the inhibition is concerned, our data are consistent with the hypothesis that theophylline inhibits some enzymic step involved in germination by L-alanine. A number of enzymes, in particular phosphorylases[4,5] and phosphodiesterases[6], are known to be affected by theophylline; however, there is so far no evidence that the germinative activity of L-alanine depends specifically on one of these enzymes. In *B. cereus* 'R' spores, theophylline inhibits the ribonucleosidase[1] but this enzyme, whose involvement in the germination by nucleosides has been questioned[1,7,8], has presumably no role in germination by L-alanine. Therefore, further studies must be carried out in order to define the specific target of the inhibitory activity of theophylline on germination.

WARREN and GOULD[3] suggested that, in *B. cereus* spores, the role played by L-alanine alone in 'triggering' germination is different from that played by this amino-acid in 'stimulating' nucleoside-induced germination. The fact that theophylline strongly inhibits the germination by L-alanine alone but does not affect the stimulation by L-alanine of inosine-induced germination, gives additional evidence in favour of the suggestion made by WARREN and GOULD.

Table II. Effect of theophylline on the stimulation by L-alanine of inosine-dependent germination

Effectors of germination	Extent of germination (E%)		Rate of germination (V)	
	−	+	−	+
Inosine	40.0	39.2	2.70	2.55
Inosine + L-alanine 3 μM	45.0	44.8	4.80	5.00
Inosine + L-alanine 6 μM	45.0	45.0	5.30	5.25
L-alanine 6 μM	0.0	–	0.0	–

Heat-activated spores (75 °C, 15 min) were incubated at 34 °C in sodium (Na/Na_2) phosphate buffer 0.033 M, pH 6.4 containing the effectors of germination cited in the Table, in the absence (–) and in the presence (+) of 10 mM theophylline. Inosine was used at a concentration of 0.1 mM. The parameters E% and V are expressed as defined in methods.

Riassunto. In spore di *Bacillus cereus*, la teofillina inibisce reversibilmente la germinazione da L-alanina ma non l'attivazione che la L-alanina esercita sulla germinazione da inosina. Il possibile significato di questi dati è discusso.

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THEORIA

Some General Criteria for the Energetical Characterization of Excitable Systems

All living organisms exhibit, at their respective levels of specialization, excitability, allowing them to function suitably in the medium with which they interact. From the physical view point, excitability represents the ability of the system to respond to a certain modification of the *intensive parameters* of the medium by a sudden release of energy, which consequently reduces its free internal energy. Man-made models of biological excitable systems[1] and some other technical devices also have this property.

In this preliminary note, I propose some general criteria for a phenomenological characterization of the functioning of the excitable systems, irrespective of their concrete nature. A more detailed analysis in terms of theoretical physics will follow.

Firstly, we have to point out that all excitable systems are *dissipative structures*[2] which are maintained only by a well-defined cooperation between the internal processes and the fluxes of energy and matter from the external world. In its resting state, the excitable system as a whole possesses a higher potential energy, due to the fact that some of its microscopical components (elementary particles and/or atoms and molecules) are on higher energy levels and prevented by the structure of the system from leaving these levels. If a number of microscopical components pass, however, to lower energy states, this represents 'leakages' which, in terms of non-equilibrium thermodynamics[3], will be denoted by the fluxes J_k^0. The fact that some microscopical components are prevented from reaching the lower energetical levels means that there are in the system generalized (thermodynamical) forces X_k which are maintained constant, despite the leakage fluxes:

$$X_k = cts = X_k^0 \neq 0.$$

This implies that, during the resting state, an energy:

$$\frac{dU_R}{dt} = \sum_k J_k^0 X_k^0$$

is dissipated per time unit. This formula represents the most general expression for the 'resting metabolism' intensity of the excitable systems.

