isotherm[6]. The 'FREUNDLICH' steady-state equations are: $C_i = 19\ C_0^{0.70}$ for α-amino*iso*butyric acid in mouse brain slices; $C_i = 15\ C_0^{0.70}$ for *cyclo*pentane-1-amino-1-carboxylic acid in mouse brain slices; and $C_i = 5.5\ C_0^{0.51}$ for L-histidine in rat small intestine, with all concentrations in μmoles/ml water. The agreement with the FREUNDLICH adsorption isotherm is almost certainly coincidental, as it is most unlikely that active transport has any fundamental physical resemblance to adsorption.

Although the 'FREUNDLICH' steady-state equation for active transport is strictly empirical and is not followed by all systems, when applicable it provides a convenient expression for the relation between internal and external steady-state concentrations. Furthermore, and equally important, the fact that certain systems obey this relation rather than equation 2 shows that the model of a combination of a Michaelis-Menten pump and simple diffusion is inadequate for some examples of active transport and suggests the possibility of regulatory mechanisms controlling uptake, exit, or both. Evidence for regulation of influx by the internal concentration has recently been found by RING and HEINZ in their study of the uptake of α-amino*iso*butyric acid by *Streptomyces hydrogenans*[7].

Zusammenfassung. Im aktiven Transport der Aminosäureanalogen (α-Aminoisobuttersäure und Cyclopentan-1-aminocarbonsäure) in Gehirnschnitten folgt das Verhältnis im stationären Zustand zwischen der intrazellulären Konzentration und der Lösungskonzentration der FREUNDLICH Adsorptionisotherme, $C_i = AC_0^n$. Dieses Verhältnis stimmt mit dem Modell einer aktiven Pumpe, die der Michaelis-Menten Kinetik folgt und mit einer passiven Ausdiffusion ausgeglichen ist, nicht überein. Es wird vermutet, dass eine Kontrolle des aktiven Transports durch intra- und extrazelluläre Konzentrationen reguliert wird.

S. R. COHEN

New York State Research Institute for Neurochemistry and Drug Addiction, Ward's Island, New York (New York 10035, USA), 3rd March 1967.

[6] H. FREUNDLICH, *Colloid and Capillary Chemistry* (translated from 3rd German edn by H. S. HATFIELD; E. P. Dutton and Co., New York 1926), p. 111.

[7] K. RING and E. HEINZ, Biochem. Z. *344*, 446 (1966).

Transplantation of Nuclei and Mitochondria of *Physarum polycephalum* by Plasmodial Coalescence[1]

The plasmodia of the coenocytic slime mold, *Physarum polycephalum*, when grown on semi-defined liquid medium on the surface of filter paper[2], are comparable, for experimental purposes, to giant, multinucleated 'cells' in which all nuclei divide in synchrony[3,4]. When plasmodia are brought into contact with one another, they coalesce spontaneously[3,5]. Once communication between them is established, plasmodial strands are formed which extend from one plasmodium into the other, and rapid exchange of components is brought about by the well-known protoplasmic streaming of this organism. This phenomenon can be employed for studies requiring transplantation of plasmodial components. Two prerequisites have to be fulfilled for such an experiment, namely, (1) that only a small amount of material from the donor is taken up by the host, and (2) that those components of the donor which we wish to study (e.g. nuclei, mitochondria) remain identifiable either by morphological criteria or otherwise, for some time after transplantation. If, for example, nuclei are transplanted at a stage in the mitotic cycle[6] which is different from that of the nuclei of the host, morphological criteria may be used for their identification during a short period after transplantation[4]. For longer periods, or if other plasmodial components, such as mitochondria, are involved, identification by label with a radioactive isotope might be necessary.

Method. Coalescence of 2 plasmodia can be conveniently obtained by sandwiching. For this purpose, the plasmodia are first placed, together with the underlying filter paper, on non-nutrient agar. Being deprived of nutrients, the plasmodia soon begin to rapidly spread over the agar. Approximately 30 min before coalescence is desired, a small piece (donor) of one plasmodium is placed, upside down (Figure 1), together with some of the adhering agar, on a considerably larger piece (host) of the other plasmodium. The small piece of agar adhering to the donor provides just enough pressure to promote close contact between the 2 plasmodial pieces.

