REVERSIBLE LINEAR CHANGES IN THE TISSUES OF PLANTS AND THEIR SIGNIFICANCE.

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From a long time it has been noticed that the autonomic movements of leaves are due to the differential rates of elongation of the tissue of pulvinus or leaf-base on the dorsal and ventral sides, brought about by the varying turgidity of the tissue not at all connected with growth. This variation is known to be reversible in its nature as also rhythmic (DARWIN, 1880, KRISHNA IYENGAR, 1942*a*). The author's work on the leaf-movements is an attempt to prove that these movements form the characteristic feature of not only *Desmodium gyrans* and *Eleiotis* sp. but also many other plants, and that these are connected with the periodically fluctuating water-content of the plant-body. By further investigation the author has tried to establish the existence of a relationship between the direction of the leaf-movement and the fluctuating weight of a plant (KRISHNA IYENGAR, 1943*a*, 1946). It has been suggested by the author that the reversibility in weight-changes noticed from time to time and the daily rhythm about it form the inherent trait of many plants. Thus, according to the author the direction of leaf-movement signified the positive or the negative aspect of water-content. Since the leaf showed not only major oscillations taking an hour or more but also minor oscillations of very short duration (less than a minute) the author felt that there was reversible change in the water-content even at short intervals. This accounts for the author's efforts to isolate the tissues and study their behaviour. As linear change was the most convenient to tackle the author has selected this feature. A preliminary note on the subject (KRISHNA IYENGAR, 1945) has been published in the Proceedings of the Indian Science Congress of 1945. An account of the author's observations is given below.

MATERIAL AND METHODS.

The sprouted bulbs of *Allium Cepa, Polianthes tuberosa* and *Zephyranthes* sp., the sprouting tubers of *Solanum tuberosum*, the storage roots of *Asparagus racemosus* and the aerial roots of *Vanda* sp. were selected for investigation. The bulbs were cut into slices, each retaining a bit of the stem. Except the leaf-base meant for observation the others were removed. In the selected leaf-base only the inner epidermis was retained. The stem part of the material was fixed to a stand with rack and pinion arrangement while the free end of the epidermis was connected to the optical lever designed by the author (KRISHNA IYENGAR, 1944). Regarding the potato, cylinders were cut with the cork-borer, the axis of these being at right angles to the length of the tuber. In the case of *Asparagus* and *Vanda,* strips of cortical tissue were selected. Just as before one end of the tissue was fixed to the stand while the other end was connected to the optical lever. The material was completely immersed in water properly aerated during the period of observation, care being taken to give the tissue enough of time for adjustment. Readings were taken at intervals of 5 minutes, 1 minute, ½ minute, 10 seconds or 2-3 seconds according to needs, employing magnifications ranging from 500 to 6,000, and from these graphs were drawn. During the periods of observation the temperature variation of water was found to be negligible. The observation was made in artificial light of 40 c.p. (at a distance of 2 feet from the water-level). Control experiments were conducted on dead tissues to make sure that the changes formed the feature of living tissue.

Observation.

Fig. 1 represents the linear changes noticed in the epidermis of the leaf-base of a fully formed leaf of *Allium*. The readings were taken at intervals of five minutes, and the magnification employed was 500. The observation extended over a period of nearly two hours. The oscillations are noticed generally at intervals of 10–25 minutes.

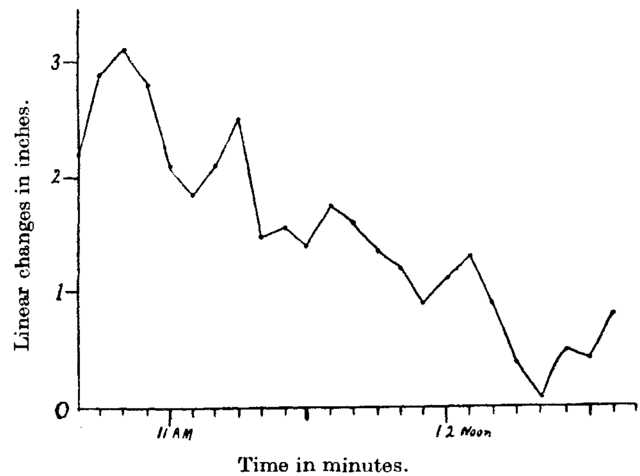

Fig. 1. Linear changes in the epidermis of *Allium Cepa*. × 500.

