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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[7] E. Guttes and S. Guttes, in preparation.
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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).

Table II. Fluorescence emission attributable to reduced pyridine nucleotide and acid secreted by 2 frog gastric mucosae

Time (min)	Fluorescence emission (relative intensity) (A)	H^+ secreted (μ Equiv.)	Fluorescence emission (relative intensity) (B)	H^+ secreted (μ Equiv.)
0	100		100	
5	95.6		97.8	
10	93.8	0.68	94.5	0.72
15	93.1		91.6	
20	92.6		90.3	
25	92	0.46	90.3	0.42
30	91.5		90.0	
35	90.8		89.8	
40	90.6	0.42	90.7	0.38
45	90.6		90.9	
50	90.6		91.4	
55	90.1	0.42	91.6	0.40
60	89.9		91.4	
65	89.4		91.4	
70	89	0.30	91.4	0.40
75	89		90.7	
80	88.5		90.5	
85	88	0.24	89.8	0.40
90	88		89.8	

in parallel with the amount of acid secreted. This may be seen from Table II, which gives the time course of the intensity of the fluorescence emission and the acid secreted by 2 mucosae.

These experiments demonstrate the possibility of studying the level of the total reduced pyridine nucleotide in intact gastric mucosa during the process of acid secretion. It would appear from the results that there is an association between the level of intracellular reduced pyridine nucleotide and the rate of acid secretion, as implied in the redox theory of CONWAY and BRADY[1]. This association deserves further investigation[4].

Zusammenfassung. In Magenschleimhaut des Frosches wird das Nicotinamid-adenin-dinucleotid nur im oxydierten Zustand gefunden. Die Absonderung der Säure in der Schleimhaut scheint von der Konzentration des reduzierten Pyridinnucleotids abhängig zu sein.

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Die Wirkung von Progesteron und Progesteronmetaboliten auf das Wachstum von embryonalem Knorpel in vitro

Nach S. MUROTA und Mitarbeitern entstehen bei Zusatz von Cortisol zu embryonalem Knorpel in vitro 11β-Hydroxyandrosten-3,17-dion, 5β-Dihydrocortisol und 3α-OH,5β-Tetrahydrocortisol[1]. Diese Metaboliten zeigen die für Cortisol charakteristischen Wirkungen auf den embryonalen Knorpel nicht mehr[2,3]. MUROTA et al. fanden weiterhin, dass Knorpel Progesteron in 5β-Dihydroprogesteron, in 3α-OH, 5β-Tetrahydroprogesteron und in 5β-Pregnan-3α, 20β-diol überführt; Angaben über die Wirkung dieser Metaboliten werden nicht gemacht[4]. Im Anschluss an Arbeiten über den Einfluss von Steroiden auf Zellen in vitro[5] fanden wir, dass Progesteron das organotypische Wachstum des Knorpels in vitro charakteristisch verändert. Wir untersuchten eine Reihe von Pregnen- und Pregnan-Derivaten, darunter zum Teil auch die von MUROTA beschriebenen Progesteronmetaboliten.

Methode. Femur und Tibia von 7 Tage bebrüteten Hühnerembryonen wurden während 8 Tagen in Carrelflaschen gezüchtet. Pro Hinterextremität wurde je eine Carrelflasche verwendet. Das feste Nährmilieu bestand aus 1,5 ml 0,5% Agar in Medium 199 (Difco) und Geyscher Lösung 1:1, und aus 0,5 ml flüssigem Milieu, Medium 199 mit Zusatz von 0,138% $NaHCO_3$. Die zu prüfenden Verbindungen wurden im flüssigen Milieu gelöst. Dieses wurde bei Versuch und Kontrolle nur einmal, nämlich am 4. Tag, erneuert. Am 8. Tag wurden die Konturen der Knorpel mit Hilfe eines Projektionsapparates gezeichnet und die Bilder ausgemessen. Nachher wurden die Knorpel nach kurzem Waschen mit Aqua bidest. während 10 Tagen bei 80 °C getrocknet. Anschliessend erfolgte die Wägung und Bestimmung des Stickstoffgehaltes nach KJELDAHL.

Es wurden 5 Embryonen pro Versuch (n) verwendet. Für die Bestimmung der pharmakologischen Wirkung wurden die Summe der Längen- und Flächenmasse, das Total des Trockengewichtes und des Stickstoffgewichtes

[1] Den in der Arbeit aufgeführten Trivialnamen entsprechen folgende Verbindungen:

5β-Dihydroprogesteron	= 5β-Pregnan-3,20-dion
5α-Dihydroprogesteron	= 5α-Pregnan-3,20-dion
3α-OH,5β-Tetrahydroprogesteron	= 5β-Pregnan-3α-ol-20-on
3β-OH,5β-Tetrahydroprogesteron	= 5β-Pregnan-3β-ol-20-on
3α-OH,5α-Tetrahydroprogesteron	= 5α-Pregnan-3α-ol-20-on
3β-OH,5α-Tetrahydroprogesteron	= 5α-Pregnan-3β-ol-20-on
20α-OH-Dihydroprogesteron	= Δ^4-Pregnen-20α-ol-3-on
20β-OH-Dihydroprogesteron	= Δ^4-Pregnen-20β-ol-3-on
11β-Hydroxyprogesteron	= Δ^4-Pregnen-11β-ol-3,20-dion
17α-Hydroxyprogesteron	= Δ^4-Pregnen-17α-ol-3,20-dion
11β,17α-Dihydroxyprogesteron	= Δ^4-Pregnen-11β,17α-diol-3,20-dion

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[4] S. MUROTA und B. TAMAOKI, Biochim. biophys. Acta *137*, 347 (1967).

[5] R. MEIER, F. GROSS, P. DESAULLES und B. SCHÄR, Bull. schweiz. Akad. med. Wiss. *8*, 34 (1952). – R. MEIER, P. DESAULLES und B. SCHÄR, Verh. naturf. Ges. Basel *67*, 447 (1956). – R. MEIER und B. SCHÄR, Pathologia Microbiol. *23*, 344 (1960). – B. SCHÄR und R. MEIER, Experientia *16*, 315 (1960). – B. SCHÄR und J. SCHMIDLIN, Eur. J. Steroids *1*, 333 (1966).