When contact is established the beginning of coalescence can be easily determined by the gradual formation of plasmodial strands extending through donor and recipient (host) plasmodium. If both donor and host nuclei at the time of coalescence are at different stages

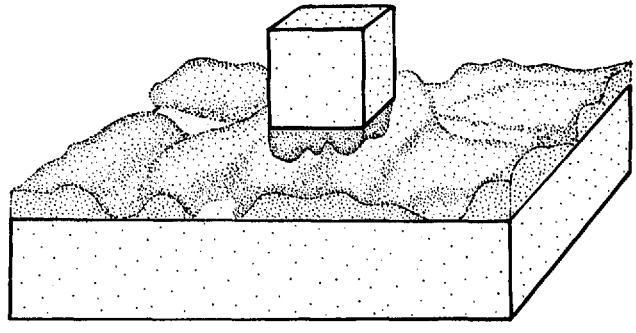

Fig. 1. Two plasmodial pieces sandwiched between agar. Both donor (upper) and host plasmodium (lower) are on non-nutrient agar.

[1] Supported by U.S.P.H.S. Grant No. 5-RO-1-GM 11949-04.

[2] J. W. DANIEL and H. H. BALDWIN, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), Vol. 1, p. 9.

[3] E. GUTTES and S. GUTTES, in *Methods in Cell Physiology* (Ed. D. M. PRESCOTT; Academic Press, New York 1964), Vol. 1, p. 43.

[4] E. GUTTES, S. GUTTES and H. P. RUSCH, Devl Biol. *3*, 588 (1961).

[5] E. GUTTES, S. GUTTES and H. P. RUSCH, Fedn Proc. Fedn Am. Socs exp. Biol. *18*, 479 (1959).

[6] With this term we denote the time which elapses from any stage of mitosis to the same stage of the next mitosis.

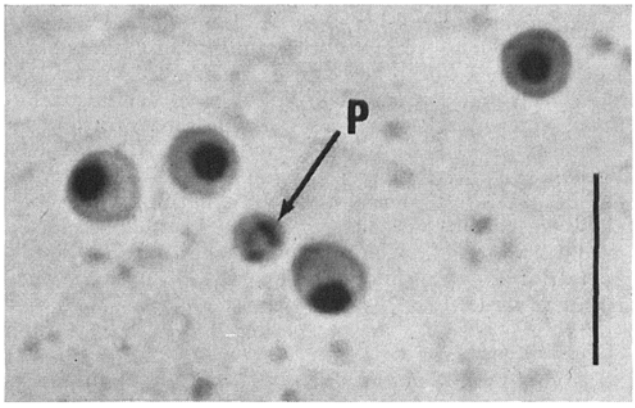

Fig. 2. Nuclear transplantation by coalescence of surface plasmodia: Postmitotic nuclei transplanted into late-interphase host. Donor plasmodium was placed, at the time when its nuclei entered early prophase, on a late-interphase host plasmodium. At time of coalescence the donor nuclei had just completed mitosis. P, postmitotic donor nucleus (dark bodies within nucleus are pronucleoli). The other nuclei are from host plasmodium. Fixation: Champy's liquid. Staining: acid fuchsin. Vertical bar: 10μ.

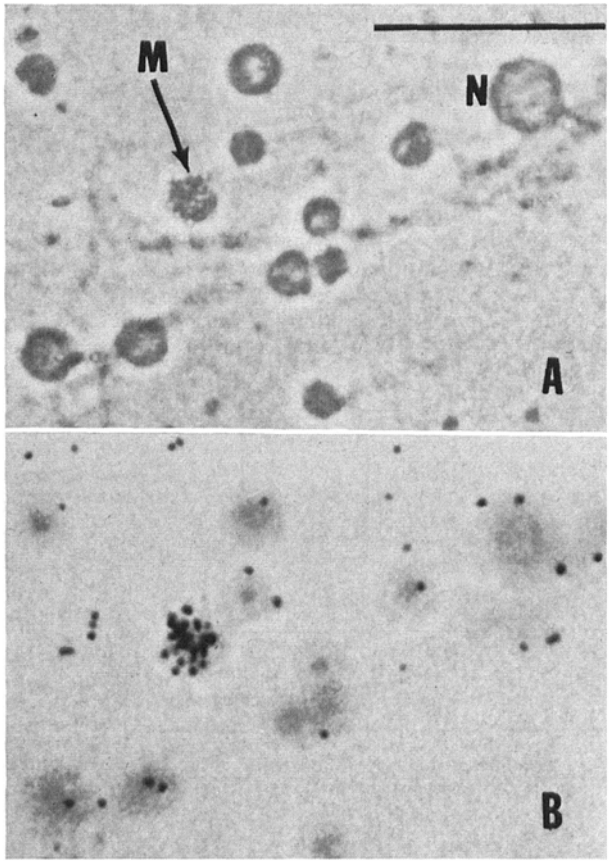

Fig. 3, A and B. 'Transplantation' of mitochondria by coalescence of plasmodial fragments. Autoradiograph (Kodak AR-10 stripping film). With (A) and without (B) phase contrast. Fragments of surface plasmodia (microplasmodia), were grown for 14 h in agitated growth medium containing thymidine (20 μc/ml; from Schwarz Bioresearch, Inc.), and in non-radioactive growth medium, respectively. After washing with non-radioactive growth medium a small number of labelled microplasmodia were allowed to coalesce on filter paper (for method see GUTTES and GUTTES[3]) with a large number of unlabelled microplasmodia. Squash preparations of explants from the resulting

