gether with suitable controls, were then sealed with paraffin and incubated at 36° C. In testing for liquefaction, each gelatin culture and also the control tube were diluted with an equal amount of distilled water and filtered through paper. A viscosimeter tube was selected of sufficient caliber so that the control diluted gelatin passed through in about four minutes. With this control time as a basis, the degree of change lnduced in the gelatin by the cultures under consideration could be readily and accurately determined.

110 (520)

On the nature of chemical stimulation and on the influence of neutral sodium salts on various forms of chemical stimulation.

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Evidence from many sides indicates that the primary change in the stimulation of an irritable tissue is a sudden increase in the permeability of the boundary layers or "plasma-membranes" of the constituent cells or elements The resistance to the escape of diffusible substances, including carbon dioxide, is thus diminished, and there results a corresponding acceleration of the energy-yielding oxidations. With increase in the permeability to ions, there is naturally also associated a change in the electrical polarization of the plasma-membrane—hence the characteristic "action-current" of stimulation. The primary and critical change, increase of surface permeability, may be produced by the electric current, by sudden changes of temperature or contact, by mechanical shock, or by the action of various chemical substances.

Chemical stimulation, on this view, results from the action of those substances which affect the constituents of the plasma-membrane in such a manner as suddenly to increase its permeability to the critical degree required. Now the plasma-membrane is primarily a colloidal structure, consisting mainly of prothins and lipoids intimately intermixed, possibly intercombined. We should, therefore, expect its structure or consistency to be altered, and its permeability correspondingly increased or decreased, by sub-

stances that influence colloidal aggregation-state; such substances ought, as a class, to show evident relations to stimulation. Again, substances with a specific action on lipoids should also show such relations. These two classes of substances, electrolytes and lipoid-solvents, do in fact show peculiar relations to the stimulation-process; their solutions affect the irritable tissue in two distinct ways; either (1) they stimulate, or (2) without stimulating directly they facilitate or hinder stimulation by other means — in other words, they *sensitize* or *desensitize* the tissue. This means, in terms of the present hypothesis, that such substances either (1) produce rapid increase in the permeability of the plasma-membrance, or (2) alter the readiness with which this change is produced by other means increasing on the one hand (sensitization), or decreasing on the other (desensitization), the liability to such sudden increase of permeability.

In normal or electrical stimulation the increase of permeability is completely and readily reversible, the tissue returning immediately to the resting state on cessation of the stimulus. In contrast to such a condition, many forms of chemical stimulation are found to be imperfectly reversible or, in some cases, completely irreversible. A distinction must, therefore, be made between *reversible* and *irreversible* chemical stimulation. This distinction is illustrated, in the case of frog's skeletal muscle, by the following classification:

A.	Reversible chemical stimulation.	Stimulation by isotonic solutions of neutral sodium salts, and by various other solutions of neutral salts of alkali metals (solutions producing spontaneous twitching).
B.	Irreversible chemical stimulation.	1. Stimulation by solutions of salts of heavy metals, strong acid or alkali, ammonia, etc. 2. Stimulation by concentrated solutions of lipoid-solvents (as chloroform). 3. Stimulation by hemolytics (as saponin), or similarly acting substances (including certain bacterial toxins, as tetanolysin).

In the solutions of Class A, the muscle typically exhibits rhythmical and often energetic twitching, *i. e.*, alternation of contraction and relaxation, and in some cases increase of tone; on return to Ringer's solution, relaxation follows promptly; the normal properties of the tissue remain essentially unaltered. In the solutions of Class B, the contraction is typically slow and steady without twitching, and the contracted state, once attained, persists

after return to the indifferent medium; loss of irritability accompanies the contraction, which is also associated with a coagulation indistinguishable from that of death rigor. In spite of the external differences between the two types of contraction, there is evidence that their fundamental conditions are identical, and that the irreversibility of the second type *depends simply on the irreversibility of the change in the plasma-membrane.* The latter loses its vital semi-permeability *temporarily* in the first, and *permanently* in the second case; the loss of irritability and the coincident rigor or contracture are consequences of this permanent loss of the normal condition of semi-permeability.

The distinction between reversibility and irreversibility in the stimulating action of salts undoubtedly has as its ground the similar distinction in the action of salts on the aggregation-state of proteins. Alkali and alkali-earth neutral salts produce reversible changes in the colloidal aggregation-state, while with heavy metal salts the aggregation-changes are irreversible (*Pauli*). In correspondence with this difference, it is found that the stimulating (or inhibiting) effects produced by the first group of salts are reversible, while those produced by the second are irreversible. In the case of lipoid-solvents in strong solution (which also produce contracture passing into rigor) it is to be assumed that the lipoids in the plasma-membrane undergo a change of state too far-reaching to be reversible (dissolving out, etc.). Hemolytics and other poisons must be assumed to act by virtue of various special peculiarities; thus saponin, *e. g.*, probably alters the condition of the cholesterin and so destroys the semi-permeability of the membrane.