Fig. 2 is part of Fig. 1, but with the readings taken at intervals of 10 seconds. The two graphs (*a*) and (*b*) show the linear changes at intervals of 1 minute and

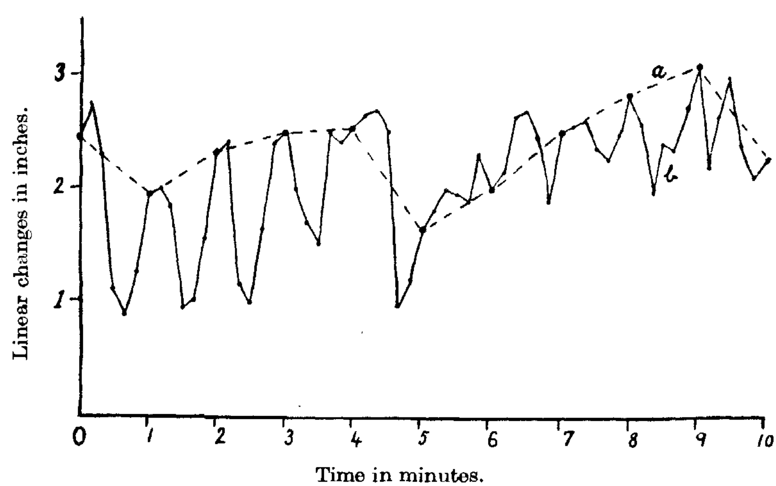

Fig. 2. Linear changes in the epidermis of *Allium Cepa* at intervals of a minute (*a*) and at intervals of 10 seconds (*b*). × 1,000.

10 seconds respectively. For proper reproduction after reduction the magnification is doubled. In both (*a*) and (*b*) oscillations are noticed, the shortest and the longest duration for an oscillation in (*b*) being 20 and 60 seconds respectively. These fine oscillations are wanting in (*a*) and Fig. 1 because of the long interval of the readings.

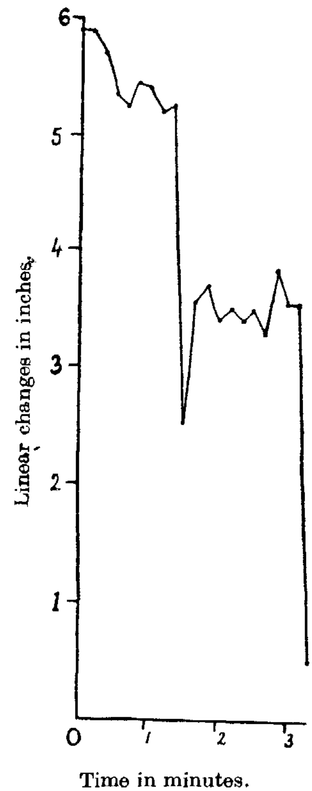

FIG. 3. Linear changes in the epidermis of *Allium Cepa*. × 5,000.

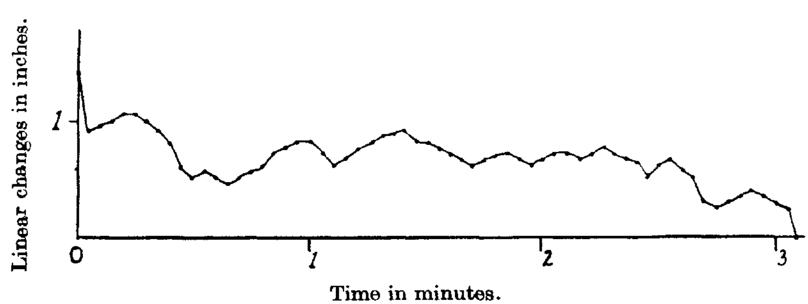

FIG. 4. Linear changes in the epidermis of *Allium Cepa* at intervals of 3 seconds. × 5,000.

Fig. 3 shows the oscillatory linear changes in the fleshy leaf-base of a sprouted bulb of *Allium*. The magnification employed is about 5,000, and the readings are at intervals of 10 seconds.

The next figure (Fig. 4) shows the changes at even shorter intervals. The material used for Fig. 3 was used for this also, but the readings are at intervals of about 3 seconds, the magnification remaining the same. The shortest time for an oscillation happens to be about 6 seconds.

