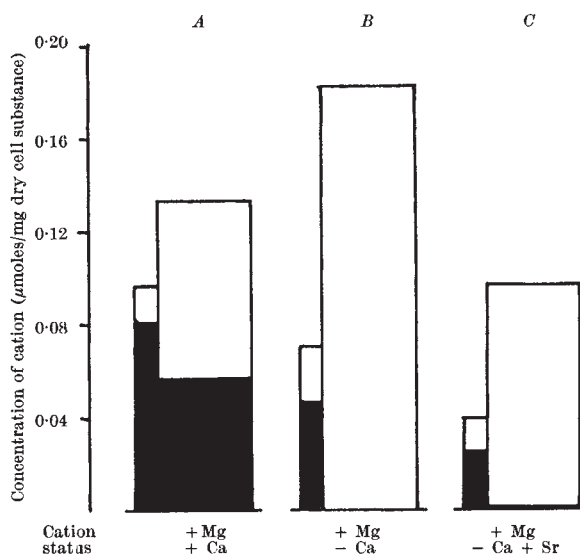


Fig. 1. Distribution of calcium and magnesium between walls and cell interior. In each histogram (A, B and C) the wall, narrow column at the left, is taken to be one-fifth the total cell mass. The height of each column represents concentration; the area, total amount contained in wall or cell. White, magnesium; black, calcium