of the mitotic cycle, the spreading of components from the donor through the adjacent host area can be monitored in ethanol-fixed smear preparations from small explants taken at a distance of approximately 5 mm from the area of contact. Exchange of components between donor and host can be stopped at any time by removing the donor piece. Thus, the amount of components that are 'transplanted' into the host can be kept low, leaving the original host environment essentially unchanged.

Prompt coalescence and homogeneous mixture of plasmodial components within a short period of time is best obtained by first converting the surface plasmodia into fragments which are adapted to growth in agitated culture (microplasmodia)[2]. As cultures of microplasmodia are not mitotically synchronized, they can be employed only in experiments which do not require transplantation of plasmodial components at a defined stage of the mitotic cycle. The plasmodial constituents which one wishes to transplant must be marked, e.g. with a radioactive tracer, before coalescence takes place. A small number of microplasmodia containing the labeled components is then mixed with a large number of unlabeled microplasmodia and this suspension is used for preparation of a surface plasmodium as described previously[3].

Applications. (I) Transplantation through coalescence of mitotically synchronized surface plasmodia can be employed, e.g., to transplant nuclei of one stage of the mitotic cycle into a plasmodium representing another stage. Figure 2 shows a stained section through a late-interphase plasmodium which had received, not more than 30 min before fixation, postmitotic nuclei. The high degree of synchrony with which all nuclei of a surface plasmodium go through the mitotic cycle, plus the characteristic morphological differences[4] between post-

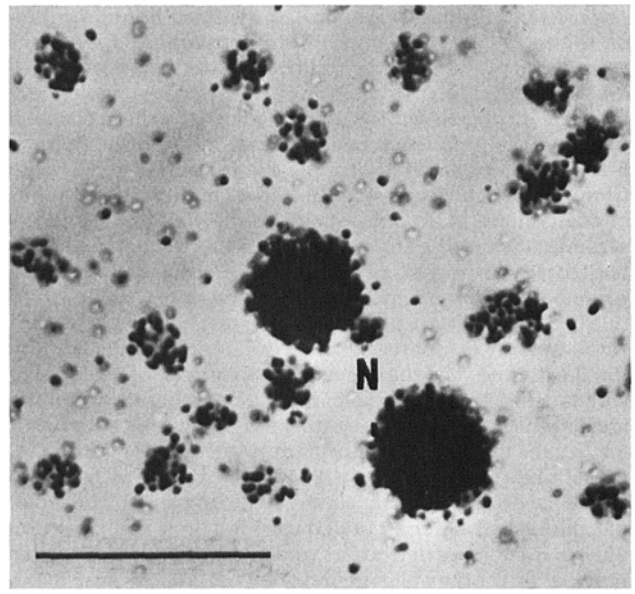

Fig. 4. Control to Figure 3, A and B: only labelled microplasmodia were allowed to coalesce. Technique and magnification same as in Figure 3, A and B. All mitochondria labelled. N, labelled nuclei.

surface plasmodium were fixed, shortly after the first synchronous postfusion mitosis[3,4], in osmic acid (1%) and processed for autoradiography. N, unlabelled nucleus. M, mitochondrion from labelled microplasmodium. Horizontal bar: 10μ.

mitotic and late-interphase nuclei make it possible to distinguish for several hours, without special label, between transplanted nuclei and host nuclei. In order to obtain the desired combinations it is necessary to have simultaneously available a number of plasmodia at different stages of the mitotic cycle. Successful are those combinations in which actual coalescence begins just before the nuclei of both plasmodia are at the desired state of the mitotic cycle.

(II) Figures 3 and 4 show an example of an experiment designed to study, by coalescence of plasmodial fragments (microplasmodia), multiplication during the mitotic cycle[7] of mitochondria that were prelabeled with tritiated thymidine[8]. The heavily labeled mitochondrion seen in Figure 3, A and B, is from a labeled microplasmodium (see control in Figure 4) and it is surrounded by an environment which is predominantly composed of components from unlabeled microplasmodia. Although the radioactivity carried over by the labeled microplasmodia after washing was sufficient to produce slight labeling of the mitochondria from unlabeled microplasmodia, the originally labeled mitochondria had incorporated enough ^{3}H-thymidine to afford their identification during at least 1 mitotic cycle.

