spherical bodies uniform in size and staining reaction, the other a homogeneous, faintly staining, amorphous material in which the minute bodies are suspended. The staining method which we have devised for this purpose is as follows:

A smear preparation dried over a flame is mordanted one minute with a .25 per cent. aqueous solution of potassium permanganate. It is then rinsed in running water and stained one minute with a few drops of the following solution: Alcohol (30 per cent.), 100 cc.; basic fuchsin, 1 gram; phenol (crystals), 1 cc.; anilin oil, 1 cc.

The stain is washed off in running water, and the preparation, blotted and dried, is ready for examination. This method of staining is not attended by the formation of any precipitate and stains the minute bodies quite sharply.

Thus from a morphological standpoint it is possible to demonstrate that the specific inclusions of fowl-pox are formed within the cells by the accumulation of a foreign material within the cytoplasm unattended by evidences of degeneration, and that these specific inclusions are constituted of minute, uniform bodies in great numbers, suspended and surrounded by a hyaline matrix. Morphologically, therefore, it is possible to demonstrate structures which resemble a minute micro-organism as the important constituent of the inclusion. These minute structures are in numbers sufficient to account for an infectiousness of material from the lesion in high dilutions. The minute bodies are small enough apparently to pass through the pores of a Berkefeld filter. It would appear from the fact that minute bodies are surrounded by a homogeneous matrix that filtration experiments might be attended with difficulties, and this experimentally is found to be the case. The incorporation of the minute structures within a hyaline matrix, which seems to be of a semipermeable nature, and appears to have a lipoid and proteid structure, might protect an enclosed micro-organism against such physical changes as dehydration by drying or glycerination.

We have demonstrated that the minute bodies are preserved in dried or glycerinated preparations after periods of many months (six) by soaking such material for twenty-four hours in distilled water, making smears and staining as above indicated. This material was infectious.

It has further been possible to show, we believe, that active virus is associated with the inclusions. By bacterial putrefaction it has been possible to soften and disintegrate the epithelial cells of lesions, leaving the inclusions apparently intact. By repeated washings and centrifuging at low speed the inclusions have been freed in large part from adherent cellular material and bacteria. A suspension of these inclusions was centrifuged at high speed. The supernatant fluid inoculated upon the skin of a susceptible fowl proved to be non-infectious, while the sediment, consisting of inclusions, proved to be highly infectious when similarly inoculated upon the skin.

From these observations we are convinced that in fowl-pox we have to do with a specific infectious disease due to a living microorganism which is visible under the microscope. This microorganism, we believe, invades the epithelial cells of the lesion; and the daughter cells, resulting from the division of an infected cell, will carry the virus by direct transmission. The microorganism proliferates within the cytoplasm of infected cells resulting in the formation of minute colonies suspended in a hyaline lipo-protein material which constitutes the specific inclusion.

The epithelial inclusions of molluscum contagiosum of man are composed essentially of the same type of minute bodies which can be demonstrated in suitably stained smear preparations and in fresh preparations with ordinary illumination. In fresh preparations the minute bodies can be seen in rapid molecular motion in every way similar to that exhibited by the bodies within the globules of swollen fowl-pox inclusions. In molluscum, however, there is not so dense a matrix; consequently, the inclusions are more diffuse and, because of their corpuscular content, appear finely granular in stained sections of the lesion. In molluscum contagiosum it is believed that we have to do with an acute infectious disease similar to fowl-pox, and caused by a visible microorganism which penetrates and proliferates within the affected cells of the lesion. This conception has been advanced by Lipschutz and by da Rocha-Lima.

Through the studies of Borrel, Paschen and others it seems possible that a similar microorganism may be the etiological factor in the diseases sheep-pox, variola and vaccinia.

This group of virus diseases then offers, we believe, a particularly favorable material for further etiological investigations.