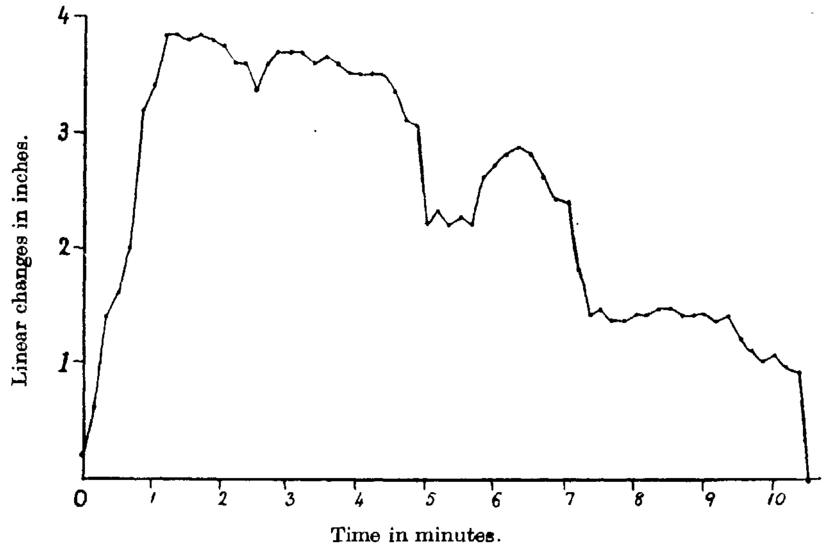

FIG. 5. Linear changes in the epidermis of *Polianthes tuberosa*. ×2,000.

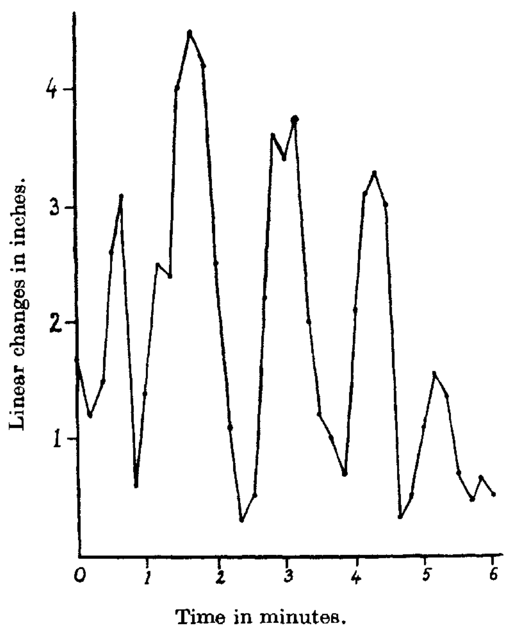

FIG. 6. Linear changes in the epidermis of *Zephyranthes* sp. ×4,000.

Figs. 5 and 6 are the records showing the oscillatory changes in the epidermis of *Polianthes* and *Zephyranthes* respectively. The magnification is 2,000 or 4,000 and the recording interval is 10 seconds.

The linear changes noticed in the tissue of potato tuber are represented in Fig. 7. The readings were taken at intervals of 10 seconds employing a magnification of 5,000.

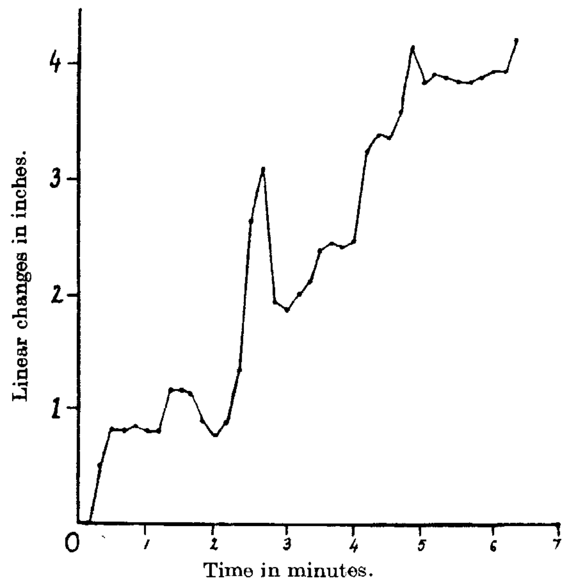

FIG. 7. Linear changes in the tissue of the tuber of *Solanum tuberosum*. ×5,000.

