ing medium (Table I) per pair of kidneys. This medium was found more satisfactory than the phosphate buffer used by WOLF et al., since it provides faster dissociation and retains more viable cells. The digestion was done over a magnetic stirrer (Bellco) at 12–15°C, maintained by a cold running water bath. The medium was changed twice in the first 2 h and replaced by fresh medium to drain any toxic material. When most tissue was dissociated, the cells were centrifuged at 1000 rpm for 4 min, washed in the Ringer's saline, centrifuged and dispersed in a growth medium (Table II) to give concentration of 3×10^5 cells per ml. They were allowed to attach in 4 oz. medicine bottles or Leighton tubes with 10×50 mm No. 1 coverglasses at 18°C. The above growth medium was used because we had found that the modified Eagle's basal medium, containing salt concentrations used by BARTH[3] for frog embryonic cells, with calf or horse plasma, gave better growth of the amphibian tissue explants than the undiluted or arbitrarily diluted mammalian media or other media.

Most of the viable cells attached within 70 h. In seven to ten days mitotically active (approx. 2.5% mitotic index) cultures were obtained. The cells form a uniform monolayer of predominantly epithelioid cells (Figure 1) containing long threadlike mitochondria (M Figure 2) and one or two juxta nuclear Golgi bodies (G Figure 2).

Frog kidney cells with Lucke's adenocarcinoma (Figure 3) have also been cultured with the above technique and have found to attach and grow faster than the normal cells.

The primary cultures have been kept as long as three months without any noticeable impairment of growth.

Zusammenfassung. Eine verbesserte Technik zur Gewinnung der ersten Einzelschichtbildungen von Froschnierenzellen wurde entwickelt. Die Zellen werden in neuem auflösendem Nährboden isoliert und wachsen auf modifiziertem Eagle-Nährboden mit 13% Kalbsserum. Nach 10 Tagen zeigt sich stark aktives mitotisches Zellwachstum in der Kultur.

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[3] L. G. BARTH and L. J. BARTH, J. Embryol. Morph. *7*, 210 (1959).

A Quantitative Micro Tube Gel Precipitin Technique

The gel-precipitin technique developed by OUDIN[1] was elaborated by OUCHTERLONY[2] on agar plates. OAKELY and FULTHORPE[3] adapted OUCHTERLONY's modifications to test tubes. Several variations of these techniques have been published[4,5]. A micro adaptation[6] of OUCHTERLONY's plate technique proved superior to conventional methods. Since the test tube technique of OAKELY and FULTHORPE is better adapted for quantitative work[4], and has been found to yield a definable pattern, where many plate modifications failed[7], and since a micro adaptation of this technique has apparently not been attempted, the following technique was devised.

The precipitin pattern is developed in a capillary tube (50 mm × 1, or 2 mm), charged as indicated in Figure 1. First, 1% melted agar is injected into the middle of the capillary with a syringe, up to the required length, and is left to set. The antigen and antibody solutions are then injected at opposite ends of the agar column, carefully avoiding air bubbles and injury to the agar surfaces. Quantities of each reactant solution, as little as 0.02 ml or less, are sufficient. Finally, both ends of the capillary are sealed with melted wax, leaving an air bubble between the wax plug and the reactant solutions.

The development of the precipitin pattern can be conveniently followed by photographic recording, or by tracing after projection through a microfilm reader. After sufficient development of the pattern, the column can be extruded unharmed, and washed into a bath of distilled water. After washing, the agar column can be repacked in a bigger capillary filled with distilled water, sealed with wax, and thus preserved undeformed permanently. Agar columns after washing can be stained, to intensify the pattern[6], or to study specific components[8].

Accurate determination of the relative positions[5,9] and intensities[10] of the various bands are required to verify and apply the theories concerning the formation of the precipitin patterns. In the technique reported here, the positions of all bands can be determined either by a travelling microscope, or an ordinary microscope fitted with a graduated stage, or simply, but with the same degree of accuracy, from measurements on a tracing of the projected pattern. The relative intensities of the various bands were evaluated by scanning the 35 mm negative of the precipitin pattern, using a paper electrophoresis scanner (Spinco Analytrol unit, Beckman Instruments, Inc., Belmont, California).

Figure 2 illustrates the degree of resolution attainable for the system tetanus toxin and tetanus anti-toxin. Successful patterns were obtained with the following antigens against their respective antisera from horses or rabbits: Diphtheria toxin, human serum, cow and other animal sera, milks of different cattle, vaccinia and influenza viruses, and also different snake venoms. This technique may well be applied in the study of other precipitin systems.