In the following experiments the sensitizing and desensitizing action of various electrolytes has been studied in relation to the above different forms of chemical stimulation. Frog's gastrocnemii have been chiefly used. The muscle, arranged to write on a drum, is brought from Ringer's solution into the stimulating solution (where it contracts, describing a curve); after a definite interval (1 to 2 minutes) it is returned to Ringer; relaxation may or may not follow, according to the character of the stimulus. The same muscle (if reversible stimulation is used), or the other muscle of the same animal (with irreversible stimulation), is then exposed

for a definite period to the sensitizing (or desensitizing) solution, *e. g.*, is placed for four minutes in $m/8$ NaBr, from which it is transferred directly to the stimulating solution; the response, if sensitization has occurred, is found to be more energetic than before; if desensitization has occurred, it is lessened or abolished.

The relative sensitizing powers of a series of sodium salts have been thus determined, using the following solutions as stimuli:

1. $m/8$ KCl.

2. Isotonic solutions of sodium salts containing potassium (to increase the twitching effect), *e. g.*, 7 vols. $m/8$ NaI + 1 vol. $m/8$ KI.

3. Pure isotonic solutions of sodium salts which produce active twitching: acetate, sulphate, tartrate, citrate.

4. Saturated solution of a typical lipoid solvent, chloroform, in Ringer's solution.

5. Solution of a typical hemolytic, 0.2 per cent. saponin, in Ringer's solution.

All of these solutions produce contraction in fresh normal muscle. The response is typically and often markedly increased after treatment with pure solutions of sodium salts, especially sodium nitrate, sodium chlorate, sodium sulpho-cyanate and sodium iodide. Magnesium and calcium chlorides, on the other hand, decrease the response *to all salts*, but not to chloroform or saponin. A difference thus appears according to whether the stimulating solution has a general action on colloids, or affects primarily the lipoids.

The order of relative sensitizing action for isotonic solutions of the following sodium salts is, in general, as follows: $NaCl < NaBr < NaNO_3 < NaClO_3 < NaI$ and NaCNS. This statement applies more particularly to stimuli (1), (2), (4) and (5); the salts in group (3), especially sulphate, tartrate and citrate (which appear to act by lowering the concentration of Ca-ions) show somewhat different relations. The above order corresponds to the order of relative action on colloids, and indicates that the salts increase irritability by altering, *in the direction of increased dispersion*, the state of subdivision of the colloidal constituents of the plasma-membrane. The desensitizing or anesthetic action of the alkali-earth chlorides is presumably dependent on an alteration of the plasma-membrane colloids in the reverse direction.

The response of a muscle to chloroform or saponin after treatment with a strongly sensitizing solution, as $m/8$ NaI, $m/8$ $NaClO_3$, $m/8$ $NaNO_3$, or $m/8$ NaCNS, approaches closely in character to the normal contraction, *i. e.*, the upstroke is rapid and accompanied by often vigorous twitching. There is, however, *no relaxation;* and the associated coagulative change in the tissue-colloids is more pronounced than in the unsensitized muscle similarly treated. In other words, *there is a correlation between the vigor of the contraction and the degree of the coagulation*, indicating that the fundamental change in contraction is of the same nature as in colloid coagulation, *i. e.*, that contraction is the expression of a coalescence of colloidal particles in the fibrillæ, due presumably to increased surface-tension of these particles. These experiments also indicate that rigor contraction is of the same essential nature as normal contraction.

III (521)

A new method for the analysis of proteins.

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The method outlined will serve to supplement the ester method, and to characterize proteins when relatively small amounts of material are available. Two and a half grams of protein are hydrolyzed by 15 to 18 hours boiling with 20 per cent. hydrochloric acid. The solution is concentrated to a syrup, then transferred to a one-liter Claissen distilling flask with 200 cubic centimeters of water. Saturated barium hydrate solution to 25 cubic centimeters excess is added and the *ammonia* is distilled *in vacuo* into $N/10$ sulphuric acid from a bath at 45° C. The residual solution is acidified with sulphuric acid and boiled while silver sulphate is added until all the hydrochloric acid is precipitated. The precipitate, which carries the *melanine* with it, is Kjeldahled to determine the melanine nitrogen. The filtrate is brought to 100 cubic centimeters and 5 cubic centimeter duplicates taken for total and amino nitrogen determinations.[1]

[1]Method for Determination of Amino Nitrogen, *Proc. of the Soc. for Exper. Biol. and Med.*, 1909, vii, 46.