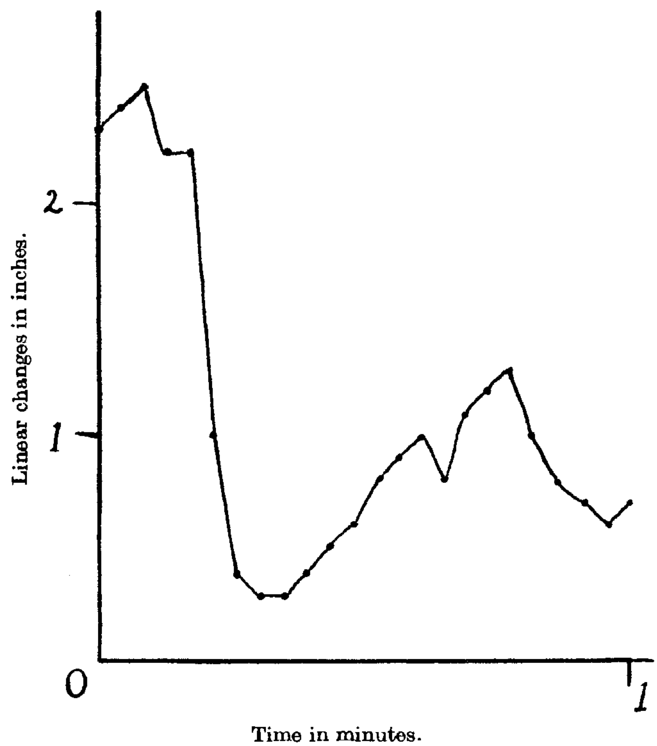

FIG. 8. Linear changes in the cortical tissue of the root of *Vanda* sp. ×16,000.

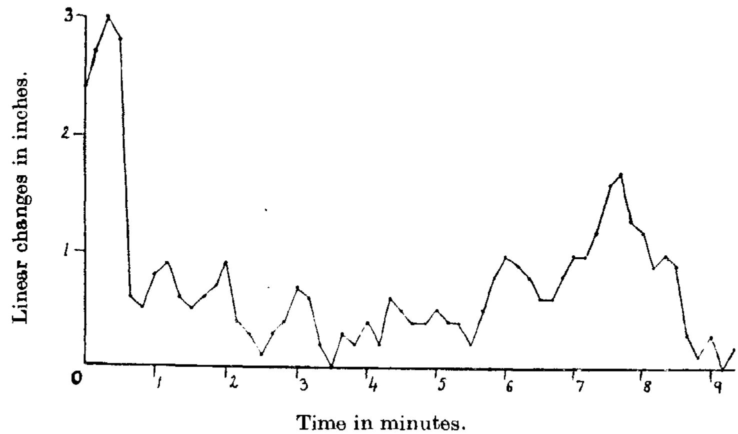

FIG. 9. Linear changes in the cortical tissue of the storage root of *Asparagus racemosus*. ×16,000.

The behaviour of the cortical tissue of roots of *Vanda* and *Asparagus* is represented in Figs. 8 and 9 respectively. In both the cases the magnification happens to be about 16,000, but the recording interval is different. In the case of *Vanda* 23 readings were taken during a minute, thus making the recording interval nearly 2½ seconds. In *Asparagus* this time-interval is 10 seconds. Oscillations are noticed in both the graphs.

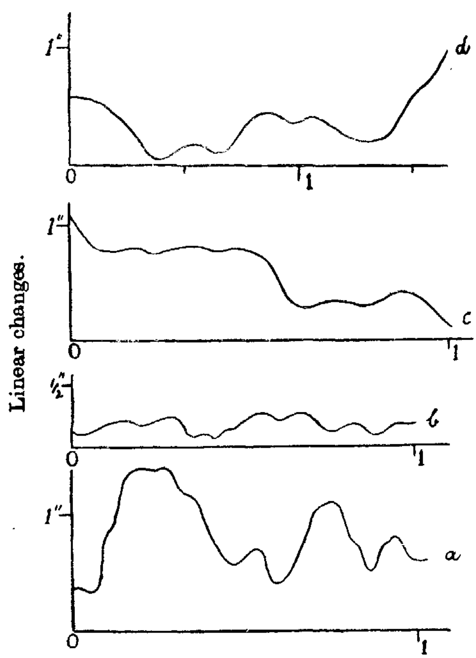

FIG. 10. Finer oscillations in *Allium Cepa* (*a* and *b*), *Solanum tuberosum* (*c*) and the thigh muscles of a frog (*a*, *b* and *c* ×5,000 ; *d* ×2,000).

