terpreted as measuring only the L isomer. Further study of this problem using *Lactobacillus fermenti* 36, which responds to both isomers, is in progress.

It will be noted that a fixed test dose of methionine was employed throughout all these experiments, regardless of variation in body size, and this probably accounts in part for the variation in absolute values here reported. However, expressing the observed rate of disappearance in terms of milligrams per hour yields values which are quite comparable. Experiments with other test dosages may further clarify this point.

The rapid initial fall in plasma methionine after intravenous injection is interpreted as due mainly to diffusion into the blood cells and extravascular spaces and, to a very slight extent, to excretion. The more gradual and regular rate of disappearance apparent in the later stages would seem to be a reflection of metabolic phenomena, and hence this has been considered more significant in the interpretation of the response of various subjects to the test dose of methionine.

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Activation of Eggs by Oxidation-Reduction Indicators

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In a previous paper (*1*) the writer suggested that the mechanism of fertilization of eggs was dependent upon the production of the appropriate redox potential of the enzymes controlling oxidations in the egg.

In order to test this hypothesis further, eggs were placed in sea water containing redox dyes whose E_0' values at pH 7.0 ranged from +.195 to −.258, giving an rH from 20.5 to 5.2. These dyes included o-cresol indophenol, methylene blue, brilliant cresyl blue, indigo tetra-, tri-, di-, and monosulfonate, Janus green, and neutral red. The concentration of the dye varied from .002 to .0001 per cent. The marine eggs used were those of *Urechis caupo* and *Strongylocentrotus purpuratus*. Since the end of the season for these eggs prevented further experimentation, this preliminary report is made.

The freshly obtained eggs were placed in solutions of dye in sea water for various periods of time from 1 minute to 24 hours and then replaced in sea water. Crowding was avoided. Samples were taken out at intervals. Activation was observed chiefly when eggs had been exposed from 1 minute to 20 minutes in the dye solution. Activation depended at different times on temperature, time in the dye solution, and concentration of dye. By activation is meant here either the formation of a fertilization membrane or cleavage with or without the membrane. In the case of *S. purpuratus*, development progressed to the pluteus stage in from 1 to 2 per cent of the eggs, in *U. caupo*, to the trochophore stage in about 50 per cent of the eggs. In other cases only the fertilization membrane appeared, the 2-, 4-, 8- or more cell stage developed, or irregular division occurred. The maximum percentage of activation varied from 70 per cent in *Urechis* to 10 per cent in *S. purpuratus*. Toward the end of the season no activation was obtained. In all cases unfertilized controls were completely free from activated eggs, while fertilized controls showed from 90 to 100 per cent activation.

In the case of *S. purpuratus*, the best results were obtained with indigo monosulfonate, which has an rH of 8.7. The rH of the sperm of this sea urchin was found to be from 9.0 to 9.5. In the case of *Urechis*, methylene blue and indigo tetrasulfonate gave the best results. The rH values of these dyes are 14.4 and 12.1, respectively, while that of the sperm of *Urechis* was found to be between 13 and 14. These results show a remarkable agreement between the rH values of the activating dyes and the respective sperms. Neutral red produced some fertilization membranes in a few cases. O-cresol and Janus green showed no results. A few cases of activation were produced by some of the dyes in the middle range of rH values. Development in all cases was slower than that of eggs fertilized by sperm, and a smaller proportion developed. These facts were also found by J. Loeb (*2*) in his experiments on artificial parthenogenesis with salt solutions. In some cases unorganized division takes place, resembling that of growth in neoplasms.

It should be pointed out that the sulfonate dyes have long been considered not to penetrate living eggs. However, since they have an activating effect on eggs in these experiments, one must conclude that either they penetrate at least far enough to produce parthenogenesis or the process of fertilization is initiated as a surface effect.

This preliminary report shows definitely that there is a direct relation between the redox potential and the fertilization of eggs. Other factors which may be involved will be discussed elsewhere.

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Irreversible Differentiation in Certain Plant Cell Lineages

