consists of material taken from sporulated agar cultures which can be kept for some weeks or months in the refrigerator or at room temperature. Although convenient, this method has some disadvantages: a) Transfer of 'dry spores' with the loop may cause contamination of the working place and also cross-contamination of cultures. b) Because of the hydrophobic nature of the spores of most streptomycetes, the inoculum is not homogenous and contains clumps of aerial mycelium. c) Streptomycetes differ greatly in the abundance of spores produced; thus the amount of inoculum taken from slant cultures may vary considerably from strain to strain; furthermore, when working with a great number of species, slant cultures have to be prepared and stored in batches of different size. d) Finally, storage on agar media over weeks or months may cause genetic instability.

Soft agar conservation. In order to overcome these disadvantages and to have always a homogenous inoculum available, we store streptomycetes in soft agar: Spores are harvested from mature cultures with sterile water containing Triton X-100 (1:10,000) as wetting agent; the heavy suspension is freed from clumps by filtration through a coarse filter paper and then centrifuged. The spores are then resuspended in water and mixed with a warm solution of agar; the final agar concentration (Oxoid No. 3) is 1.25 g/l. These 'soft agar spores' are stored in 10 ml screw cap bottles and kept in the refrigerator. For subculturing a small drop is withdrawn with a capillary pipette. At present, about 300 strains of *Streptomyces* are stored in this way, the oldest preparations being now three years old. – These soft agar spores have served throughout this period as a very convenient and reliable inoculum for many media in our studies on various physiological tests[13,14]. Growth appeared to be quite normal and sporulation was luxurious on suitable media.

Discussion. Although we cannot offer quantitative data on the survival of spores stored in soft agar, we find this method very useful for short term storage of an 'instant inoculum'. Microscopical examination showed that some spores germinate under these conditions and even microcolonies may develop on prolonged storage. However, this did not seem to lessen the usefulness of the method. Occasionally a flask became contamined by a mould; these organisms grow out to rather large colonies which can easily be recognized.

Storage of microorganisms in the 'wet state' appears to be quite contrary to what is usually advocated for the conservation of living cells. Nevertheless, the method seems to be useful for short-term preservation of streptomycetes. The report by KOKOLIOS et al.[15], which appeared a year after our first soft agar spores were prepared, indicates that the method may even be applicable for long-term conservation of bacteria.

Zusammenfassung. Luftmycel-Sporen werden mit Hilfe von Triton X-100 als Netzmittel suspendiert und die filtrierte, homogene, sehr dichte Suspension mit einer Agarlösung vermischt (Endkonzentration 1.25 Agar/l). Diese Sporenkonserve in Weichagar wird bei 5 °C aufbewahrt und kann über Monate und sogar einige Jahre als Impfmaterial dienen. Es wird über Erfahrungen mit anderen Konservierungsmethoden für Streptomyceten berichtet.

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A Simple Method for Heme Isolation

Various methods for heme isolation have been described[1–3]. Of these the procedure of LABBE and NISHIDA[3] is the most widely used. Though this method is simpler than the previous ones, it still implies difficulties and cannot be easily carried out in routine work with a great number of samples. It requires relatively large amount of minimal starting material (1 ml whole blood).

The method to be presented is very simple and reliable. The isolation procedure can be performed at room temperature with the minimal amount of starting material being not more than 0.2 ml whole blood or other similar heme containing systems. In the first step acetone is added in 10 volumes to 0.2–1.0 ml whole blood or other similar heme containing system (pH $\geq$ 7.0). After 30 min standing the precipitate is centrifuged. The supernatant is discarded and the sediment is homogenized in 5 ml ethylacetate-glacial acetic acid (3:1) with a glass rod. After extraction the mixture is filtered (by gravity) and the filtrate is used. The whole amount of filtrate or its aliquots (if not the whole heme is needed but the similarity of the samples should be controlled) is vigorously shaken with 10 volumes of distilled water. In this proportion the ethyl acetate dissolves in water. In contrast heme becomes insoluble and is quantitatively precipitated in crystalline form immediately after mixing. It is noteworthy that the same process was observed by THUNELL[4], who described as a concomitant phenomenon in his procedure a dark precipitate that developed on the borderline of the organic and aqueous phase while standing.

The crystals are then centrifuged or filtered by suction through a fine quality filter paper and washed with distilled water. If recrystallization is needed, the crystals are dissolved in small amounts of 0.1 *M* Na_2CO_3 and 5 volumes of ethyl acetate-glacial acetic acid (3:1) are added. After mixing with 10 volumes of distilled water heme crystallizes again. In general, recrystallization is not needed, since after the simple isolation procedure practically no radioactivity can be measured in the heme fraction of a hemoglobin solution contaminated by inorganic ^{59}Fe (10^6 cpm/ml blood). The procedure can also be carried out on a large scale.