Fig. 10 represents the finest oscillations in *Allium* (*a* and *b*) and *Solanum* (*c*). The movement of the beam of light was traced on the graph paper mounted on a revolving drum. For the sake of comparison, record (*d*) showing the oscillations in the thigh muscles of a frog is introduced. In all these figures the finest oscillations are noticed, the duration of an oscillation being 6–10 seconds. Even finer oscillations than these were noticed but these could not be recorded. The approximate time for this type happens to be 2-3 seconds.

During various hours of the day the tissues were studied and throughout the period of observation the oscillations manifested themselves. Experiments conducted on the bulbs of *Allium* during different periods of the year have revealed (1) that the oscillations are noticed in the resting bulbs also, but are of lower magnitude, (2) that the leaf-bases about the middle of the bulb are the best for observation and (3) that the seasonal rhythm of the plant is likely to determine the magnitude and frequency of oscillations.

DISCUSSION.

The observations recorded above reveal that all living tissues are capable of oscillatory linear changes. While the author has tried to show the behaviour of homogeneous tissues the previous work (MAC DOUGAL, 1921, 1925 and MAC DOUGAL and SHREVE, 1924) shows that there are reversible daily changes in the diameter of trunks and branches, signifying thereby the rhythmic volumetric changes in these parts. There are major and minor changes or oscillations, the former taking an hour while the duration of the latter may be a few minutes or a few seconds. Just as in growth the linear changes take place by a series of stages, each stage being sigmoid in its nature. Short-interval readings taken with the help of a very high magnification reveal that a period of elongation is invariably followed by a stage

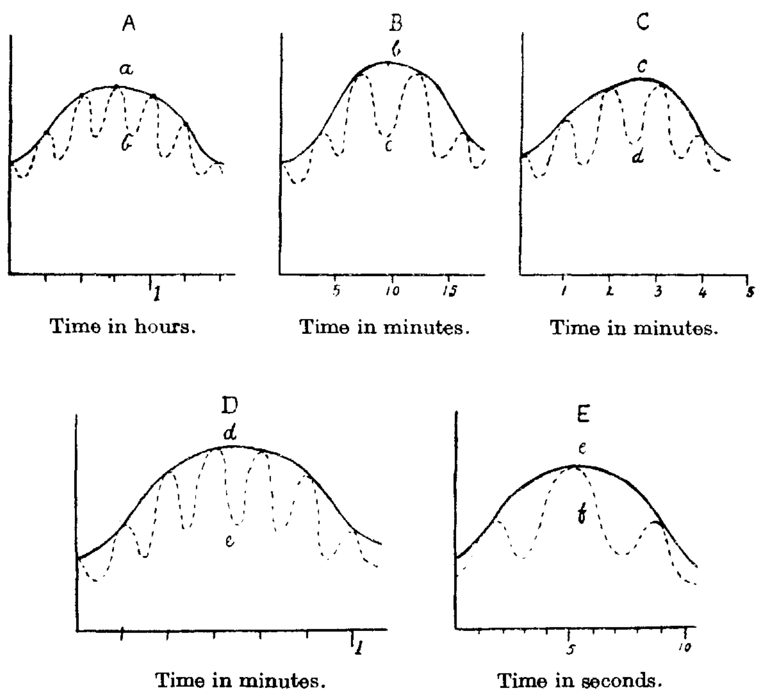

FIG. 11. Diagrammatic representation of the different kinds of oscillations.

of contraction. When the linear changes are almost negligible there are very fine oscillations, the shortest time for one of these being about 3 seconds. As already mentioned the magnitude of these oscillations depends on the kind of plant and its seasonal rhythm, nature of the tissue, its age and tone. In several cases the oscillations requiring about 8–10 seconds were composed of two or three finer oscillations. These fine oscillations are likely to be missed when the employed magnification is low and the recording interval is longer. Fig. 11 is introduced here to stress the importance of short-interval recording and to explain the relationship of the different kinds of oscillation. In connection with growth the author (KRISHNA IYENGAR, 1943*b*) has stressed this point.