Zusammenfassung. Die Plasmodien des vielkernigen, mitotisch synchronen Schleimpilzes *Physarum polycephalum* zeigen bei Berührung Fusionstendenz. Der so erfolgende Protoplasmaaustausch erlaubt die Transplantation der Zellkerne, Mitochondrien usw. von einem Plasmodium in das andere und, nach Vormarkierung, die weitere Verhaltensbeobachtung.

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Department of Biology, Loyola University, Chicago (Illinois 60626, USA), 24th January 1967.

[7] E. Guttes and S. Guttes, in preparation.
[8] E. Guttes and S. Guttes, Science *145*, 1057 (1964).

The Oxidation-Reduction State of Pyridine Nucleotide in Isolated Frog Gastric Mucosa

The redox theory of acid secretion, as formulated by Conway and Brady[1], implies that reduced pyridine nucleotide is the source of the hydrogen ions secreted by gastric mucosa. The present experiments were carried out to examine this hypothesis.

The oxidation-reduction state of nicotinamide-adenine dinucleotide (NAD) in isolated frog gastric mucosa was determined by direct assay of the levels of the oxidized (NAD^+) and reduced (NADH) forms. Similar determinations were carried out on frog livers for the purpose of comparison. The tissues were obtained from starved frogs of the species *Discoglossus pictus*. The tissue levels of NAD^+ and NADH were determined by the method of Villée[2]. The assays had a coefficient of variation of 3%.

The levels of NAD^+ and NADH observed in 5 gastric mucosae and 5 livers are given in Table I. The results show that in the gastric mucosa of starved frogs, NAD is present mainly in the oxidized form. It is oxidized to a greater extent than in liver, the NAD^+/NADH quotient being approximately twice that of liver.

Fluorescence emission spectroscopy was employed to look for changes in the total intracellular pyridine nucleotide in gastric mucosa during the process of acid secretion. The experiments were carried out with an Eppendorf fluorimeter. An excitation wave-length of 366 nm was used. The light impinged on an area of approximately 38 mm^2 on the secretory surface of an everted sac of frog gastric mucosa. The sac was prepared as described in a previous publication[3]. It was kept in a quartz cuvette containing incubation medium. The fluorescence emission passed through a filter transmitting only above 420 nm.

The sac of mucosa and the cuvette were filled with the incubation medium described previously[3]. The mucosa secreted acid into the medium in the cuvette. In order to determine the acid secreted, the cuvette fluid was circulated by means of a roller pump through a Pye Ingold capillary flow pH measuring assembly (Pye, Cambridge, England). pH was measured by means of a Model 48B Vibron pH meter (Electronic Instruments, Surrey, England). The total volume of fluid in the cuvette and outside it was 6 ml. The top of the cuvette was partially open to permit continuous equilibration of the circulating fluid with the air. In order to ensure adequate aeration, a flow rate of 6 ml/min was maintained.

The experiments were carried out at room temperature (about 20 °C). Simultaneous measurements of fluorescence emission and pH were made at regular intervals. The quantities of acid secreted were subsequently determined from the titration curve of the incubation medium. The measurements of fluorescence emission were corrected for decrease in the sensitivity of the fluorimeter with time.

The mucosae, which were obtained from starved frogs of the species *D. pictus*, showed spontaneously 2 kinds of acid secretory behaviour over a period of 90 min in a series of 20 experiments. There was either a slow progressive decrease in the rate of acid secretion with time, or an initial fall after which a steady rate of secretion was maintained. The intensity of the fluorescence emission attributable to reduced pyridine nucleotide tended to vary

Table I. NAD^+ and NADH levels in frog gastric mucosa and liver

	NAD^+ (μg/g wet wt.) (Mean ± S.E.)	NADH (μg/g wet wt.) (Mean ± S.E.)	NAD^+/NADH (Mean ± S.E.)
Gastric mucosa	97 ± 14 (5)	48 ± 19 (5)	2.3 ± 0.5
Liver	200 ± 32 (5)	191 ± 16 (5)	1.0 ± 0.1

Number of tissues assayed is given in parentheses.

[1] E. J. Conway and T. G. Brady, Nature *162*, 456 (1948).
[2] C. E. Villée, Biochem. J. *83*, 191 (1962).
[3] W. H. Bannister, J. Physiol. *177*, 429 (1965).