The yield of the method – checked by ^{59}Fe-labelled heme – is 80–85%. The same procedure can be adapted for protoporphyrin preparation. If protopophyrin and

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heme are to be separated, heme can be extracted from the ethyl acetate-glacial acetic acid (3:1) phase by 15% HCl. The protoporphyrin-free heme can be crystallized from this solution in the manner described above after neutralization and shaking into an ethyl acetate-glacial acetic acid (3:1) phase.

In conclusion, the procedure described above is suitable for the isolation of the final product of both heme and protoporphyrin synthesis[5].

Zusammenfassung. Neues Verfahren für Häm-Isolierung durch Aceton-Fällung: Lösung in Aethylacetat-Eisessig und Auskristallisieren mit Wasserzugabe.

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Membrana testacea: A Support for Organ Cultures

Organ-culture method is a powerful tool for biological investigations and so it has been extensively used in several fields (e.g. embryology, genetics, cancer research)[1]. The usefulness of this methodology is largely dependent upon many factors such as composition of nutritional media, nature of supports. As concerns the support, the vitelline membrane (VM) is the most frequently employed one, since it favors adhesivity, flattening and nutrition of the explants[2]. On the other hand, its preparation is difficult and time consuming. The research for supports is therefore advisable.

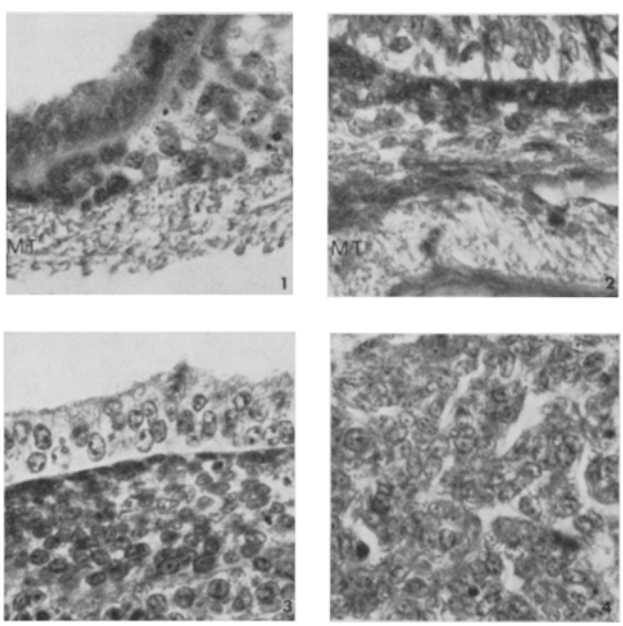

Fig. 1. 6-day lung rudiment after 5 days of incubation. Note the adhesivity of the mesenchyme over the membrana testacea (MT). ×1,500.

Fig. 2. 7-day stomach rudiment after 4 days in culture. ×1,500.

Fig. 3. 6-day intestine rudiment after 4 days of incubation. ×1,500.

Fig. 4. 6-day liver rudiment maintained in culture for 5 days. Note the well preserved structure of epithelial cords. ×1,500.

We have observed that the membrana testacea (MT), a proteinaceous framework placed inside the egg-shell[3], provides a natural support as well and easier to handle than the VM.

MT is removed under sterile conditions from unincubated eggs, rinsed in Tyrode's, cut in small pieces and then placed upon the solid media. Nutrient medium consisted of: 6 drops gelose 1% in Gey fluid, 3 drops chick embryo extract (Difco; 50% in Tyrode's), 3 drops chicken serum (Difco), and Tyrode's containing penicillin 1 drop[4]. Lung, liver stomach, intestine, heart, skin, kidney, limb bud rudiments removed from 6–7-day-old chick embryos have been examined both upon MT and VM. Cultures were incubated at 37 °C; fixed in Bouin fluid; serial sections were cut at 8 + 10 μm and stained with hematoxylin-eosin.

The different anlages undergo a fairly good morphogenesis and differentiation over the MT (Figures 1–4). As shown in Figures 1 and 2, adhesivity to the MT is very good. Morphology of those parts which directly rest upon this support is much better when compared with the VM. One can therefore conclude than nutrition of explants is possible through the MT and occurs at a very good rate[5].

Riassunto. È stato dimostrato che la membrana testacea costituisce un substrato idoneo e di agevole preparazione per culture organotipiche.

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Zur Bestimmung der Katalaseaktivität in Bodenproben

Die Bestimmung der Aktivität mikrobiell gebildeter Enzyme im Boden ist ein häufig angewendetes Verfahren zur Charakterisierung der biologischen Aktivität des Bodens[1, 2]. Es muss jedoch festgestellt werden, dass für viele interessierende Enzyme keine geeigneten Methoden beschrieben sind, die ihre Aktivitätsbestimmung im Boden ermöglichen.

Die Katalase, ein weit verbreitetes Enzym, das die Spaltung von Wasserstoffperoxyd in Wasser und Sauerstoff katalysiert, kann zwar recht gut über die spektralphotometrische und titrimetrische Bestimmung des Substratverbrauches untersucht werden[3], doch sind beide Methoden nicht anwendbar, wenn die Aktivität des Enzymes in Bodenproben gemessen werden soll, da in diesem