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ANALOGIES BETWEEN PHYSIOLOGICAL RHYTHMS AND THE RHYTHMICAL REACTIONS IN INORGANIC SYSTEMS

THE periodic or rhythmical reactions of inorganic chemistry are surface reactions, characteristic of poly-

phasic systems of a somewhat special type.[1] Typically they occur at the boundary between certain metals (iron, chromium, nickel and others) and electrolyte solutions, usually acids, and are always associated with variations in the electrical potential of the metallic surface. In some cases the metal shows a spontaneous rhythmical reaction with the solution (e.g., iron in nitric acid, mercury in hydrogen peroxide); in others the rhythm occurs only during electrolysis, usually with the metal as anode (e.g., periodic sulphide or iodide electrode reactions). A constant feature of each single reaction in the rhythmical sequence is the formation of an insoluble reaction-product which is strongly adsorbed by the metallic surface, forming over the latter a thin impermeable coating or film. This film limits or prevents the reaction between the phases and at the same time alters the interfacial potential; the reaction then ceases (or its velocity is greatly reduced) until it is renewed by some secondary reaction which removes or alters the film. In the spontaneous rhythms, e.g., of iron or mercury just cited, the removal or dissolution of the film occurs rapidly as the result of a local electro-chemical reaction which is propagated over the whole surface; the film is then reformed and the cycle is repeated. Under uniform conditions the succession of reactions exhibits a rhythm of remarkable regularity. From the standpoint of the physiological comparison it is of special interest to note that the formation of interfacial films and their dissolution under a transmitted electrochemical influence are the essential factors governing the peculiar behavior of such systems.

The parallels with the rhythmical reactions of living tissues (of the heart, nerve centers, cilia) have long attracted attention; and some years ago Bredig and his students,[2] in a series of studies on the rhythmical catalysis of hydrogen peroxide by mercury, pointed out various features of special physiological interest, such as the synchronism between the rhythms of reaction velocity and electromotor change, the dependence of the rhythm on the alternate formation and dissolution of an interfacial film, the high temperature coefficient, the sensitivity of the rhythm to H-ion concentration and the presence of foreign substances, and the part played by local circuits in electrolyzing and removing the film. Such striking resemblances point to some fundamental identity in the general conditions controlling the reaction velocities in both types of system.

[1] A comprehensive account of these reactions is given in the monograph by R. Kremann, ''Die periodischen Erscheinungen in der Chemie,'' Ahrens Sammlung chemischer und chemisch-technischer Vorträge, 1913, Bd. 19, p. 289.

[2] Cf. Bredig and Wilke: *Biochem. Zeitschr.*, 1908, Vol. 11, p. 67; Antropoff: *Zeitschr. f. physik. Chem.*, 1907, Vol. 62, p. 513.

The rhythmical reaction of pure iron wire in HNO_3 exhibits under certain conditions even more detailed analogies with the physiological rhythms. The tendency of iron to react rhythmically with nitric acid and the similar behavior of iron anodes in sulphuric acid have long been known; the precise conditions of the rhythm have, however, not been clearly analyzed; in particular its relation to the varying properties of the surface-film has been insufficiently considered. This relation is important because the whole resemblance to the physiological rhythm depends on the control of the reaction cycle by periodic variations in this film. It is now widely recognized that variations in the semipermeable protoplasmic membranes or surface-films underlie the response of irritable tissues to stimulation. The primary reactions in stimulation are surface reactions, as is shown clearly by their sensitivity to changes of electrical polarization and to the presence of surface-active compounds. The bioelectric variations of stimulation are also evidence of the part played by surface processes; these variations are now very generally referred to changes in the polarization of the protoplasmic membranes (membrane theories), and there is independent evidence of parallel variations in permeability. The well-known dependence of electrical stimulation on the polarizing action of the current (shown by Nernst and others), and such general physiological facts as chronaxie, polar stimulation and inhibition, together with the temporary existence of a non-reactive or refractory period following excitation, are other features of agreement between the living system and the inorganic model.

The rhythmical reaction of iron in nitric acid consists essentially in an alternation of active and passive periods, the latter corresponding to the film-covered condition. Not all specimens of iron show a regular rhythm in nitric acid; an irregular or local rhythm is common, but a rhythm in which all parts of an extensive surface—e.g., of a long wire—are simultaneously active is found only in iron having special properties, the chief of which are a rapid and complete transmission of the active state over the whole surface when the metal is locally activated (as by scratching or touching with zinc) and a rapid return of transmissivity after activation. Such properties are shown most completely in soft iron of low carbon content; pure iron wire prepared by electrolysis[3] and the pure commercial wire known as Armco[4] exhibit an especially beautiful and regular rhythm under the conditions described below. In hard steel wire (piano

[3] Kindly supplied by the U. S. Bureau of Standards, Washington.

