Human plasma renin concentration[a], theoretical activity[b] and actual activity[c] in normal, anephric and renal hypertensive patients

	Mean plasma renin concentration[a] (μg standard renin per ml plasma)	Mean theoretical renin activity[b] (ng angiotensin per ml plasma per h)	Mean actual renin activity[c] (ng angiotensin per ml plasma per h)
Normal[3]	5.8	18.2	1.15
Anephric[2]	0.0	0.0	0.0
Renal hypertensive[3]	1.2	3.7	4.45

[a] Measured by line electrophoresis
[b] Calculated from renin concentration and the activity of the standard human renin preparation
[c] Measured by bioassay
Individual assays were done in triplicate

mean renin concentration of 1.2 μg standard renin-ml, equivalent to a theoretical plasma renin activity of 3.7 units. The mean plasma renin activity was estimated to be 4.5 units. Anephric human plasma contained no renin detectable by these techniques.

Discussion. These initial results suggest two things. First that the production of angiotensin II from semi-purified dog substrate by human renin is considerably inhibited in normal plasma. This is shown by comparison with the production of angiotensin II during incubation of highly purified human renin in similar conditions. Second that patients with renal hypertension have a lower concentration of renin than normal, yet, in the few cases studied show a higher renin activity. These observations could be explained in two ways. Either the renin is in a more active form, or there is less inhibition of angiotensin production in the plasma of patients with renal hypertension. The possible existence of an inhibitory system has already been postulated[6].

Résumé. Nous avons institué une nouvelle méthode directe pour l'estimation du rénin par l'immunoélectrophorèse. Les résultats préliminaires obtenus par cette méthode indiquent que des changements importants peuvent se faire dans l'activité enzymatique du rénin dans le cas de patients ayant de l'hypertension rénale.

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Storage of Streptomyces in Soft Agar and by Other Methods

Long term conservation. There are numerous methods available for the long term preservation of microorganisms[1,2]. We have employed several of them for the maintenance of a rather large collection of streptomycetes and found the following quite useful: 1. Soil culture – a method recommended repeatedly for the conservation of fungi and streptomycetes[3,4]: Most of our 165 soil cultures prepared in 1958[5] could be subcultured after 8 years of storage and even now many of them give a high viable count. 2. Deep freeze: Our observations on the viability of 400 cultures kept at −20 °C for 5 years agree well with those made by other investigators[6,7]. In order to save storage space in the deep freeze unit, the streptomycetes were grown in ampoules which were sealed after the development of a mature aerial mycelium had taken place. 3. Lyophilization – the method most widely employed: The procedure suggested by Hopwood and Ferguson[8] has been used. Our liophils – prepared $1^1/_2$ years ago – give heavy growth. 4. Drying in nutrient gelatin discs[9]: Of 50 streptomycetes dried 2 or 3 years ago 35 yielded a high viable count recently, whereas the others gave only few colonies per disc or had died. 5. Drying on unglazed porcelain beads[10,11]: 60 stains – preserved by this method $1^1/_2$ years ago – are easily revived by placing a bead charged with spores on a nutrient medium. – A similar method suggested by Perkins[12] using anhydrous silica gel as acceptor of the spore suspension proved to be unsuitable for streptomycetes as these organisms could not be recovered after a short time of storage.

With most methods for long term conservation, the organisms have to be revived and propagated before they can be used in the experiments intended; soil cultures and agar cultures stored in deep freeze may be exceptions. Therefore, the inoculum for 'experimental media' usually

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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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