During the past 5 years a large number of such cases have been recorded photographically by means of the technique previously described.[2] These are being studied with a view to correlating all the phenomena since, to arrive at a theory of living matter, it is necessary to have accurate observations of its fluctuations under normal conditions. It would seem that such alterations might be due to changes in the permeability of the surface or in resistance elsewhere. Such changes in the surface might be essentially structural (*e. g.*, due to the temporary formation of openings in certain layers) or chemical in nature.

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Predicting Penetration of Dyes into Living Cells by Means of an Artificial System.

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The penetration of dyes into the vacuoles of living cells of *Nitella* and *Valonia* proceeds as though the cell consisted of a non-aqueous layer lying between the external aqueous dye solution and the internal aqueous sap of the vacuole. On this basis it should be possible to predict the relative rate of penetration of dyes into the vacuoles of living cells if we know their rate of diffusion into the "vacuole" of an artificial system representing the 3 phases just mentioned.

To test this an artificial system was constructed consisting of a horizontal glass tube with 3 vertical arms. (1) To the left arm is added the dye solution (identical with the one in which cells are placed, *i. e.* sea water for *Valonia* and buffer solution for *Nitella*); (2) to the central arm is added chloroform (representing the non-aqueous layer of the living cell) until it fills the horizontal portions and the lower part of each upright tube. (3) Upon the chloroform in the right arm is poured some of the sap, artificial or freshly extracted from the living vacuole of *Valonia* or *Nitella* as the case may be (this will be called the artificial "vacuole"). Each one of these 3 phases is stirred by a separate glass stirrer entering through the corresponding upright arm. All the stirrers revolve at a uniform rate. Dye which penetrates into the chloroform at the phase

[2] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, xi, 391, 673.

boundary is rapidly distributed uniformly throughout the mass of chloroform. The rate of passage of the dye from the dye solution through chloroform into the sap (the artificial "vacuole") is chiefly determined by the rate of diffusion at each phase boundary, which in turn is partly determined by the partition coefficient of the dye between the non-aqueous and the aqueous phase.

The concentration of dye in the left arm was kept as constant as possible. The system was stirred for one hour after which the concentration of dye was colorimetrically determined in the dye solution (left arm) and in the sap (right arm).

The corresponding rate of penetration of dye into the vacuoles of cells in normal condition was determined as previously described.

A striking similarity exists between this artificial system and a living cell in respect to the control of the rate of penetration. (1) Cresyl blue, azure B, basic fuchsin, toluidine blue, new methylene blue, methylene green, penetrate the living and the artificial "vacuoles" more readily at pH 9.5 than at pH 5.5. This is also true of *Nitella* and *Valonia*. (2) Trypan blue, trypan red, Congo red, alizarin S, methyl blue, and Bordeaux red penetrate neither the living vacuole nor the artificial "vacuole". With acid fuchsin a trace is found in the artificial vacuole and none in *Nitella* and *Valonia*. (3) *a*. With crystal violet and methyl violet penetration was very slow in all cases. The rate was not appreciably altered with a change in the pH value. *b*. With methyl green a trace of green dye penetrated into the artificial "vacuole" and into the living vacuoles of *Valonia* and *Nitella*. The methyl green solution was green in color but a violet dye was readily absorbed by chloroform. Spectrophotometric measurement showed that this violet dye was crystal violet while the green dye was methyl green. *c*. With thionine in sea water a trace of a pale violet pink dye penetrated the artificial "vacuole" containing *Valonia* sap and into the living vacuole of *Valonia*; with thionine in buffer solution a trace of pale violet dye penetrated into the artificial "vacuole" containing artificial *Nitella* sap and into the living vacuole of *Nitella*. More dye penetrated at pH 9.5 than at pH 5.5. The thionine solution appeared violet but the dye absorbed from it by chloroform gave a pinkish orange color to the chloroform. Both these dyes were found to give absorption curves characteristic of thionine. The amount absorbed by the sap was too small to permit spectrophotometric measurement.

Overton's theory,[1] which involves only one partition coefficient

[1] Overton, E., *Jahrb. wissensch. Bot.*, 1900, xxxiv, 669.

and which states that only substances soluble in lipoid can penetrate living cells might explain the penetration of dyes in the first 2 groups (with the exception of acid fuchsin and alizarin S). But the behavior of the third group cannot be explained by his theory. For example, crystal violet and methyl violet are readily soluble in chloroform and yet they penetrate the vacuole very slowly.

On the other hand the behavior of all the groups may be explained by the theory[2] which postulates 2 or more partition coefficients and which is illustrated by the artificial system.

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Is Respiratory Anaphylaxis (Asthma) the Result of a Local or General Sensitization?*

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When we made our investigations[1] on respiratory anaphylaxis (asthma) we did not know whether the sensitization brought about by the inhalation of a dry antigenic dust was general or purely local in character.

If sensitization and shock are brought about through inhalation, and, anaphylactic death demonstrated by a subsequent intravenous injection, it is plausible to assume that this sensitization might be relegated exclusively to the respiratory tract. However we have no definite evidence to support either the local or general character of this particular form of hypersensitiveness.

More recently[2] we showed that a pregnant guinea pig who had demonstrated respiratory anaphylaxis throughout pregnancy actually transmitted similar hypersensitiveness to her offspring *in utero*. This points to the assumption that inhaled antigens do circulate in the blood of the sensitized animal. In order to show further proof for this fact we have carried out the following experiments.

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[2] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1927, XXV, 127.

* This work is being carried on under "The Crane Research Fund for the Study of Allergic Diseases in Children."

[1] Ratner, B., Jackson, H. C., and Gruehl, H. L., PROC. SOC. EXP. BIOL. AND MED., 1925, xxiii, 16; *ibid*, p. 17; *Am. J. Dis. Child.*, 1927, XXXIV, 23.

[2] Ratner, B., and Gruehl, H. L., PROC. SOC. EXP. BIOL. AND MED., 1928, XXVI, 8.