[4] American Rolling Mill Co. The carbon content is ca. 0.1 per cent., according to analyses furnished by the Company.

wire), on the other hand, the tendency to rhythm is almost entirely absent. In this case the non-transmissive period ("refractory period") following activation lasts for several minutes (in 70 v. per cent. HNO_3 at 20°), indicating the presence of a relatively thick or resistant passivating film; while in Armco wire under the same conditions complete transmission is again possible within less than one second after the passage of an activation wave. The remarkable rhythmic properties shown by this wire are closely connected with its power of rapid recovery, the rate of rhythm being directly determined by the duration of the brief non-transmissive period following each activation. Armco wire was used in the following experiments.

When short pieces of iron wire (1 to 2 mm thick, 1 to several cm long), previously passivated, are placed in a flat-bottomed vessel containing nitric acid of 60 to 75 v. per cent. concentration[5] and activated by a single brief contact with zinc, usually a rhythmical reaction begins at once. At regular intervals (of one half to one second) the dark effervescent surface of the metal becomes momentarily bright and inactive, indicating the passive state; in 70 v. per cent. HNO_3 the passive period is brief as compared with the active period; as the strength of acid is increased up to 80 per cent. its relative duration becomes longer.[5a] If the acid is well stirred during the reaction the rhythm continues uniformly until the metal is largely dissolved away; if stirring is omitted the reaction soon becomes irregular and passes into a continuous effervescence.

Further study of this phenomenon soon showed that the essential condition determining the persistent rhythm is the presence of some local area or areas in which the reaction of the metal with the acid is continuous. From such an area waves of activation travel at intervals over the whole wire. Such an active region is formed wherever a sufficient surface of the metal is in contact with the glass or is otherwise protected against free renewal of the acid; the latter is there depleted by the reaction and soon becomes too weak to repassivate the iron. When the wire is suspended freely in the acid (by thin glass threads) no rhythm is shown; a touch with zinc then elicits a single reaction which is transmitted rapidly

[5] Volumes of HNO_3 (C. P., sp. gr. 1.42) in 100 volumes of solution.

[5a] This variation in the relative durations of active and passive periods is clearly shown in photographic records of the electromotor variation, taken with the string galvanometer. The current was led to the string from the sliding contact and terminal of a low resistance tube rheostat connected with the pulsating wire and an indifferent platinum electrode immersed in the acid.

over the whole wire; the latter then immediately becomes again passive and remains so until again activated. The interval between such successive activations may be less than one second; at every touch the wire gives a single reaction and reverts automatically to the passive state. Such experiments illustrate the brevity of the period of recovery in this wire; they also show that the passive state is the state of equilibrium in acid of more than a certain critical concentration. In acid of less than this concentration (*ca.* 55 v. per cent.) automatic repassivation does not occur; the wire when activated continues to react until it is completely dissolved. This is the condition in the local protected area of the rhythmical wire. Such a continually active region may be compared with the nodal or pace-making region of the heart, or with the basal body (or "blepharoplast") of a cilium; it exerts a constant activating influence to which the external passive part of the wire responds as soon as it is sufficiently recovered. The rate of the rhythm is thus dependent on the duration of the temporary non-transmissive period, which is comparable with the refractory period of the living irritable tissue.

The chief conditions for the maintenance of a regular rhythm may be defined as follows: (1) acid of uniform concentration sufficient to reform the passivating film promptly after each activation (60 to 80 v. per cent.); (2) rapid recovery of transmissivity by the wire; and (3) presence of a local continuously reacting region sufficiently large and active to exert a constant activating influence[6] on the adjoining passive regions. These conditions were satisfactorily met by the following simple arrangement. The wire was supported horizontally, about 0.5 cm from the bottom, in a crystallization dish 10 cm wide, furnished with an outflow tube inserted into its side *ca.* 1.5 cm above the bottom. The support was furnished in some experiments by two thin glass threads suspended between the two limbs of a U-shaped glass rod lying on the bottom; in other experiments a bent wire was used, one end of which was attached to a key for making electrical connections; in this case a straight length of wire, 4 to 6 cm long, was exposed to the acid, while the bend (where the wire passed into the air) was coated with paraffin to prevent irregular action. Acid was led into the dish by a siphon connected with a large supply bottle; the slow flow of acid provided the necessary stirring.