These oscillations signify that living cells show rhythmic contraction and expansion. Regarding this feature it may be mentioned that till now more work has been done on animal tissue than on plant tissue. In his Presidential Address to the Physiology section of the Indian Science Congress of 1944 MATHUR has discussed the causes and significance of the rhythmic linear changes in the involuntary muscles. According to him and several others carbon dioxide has been the 'maintainer of life', the rhythmic changes noticed therein being brought about, though indirectly, by the varying concentration of CO_2. The presence of traces of this gas within the protoplast is said to act as a stimulant resulting in the stretching of the muscle, while a higher concentration of the same brings about the contraction, the two phases being responsible for the systole and diastole of the heart and the rhythmic respiratory action. According to DARWIN (1898) and LLOYD (1908) the closure and the opening of the stomata may be brought about by varying the concentration of CO_2 in the vicinity of the stomata, the two processes being due to the contraction and expansion of the guard cells as a result of elimination or absorption of water. The daily rhythm manifested by the guard cells and the rôle of *p*H in the same have been noticed and reported by many. In the light of this the author is strongly inclined to believe that the oscillations in length manifested by the plant and animal tissues are traceable to the varying concentration of CO_2 and *p*H variation from time to time. It can thus be stated that the varying concentration of CO_2 brings about an alteration of the *p*H of the protoplast, which in its turn affects the rate of absorption of the materials, the resulting reversible variation in the turgidity manifesting itself in the contraction or elongation of the cell.

The manifestation of rhythmic linear or volumetric changes (or oscillations) so characteristic a feature of all living cells forms an important clue to the changing metabolic activities of the living cell. The *p*H alteration of the protoplasm changes its structure resulting in its stretching or contraction and permeability variation. It is quite possible that as a result there is significant positive or negative intracellular pressure which in combination with the varying permeability of the protoplasm brings about a high rate of absorption or elimination, forming thus a powerful mechanism of a living cell. This may be termed as the 'vital force' enabling a living cell to discharge its general and special functions at a rate not at all possible if the cell has to depend only on the physical laws to determine its activity. The rapidity with which the guard cells change their size is an instance of this. Regarding the absorption of water by plants MILLER (1931) points out that it is rather doubtful if forces like imbibition, osmosis, etc. are adequate enough to supply sufficient water to replace that lost during transpiration. In connection with photosynthesis MILLER hints that the 'volume of CO_2 that can be utilized by the leaf according to physical laws seems to be an insignificant one compared with what is taking place'. The inadequacy of the physical laws to account for the high rate of translocation of materials has been sufficiently stressed by not only MILLER (1931) but also MAC DOUGAL (1925). In view of all these it seems quite possible that the 'vital force' whose presence is strongly suggested by the oscillations, is responsible for the high rates of activities, and acts in the manner already explained, being thus of very great importance in all the vital activities of a living organism.

SUMMARY.

Tissues from the bulbs of *Allium Cepa*, *Polianthes tuberosa* and *Zephyranthes* sp., from the tubers of *Solanum tuberosum* and from the storage roots of *Asparagus racemosus* and aerial roots of *Vanda* sp. were prepared for observation, and linear changes were studied at short intervals employing a very high magnification.

Irrespective of the general tendency of the tissue towards contraction or elongation the materials showed rhythmic reversible linear changes or oscillations, the major oscillations taking an hour or more, while the minor oscillations were over within a few minutes or a few seconds. The shortest time taken by the finest oscillation is about 3 seconds.

The magnitude, duration and frequency of these oscillations depend on the type of plant, its seasonal and daily rhythm, nature of the tissue and its tone.

Since animal tissues also show a similar feature the author is inclined to feel that the manifestation of these oscillations is an inherent quality of a living cell, signifying though indirectly the frequent reversibility in its metabolic activities.

The stimulation or depression due to variation in the intracellular concentration of CO_2, changing *p*H of the protoplast and the stretching or shrinking of the protoplasm, the succeeding alteration in its structure and permeability and internal pressure are probably the successive stages preceding linear or volumetric changes, forming thus a powerful mechanism or the 'vital force' of a living cell.

It seems highly probable that this 'vital force' enables absorption, elimination, translocation of materials or diffusion of gases at a rate not at all possible by physical laws alone.

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