The stimulus changes the structure of the excitable system, so that the forces X_k will be reduced from their resting values X_k^0 to some lower values X_k'. If the system acts according to the 'all or none' law, the forces will vanish, so that an overall energy:

$$U_A = \int_0^\tau \{\sum_k J_k(t) \cdot X_k(t)\}\, dt$$

will be released. The time interval τ represents the refractory period given by the condition:

$$\sum_k X_k(\tau) = 0.$$

The excitable system obeys the 'all or none' law when the stimulus produces a high *cooperative* change of the structure, which means that a local change is propagated by a resonance mechanism, so that there is no equivalence

between the energy of the stimulus and U_A. In the case of the axon membrane, the structural modifications are vibro-rotational transitions of the protein macromolecules[4]. In a system which reacts according to the 'all or none' law, a subthreshold stimulus will possibly reduce the forces X_k from X_k^0 to some lower values X_k^1. In this case, in response to a consecutive stimulation, the system will release the energy:

$$U_A^1 = \int_{t_1}^{\tau} \{\sum_k J_k(t) \cdot X_k(t)\}\, dt$$

$$[X_k(t_1) = X_k^1].$$

This last relation contains, in a 'implicit' manner, the adaptation phenomena.

The particularization of these general ideas could lead to a concrete energetical analysis of a given excitable system[5]. It is also an essential step in the calculation of the ratio between the information transmitted and the energy dissipated by an excitable system. This will be the subject of a further communication.

Résumé. On présente une brève caractérisation générale des systèmes excitables, considérés du point de vue énergétique.

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DISPUTANDUM

Non-Paramagnetism of Human Serum Proteins

Commencing with SMITHIE and POULIK's report[1] on paper starch gel two dimensional electrophoresis technique, both they and a number of others have contributed advances to the fundamental method which today has made possible exceedingly precise separation of human serum proteins. A good example of such work is the recent publication by MARGOLIS and KENRICK[2], in which they used a combination of polyacrylamide disc and polyacrylamide gradient electrophoresis for their two dimensional system. Separation of proteins by means other than electrophoresis to be followed by electrophoretic separation in the second dimension has also been reported[3,4]. The report that serum proteins could be separated by a magnetic force of 'about 25×10^6 Oersteds/cm²' acting over a period of 1 h[4] seemed to offer an interesting new approach.

In an attempt to repeat and improve on this work, we created an electromagnetic field measured at 30×10^6 Oersteds/cm². In a typical experiment, a small droplet of human serum was placed on a piece of cellulose acetate which had been submerged in a *tris*-barbital buffer, pH 8.8 (Gelman high resolution buffer), and blotted. The cellulose acetate strip was then sandwiched between 2 layers of polyacrylamide gel. This 'sandwich' was then inserted into a glass vial measuring 1×3 cm which was capped and placed in the electromagnetic field for 1 h. Evidence for the migration of proteins under the influence of this force was tested in 2 ways. In some experiments, the cellulose acetate strip was itself applied onto the surface of a gradient polyacrylamide gel measuring 3 mm × 10 cm at the point of application. A previously poured cap gel was placed against the strip and electrophoretic separation of the proteins was carried out using a pulsed constant power supply and a commercially available flat bed gel system[5]. The system was operated, except for the aforementioned method of applying the sample, as described by SCHIFF et al.[6]. A second test was simply to stain the cellulose acetate strip with Ponceau S to observe the protein spot which ranged in size from 1 to 3 mm in diameter. If magnetic force had moved all or an appreciable number of the serum proteins, it is to be expected that the spot would change from its typical round form to an oval or elongated shape. With both tests we were not able to discern any evidence to suggest that any protein had moved even 1 mm as a result of the magnetic force applied.

Since proteins would be expected to move more easily in cellulose acetate than in polyacrylamide gel, we conclude that the migration of protein through a distance of several centimeters as pictured in the previous publication[4] was due to a force other than magnetic. Under the conditions of our experiment, no effect of the movement of human serum proteins by magnetic force is observed[7].

Zusammenfassung. Normale Serumproteine wurden mittels Elektromagnetophorese in Zellulose-Azetatgel ausgeführt, wobei keine Trennung der einzelnen Proteine erfolgte. Es stellte sich heraus, dass Serumproteine keine paramagnetischen Eigenschaften aufweisen.

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