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One fundamental problem in the morphogenesis of multicellular plants and animals is the mechanism which produces cellular diversity, and various theories as to the principal causes of histological differentiation have been proposed. Weismann's original idea of a progressive sorting out of nuclear determinants cannot be accepted. Nuclear changes of mutational character apparently play only a minor role, and at present there is general agreement that the locus of differentiation must be the cytoplasm itself. There is some difference of opinion among biologists as to just how this is brought about. It is characteristic of multicellular plants that they respond more directly than animals to influences from the outside; their cells, both in normal and regenerative ontogeny, preserve a high degree of totipotency, and, unless they have become very specialized or possess aged cell walls, they differentiate, dedifferentiate, and redifferentiate in relation to their position and to factors of both the internal and external environment (*3, 9*). The specific character of animal cells, on the other hand, is often determined very early, and it is maintained in nonspecific environments such as tissue cultures; if dividing, such cells may "breed true"—that is, produce only their own type. Botanists have thus come to regard the environment as a factor of major importance in many phases of histological differentiation, and much of the work has therefore been concerned with environmental modifications and effects on the cells. Zoologists, in several recent discussions, have developed a principle which might provide an understanding of the phenomenon of determination and stabilization of specific cellular differentiation in the organism. This assumes the presence in the cytoplasm of determinants genic in nature—the plasmagenes—which would account for the persistence of a particular type of cell character. It has been suggested that such plasmagenes are replicas or partial replicas of nuclear genes, nucleoprotein in nature, the activity and competition of which are subject to selection and mutational modification under the influence of variable local conditions and nuclear products. The capacity of autonomous, persisting duplication of such entities forms the basis for the transmission of specific cellular differences along cell lineages in somatic differentiation (cf. *11, 12*). Plasmagenes seem to bear close relation to viruses (cf. *6*) as to origin and chemistry, and it has been suggested that they are capable of being transmitted by grafting (*2*). Cytoplasmic inheritance is known in plants and has been demonstrated, for instance, for visible cytoplasmic constituents such as plastids (e.g. *7*), but there has been little evidence of persistence of cellular character in somatic cell lineages such as described for animals. The following observations, however, suggest that similar phenomena occur in plants which may serve as a basis for further work in this direction.

In the seedling and mature plant of *Ricinus communis* various kinds of cells (idioblasts) occur, notably those containing calcium oxalate and secretory cells whose contents give the reactions of unsaturated fats and tannins. A general survey of the distribution of both crystal and secretory cells was given by Scott (*8*). In some varieties of *Ricinus* the latter develop a natural red pigment (*1*). In the stem they are found in epidermis and ground parenchyma, especially of the pith. The writer has observed that the initials of these secretory cells are differentiated from cells of the ground tissue during early development. Their earliest appearance may be noted within the small-celled meristematic cone of the growing point itself, and they can be clearly seen at the level of the nodes of even the youngest portion of the stem. Once differentiated, they continue to divide and give rise only to secretory cells, which finally form long, vertical rows, one or several cells wide, among the ordinary cells of the ground parenchyma. In addition, new secretory cell initials are being differentiated here and there from larger and older ground parenchyma cells which develop in a manner analogous to those formed in the apical meristem. Between secretory cells in different parts of the plant some differences in the microchemical reactions were noted; in the red varieties, for example, the pigment does not appear in the youngest cells, but in those at some distance from the apex. Internodes were wounded by lateral cuts in both the youngest and fully differentiated older region of the stem, and a wound meristem was formed through cortical and pith parenchyma in which both parenchyma and secretory cells divided vigorously. The secretory cells, wherever present, produced only secretory cells. Thus, neither active cell division and growth nor altered position at the surface of the wound meristem had an effect on their anatomical and physiological character.

In other species of plants there occur various kinds of early differentiated idioblasts—for example, the so-called oil cells, tannin cells, and cells containing crystals of calcium oxalate. Cells of this kind were never observed to dedifferentiate in regenerating tissues. Some retain for a considerable time their power of multiplication; in air roots of *Cissus gongylodes*, for example, near the growing point, raphide cell initials divide several times, giving rise to rows consisting of crystal cells. Additional evidence as to the stability of cell character of the secretory cells in *Ricinus* and other similar cells might well be obtained from tissue cultures.

These observations indicate that in the differentiation of plant tissues certain cells become determined relatively early in their character and give rise subsequently to cells of the same character only, even under conditions which normally induce dedifferentiation, such as wounding and alteration of their correlative relations. The majority of plant cells do not react in this way. They remain, if they stay reactive at all, in a more or less undetermined state in which their development can be tipped one way or another under the selective control of the environment; cells can often be induced to dedifferentiate, or to differentiate in a specific fashion corresponding with their position and the particular state of reactivity which they possess at a given time and locus (*3*).

One may thus tentatively suggest that the behavior of the secretory cells in *Ricinus* involves a change in their cytoplasmic system in which control exerted by determinants in the nature of plasmagenes plays a role. The result is both a limitation of cellular totipotency and stabilization of cellular character in somatic differentiation. The cytological mechanism by which determinants of this kind come into control in definite cells is at present a matter of conjecture. It may well be that polarity plays a part. At the cell division which precedes the differentiation of a secretory cell initial, an unequal distribution of cytoplasmic material might take place, resulting in a differential concentration of determinants or determinant-precursors in the two daughter cells. Such division would be differential, though not necessarily visibly so. A number of typically differential divisions, as a result of which the two daughter cells become different in size, cytonuclear ratio, meristematic condition, and subsequently in their structure have been described previously (root hair cell, trichosclereid, and hypodermal passage cell division, *5, 10*). In each of these cases the ultimate fate of the two daughter cells is very unequal, although both cells are side by side and probably under identical environmental conditions. In *Ricinus* the capacity of cells to divide differentially obviously extends over a considerable time, since they occur both in the young and old parts of the stem and in the secondary tissue formed after wounding.