[6] *I.e.*, An influence similar to that which the continual contact of zinc would have. In fact, contact with zinc will maintain a rhythm in an iron wire suspended in 70 per cent. HNO_3, but it is impossible to keep the conditions constant in such an experiment and the rhythm is irregular.

Opposite the free end of the wire was placed a short glass tube of caliber slightly wider than the wire. When the wire is inserted into this tube for a short distance (2 to 4 mm) and activated by touching with zinc a continuous reaction is readily started in the enclosed region, which is protected from free renewal of acid. The area of this active region can be adjusted by inserting the wire more or less deeply into the tube. From this region waves of activation pass in regular succession over the whole wire. The rhythm shown under these conditions is remarkably regular and continues indefinitely, *i.e.*, until the enclosed end of the wire is dissolved away. At any time the reaction may be brought to a stop by removing the tube from the wire; the latter then immediately becomes passive.

Since complete transmission and rapid recovery are necessary conditions for the rhythm, their general conditions should first be considered. The transmission itself, as is well known, is an effect of the local circuit formed between the active and the passive areas, the latter being cathodal; the film is there broken down by electrolytic reduction and the process of disruption spreads automatically, since it is repeated wherever anodal and cathodal areas adjoin each other. The process may be regarded as in the nature of a two-dimensional explosion, *i.e.*, an explosive reaction confined to the thin layer (probably monomolecular) of reactive material at the phase boundary. According to the usual conception of explosions, a reaction becomes explosive when heat is produced locally more rapidly than it is conveyed away. The film-conditioned type of chemical transmission differs, however, from the three-dimensional explosion in that the essential factor determining the spread of reaction is not a certain critical local heat-production, but a certain critical intensity of electric current between the reacting area of the surface and the passive region adjoining. If the current intensity (or density) is insufficient, the reaction fails to spread; in other words, a certain threshold must be overpassed. When the current intensity exceeds the critical level required, there is an automatic spread of the reaction to an indefinite distance, *i.e.*, over the entire area of the film—assuming the latter to have uniform properties throughout.

In another respect this type of explosive process differs from that in three-dimensional masses of material, such as explosive gas mixtures or compounds: *i.e.*, materials are at hand, in the direction of the third dimension, for replacing the film, so that a second reaction may become immediately possible. A certain interval is required, but this may be brief; in pure iron wires in 70 v. per cent. HNO_3 it is about one half second at 20°; in living systems, such as nerve,

it may be much less; the system can then react as before. The interval represents a temporary non-transmissive or "refractory" period, during which the reactive molecules are to be regarded as assembling at the surface, and presumably there undergoing orientation or other change favorable to reaction. The refractory period thus corresponds to the interval required for the formation of an adsorption film having definite characteristics. In general it should be noted that such surface reactions, since they depend on the conditions present in thin layers—where distances are small and the time required for access of material from either adjoining phase is correspondingly brief—are subject to rapid fluctuations of velocity. The quick initiations and arrests shown in the stimulation processes of living organisms—as well as their electrically conditioned character—are in themselves evidence that the controlling chemical reactions are surface reactions. Similar considerations apply to the physiological rhythms, which represent automatic and regularly repeated stimulations.

CONDITIONS DETERMINING THE RATE OF RHYTHM

Extent of pace-making area: In general any increase in the area of the continuously active or controlling region is found to accelerate the rhythm, while any decrease retards it. The rate can thus be regulated by inserting the wire for a greater or less distance inside the tube. If the active region be decreased beyond a certain length (of 1 or 2 mm) it becomes suddenly passive, by spread of the external passive area, and the rhythm ceases. There is thus for each concentration of acid a certain minimal area of the controlling region, corresponding to the slowest rhythm which is stable under the conditions. The rhythm can readily be doubled by inserting the wire for 4 to 5 mm into the tube; this increases the anodal area of the local circuit, and hence also the intensity and reducing effectiveness of the current at the adjoining passive area. A dependence of the rhythm in the rate of the local cathodal reduction is thus shown. This effect is readily understood when we consider that the film at its first deposition is refractory to reduction and attains its final state of maximum reactivity by a progressive process.[7] Hence at a given interval a stronger local current may cause activation while a weaker one is ineffective.

Concentration of Acid: Within the range of concentration in which a regular rhythm is shown (60 to 80 v. per cent.) the rate increases with concentration, in a manner approximately linear. With the controlling region kept at its minimal area, as above de-

[7] Following a curve of the type $R_t = R_o (1 - e^{-kt})$, as indicated by my earlier results on steel wires (*Journ. Gen. Physiol.*, 1925, vol. 7, p. 493).

scribed, the following rhythms were found characteristic (at 22–23°).

Concentration (v.per cent. HNO_3)	Rate per minute
60	34–36
65	50–56
70	62–70
75	80–90
80	> 100 (becomes irregular)

In 80 v. per cent. acid a regular reaction is difficult to maintain, probably because of too great heat-production at the site of the controlling reaction.

Temperature: The temperature of the acid was controlled by a water bath surrounding the supply bottle. The following rates are typical for 65 v. per cent. acid at temperatures between 10° and 30°. Below 10° the rhythm is uncertain and above 30° it tends to accelerate and become irregular. As before, the wire was adjusted to give the slowest rhythm that was stable under the conditions.

Temperature	Rate per minute
10°	14– 16
15°	28– 32
20°	46– 50
25°	84– 88
30°	122–128

The range of temperature within which the rhythm is stable is not large and is similar to the physiological range, although the precise significance of this correspondence is not clear. It may be that the film is not sufficiently stable at higher temperatures, while at lower temperatures the reaction is too slow for effective reduction.

The correspondence of the temperature coefficient with that of the physiological rhythms is striking; the Q_{10} value is 2½ to 3, equivalent to a value of 17,000 to 22,000 for μ in Arrhenius' exponential formula, $\dfrac{k_2}{k_1} = e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$ where k_1 and k_2 are the velocity constants at the absolute temperatures T_1 and T_2. As already pointed out, the interval between successive reactions represents the time required for the surface-film to attain a certain degree of reactivity, *i.e.*, of reducibility by the local active-passive current. In the case of steel wires the rate of change in the film at different temperatures has been determined, using the recovery of transmissivity as criterion; the temperature coefficient of this recovery process is the same as that of the rhythm.[8] This correspondence is further evidence that the chemical change in the film determining the recovery of transmissivity is also the condition determining the rate of

rhythm. This is apparently also the case in the cardiac rhythm, where the temperature coefficients of refractory period and of rhythm are closely similar or identical.

Other factors influencing the rhythm: As in the case of the heart beat, the rhythm of passive iron is affected by electrical polarization. When the pulsating wire is connected through a rheostat to an external source of current, making the wire anode has a retarding or inhibiting effect, while making it cathode causes acceleration. These effects vary characteristically with the intensity of the polarizing current; sufficient anodal polarization stops the reaction, while sufficient cathodal polarization renders it continuous. Polar effects of an analogous kind are well known in living tissues. A more detailed description of these and other parallels will be given elsewhere.

There is also a remarkable relation between the rate of rhythm in a pulsating wire and the total length of the wire, the rate decreasing at first rapidly, then slowly, with increase in the length. The following rates were found characteristic for different lengths of regularly pulsating wires, at one end of which a pace-making region was established by encircling the wire by a glass ring 3 mm long. The same ring was used in all experiments; the concentration of acid was 70 v. per cent. and the temperature 20°–21°.

Length of wire (cm)	Rate per minute
30 and 18	40– 46
12	42– 46
8	46– 50
6	48– 54
4	60– 64
3.5	64– 68
3	66– 72
2.5	68– 74
2.0	82– 92
1.5	84– 96
1.0	100–108
0.75	> 120 (less regular)

This phenomenon appears to be an expression of the mutual influence which the active and passive areas of the same wire exert upon one another.[9] The presence of a passive region has a restraining or inhibiting effect upon activation in other regions of the wire; reactivation at the region adjoining the pace-making area is thus delayed until the activation wave has extended over the whole length of the wire. There are biological analogies here also; in general it is observed that the frequency of ciliary beats or heart beats decreases as the linear dimensions of the tissue increase. In such cases it seems clear that a dis-

8 *Journ. Gen. Physiol., loc. cit.*, p. 500.