The complex patterns of structural differentiation and physiological reactivity which the observer encounters everywhere appear to be the outcome of most varied processes and forces. Some structural changes in the cells can be correlated rather directly with physicochemical factors in external and internal environment, as in the case of cells located at surfaces

(*3*). Doubtless polarity, which is basic in the elaboration of many patterns of growth and differentiation in both unicellular and multicellular forms (*4*), is another factor. It may control the orderly distribution of cytoplasmic material and various substances. Finally, as in the cells here described, there may be specific, qualitative alterations in the reactivity systems of the cells, due to permanent changes in the genetic make-up of the cytoplasm itself.

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Separate Localization in the Medulla Oblongata of the Vagal Inspiratory and Expiratory Reflex Centers

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Stimulation of the afferent vagus nerve acts upon respiration in two different ways. With low stimulus frequency corresponding to the low rate of afferent impulses elicited by lung deflation or small lung volume, the effect on respiratory posture and breathing movements is mainly inspiratory, whereas with higher stimulation rates corresponding to the higher afferent impulse frequencies produced by lung inflation or great lung volume, breathing becomes predominantly expiratory. It must be emphasized, however, that there is no hard, clear-cut line between the low-rate inspiratory effect and the high-rate expiratory effect, and that they both exist simultaneously over a wide range of intermediate frequencies. Thus, within physiological limits, a dual control of respiration is accomplished by these two antagonistic vagal tendencies, inspiratory and expiratory, the former prevailing with a decrease, and the latter with an increase, of the frequency of centripetal impulses emerging from the pulmonary stretch receptors. From this statement it may be inferred that posture as well as movement of the respiratory effector system, which is mainly or even exclusively an inspiratory one, results at any moment of the breathing act from the combined influence of the two opposite vagal drives, excitatory and inhibitory. Perhaps only in the extreme conditions of the vagus nerve artificial stimulation, with adequately chosen rates and intensities of stimuli, may pure inspiratory or expiratory effects be obtained, appearing then as inspiratory or expiratory standstill of breathing.

In order to determine whether separate reflex centers for inspiratory and expiratory effects, respectively, can be distinguished at the level of the medulla oblongata, small circumscribed lesions have been made by means of nonstimulating high-frequency coagulation, in the region of the bulbar respiratory center. It was first observed that suitable lesions situated at a more cranial level can destroy only the expiratory drive without any weakening of the inspiratory one. On the other hand, a somewhat lower, *i.e.* more caudally located lesion, abolishes only the inspiratory component of the vagal respiratory reflex, leaving the expiratory effect intact. Thus, the existence of two different central pathways involved in vagal respiratory reflexes and corresponding to the two antagonistic reflex components has been proved and has later on found further and more detailed anatomical confirmation.

Selective elimination of either the inspiratory or expiratory effect of vagal stimulation demands a small destruction lying in the tractus solitarius, its nucleus, and the adjacent dorsal part of the lateral reticular formation. For the expiratory component, this critical lesion occupies the tractus solitarius system at a more cranial level, *i.e.* just caudal to the entrance of the vagal respiratory fibers. But this expiratory lesion does not interfere with all the descending fibers of the tractus solitarius, unless the inspiratory effect be also abolished, and "central vagotomy" ensues. Some lateral portion of the solitary system, including mainly tractus fibers, must be spared, which seems to indicate that impulses mediating the inspiratory effect are conveyed in those descending fibers. For the inspiratory component of the vagal respiratory reflex, the region responsible lies in the caudal part of the tractus solitarius system, at a distance of about 2 or 3 mm. This inspiratory lesion is much less critical than the expiratory one, involving the entire transverse section of the solitary complex.

The related experimental findings lead to the following anatomical considerations: All the afferent respiratory fibers of the vagus nerve enter the medulla oblongata through the cranial portion of the vagus root and join the tractus solitarius, running down into the latter, but leaving it again at a higher level to form the expiratory and, at a lower level, the inspiratory reflex pathways. Assuming that these fibers leaving the tractus solitarius terminate mostly in its nucleus and the adjacent part of the reticular formation, where they come in synaptic contact with internuncial neurones, the column of gray matter accompanying the tractus solitarius may be considered as the locus proper of the vagal respiratory reflex centers. It is worth noting that central lesions affecting one or the other of the two antagonistic components of vagal respiratory control include besides, or perhaps rather than the tractus solitarius itself, these adjoining cellular structures. There is reason to believe that the above-described selective interruptions of the intrabulbar reflex pathways are located at those places where afferent fibers, or fiber collaterals, converge toward an interneuronic relay station, and that no selection would be obtained by the destruction of fiber tracts only. The same may be true with regard to the continuing fiber pathways, whether they be regarded as further intracentral connections of the afferent system or as the descending efferent paths of the "reflex arc." In this latter case, which appears the most likely, the selective suppression of central reflex transmission would thus be located at the origin of the internuncial neurones acting on the respiratory motoneurones of the spinal level. Definite conclusions, however, will have to be postponed until further anatomical and experimental studies have been made.

As far as our present state of knowledge permits inferences concerning the central mechanism and structural arrangement