9 This influence was early noted by Schönbein, *Philos. Mag.*, 1836, Vol. 9.

tance action, possibly of the same kind as that observed in the experiment just described, plays a controlling part in the determination of rhythm: *i.e.*, the latter depends on a coordinating influence, in the general nature of a removal of restraint, transmitted at a finite velocity from the pace-making region. Correspondingly, we find that when transmission is locally impaired in the heart, fibrillation often occurs, at a rhythm typically faster than the main rhythm. In such cases local pace-making regions are to be assumed, each controlling the rhythm over a relatively small area.

<div style="text-align:right">RALPH S. LILLIE</div>

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JOSEPH NELSON ROSE

1862–1928

ON the afternoon of May fourth an assembly-room in the United States National Museum was filled to overflowing in response to a call issued that morning by the secretary of the Smithsonian Institution. The gathering was called as a means of showing and recording his late associates' appreciation of Dr. Rose, who had left his desk in order late the preceding afternoon—never to return to it.

The meeting was impressive. To those of us who listened, as speakers rose here and there in the room, the kindly personality of a friend and the talented devotion of an able man to earnest work unfolded. To those of us who spoke, the sadness of the occasion was blended with a consolation born of the knowledge that a well-rounded-out life had come to ripe fruition.

I recalled that last winter Dr. Rose had reminded me of the fact that over forty years ago he had asked to do the work for his doctorate with me, but had been prevented by circumstances from doing so; and I thought of the original and thorough-going study of a difficult group of plants—the Umbelliferae—that came out of his candidacy under Professor Coulter. I remembered that when a preliminary dip into the Crassulaceae, which are ill-preserved in herbaria, had convinced me that they were beyond my own understanding, he disentangled them with masterly skill. There came to my memory a long day's tramp with him down a lava-covered mountainside bordering the valley in which the City of Mexico lies, and the keen, detailed and comprehensive way in which he examined the many agaves that we found—in which at that time he was more interested than I. Even a few days before his death he had shown me a collection of specimens and full-sized photographs of what passes for *Acacia Farnesiana,* and which for years he has known to comprise more than one species.

Interwoven with these memories were many others, like them indicative of a close observer, an energetic worker, a deliberate thinker and a friendly man even when critical.

Except for two years before attaining the doctorate, Rose was not a teacher—unquestionably to the loss of young men in whom his deliberate weighing of questions and facts would have conduced to the early formation of a judicial habit.

For forty years his connection has been with the government botanical service—first in the Department of Agriculture and later, when the present national herbarium was established under the National Museum, in this institution. To his efficiency in building up and using this great collection his associates all bear witness, and the collection itself and the long series of published "Contributions" are in evidence to the same effect; but of recent years the brunt of this responsibility has been borne by his coadjutor, Mr. Maxon.

Custodianship of a large herbarium, with an impulse to investigation, not only offers great possibilities to one who knows how to use them, but almost of necessity drives one afield. With familiarity with the contents of the larger European collections Dr. Rose also came to know many of the objects of his study in their haunts—Central and South America; and his work, especially on succulent plants, never could have acquired its lasting value in any other way. The necessary routine handling of the accessions in such an establishment as the National Herbarium affords in itself the basis of a liberal education to one keen on floristic and taxonomic studies. New material comes in from unfamiliar regions and the specimens must be named.

Among Dr. Rose's earlier tasks was naming several west-Mexican collections made by Edward Palmer, a pioneer in that field, and enumerations of such collections are among his earlier publications. It probably was in doing this work that he formed a habit of which he once spoke to me—that of synoptically bringing under his eye the characters of all the known species of a group, preliminary to naming adequately those before him; and this was a most valuable habit in his later and more difficult studies.

Though his most monumental work was on the Cactaceae, Crassulaceae, Umbelliferae and Amaryllidaceae, his interests were broadly distributed over the flowering plants. This is not the place for an enumeration of his publications, but they covered the North American representatives of Burseraceae, a considerable series of "Studies of Mexican and Central American plants," often from an economic standpoint, and a carefully executed study of the anatomical characters of certain pines applicable to their