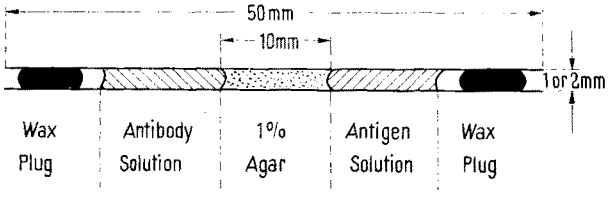

Fig. 1. The capillary precipitin set-up

[1] J. OUDIN, Ann. Inst. Past. *75*, 30 (1948).
[2] Ö. OUCHTERLONY, Acta path. microbiol. scand. *26*, 507 (1949).
[3] C. L. OAKELY and A. J. FULTHORPE, J. Path. Bact. *65*, 49 (1953).
[4] C. L. OAKELY, Discussions, Faraday Soc. *18*, 358 (1954).
[5] A. POLSON, Science Tools *5*, 17 (1958).
[6] A. J. CROWLE, J. lab. clin. Med. *52*, 784 (1958).
[7] W. A. PIERCE, Jr., J. Bact. *77*, 726 (1959).
[8] L. KORNGOLD and R. LIPARI, Science *121*, 170 (1955).
[9] E. L. BECKER and J. C. NEFF, J. Immunol. *83*, 571 (1959).
[10] A. HAYDEN and E. L. BECKER, J. Immunol. *85*, 591 (1959).

The technique reported above, presents some new features over the conventional techniques. The thin agar layer used makes the detection of faint bands easier, thus it is possible to locate the bands at an earlier stage in their formation. The extrusion of the column after the development of the pattern allows its washing, which increases considerably its clarity, specially when one of the reactants is heavily pigmented, thus facilitating its accurate photography, and quantitative determination of relative intensities of the various bands. It allows further its staining and its preservation undeformed. The use of both antigen, and antibody in a solution phase imposes no limits to the range of concentrations used for obtaining the precipitin pattern. The charging of the tube does not expose any of the reactant solutions to conditions which might cause slight denaturation, such as mixing with melted agar. Both reactant reservoirs are fully exposed, this allows the detection of any precipitate developing, which may be due to a component of a reactant diffusing right through the column. The successful development of the precipitin patterns with this set-up in the fridge might prove of importance in the study of special systems. No special treatment[11] was found necessary for the agar used, or the capillary tubes. Finally, the 0.02 ml does not represent the smallest volume of a reactant solution, as smaller volumes can be used, and even finer capillaries have been utilised when necessary[12].

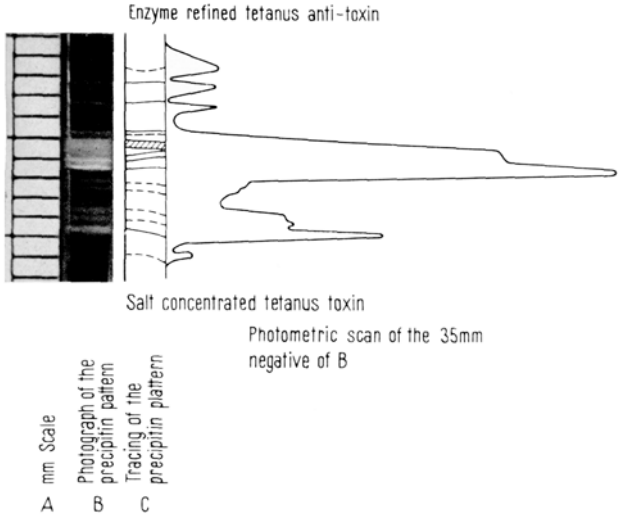

Fig. 2

Zusammenfassung. Neue Mikroröhrchenmethode für die Gel-Präzipitation. Vorteile der Methode: Geringer Bedarf an Reagenzien und Möglichkeit quantitativer Auswertung der relativen Intensitäten der Präzipitationsbanden.

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Research Department, Production Laboratories, Ministry of Public Health, Agouza (Cairo U.A.R.), November 22, 1961.

[11] J. Oudin, Methods in Medical Research *5*, 340 (1952).

[12] We wish to thank Professor F. Cramer (Darmstadt (Germany) and guest Professor at the National Research Center (Cairo)), for his encouragement and great help during the preparation of the manuscript of this paper.

STUDIORUM PROGRESSUS

Structure and Chemical Composition of Collagen Gel

The precipitation of typical collagen fibrils from acid solutions of the protein is due to the presence in them of minimum quantities of regulator colloids (Highberger, Gross, and Schmitt[1]; Schmitt[2]; Fitton Jackson and Randall[3]; Schmitt, Gross, and Highberger[4]; Gross[5]; Glimcher[6]). When these are lacking, the precipitation of the protein, using salts, gives rise to a gel formed by thin filaments without periodic structure, but endowed with physio-chemical reactions of collagen.

Since the study of the above gel has been practically neglected until the present time, we carried out a screening of its submicroscopical organization and chemical features; for comparison, results obtained from collagen solutions, devoid of regulator colloids, were compared with those obtained from separated samples of the same solutions, to which were added mucopolysaccharides isolated from human silicotic masses, as described by Schiller, Mathews, Jefferson, Ludowig, and Dorfman[7].

Two samples A and B of a homogeneous suspension of isolated fibrils of rat's tail tendon were prepared as described by Bairati, Clerici, and Esposito[8]. The total nitrogen of each sample was estimated by the Microkjeldahl method, amino acid composition was determined by means of paper chromatography as described by Dustin, Schram, Moore, and Bigwood[9], on a sample hydrolized with 6N HCl under reflux for 24 h, containing nearly 200–500 µg of nitrogen, determined according to Moore and Stein[10]. The chromatograms were developed as described by Pernis and Wunderly[11] and the concentration of each amino acid was referred to a standard leucine sample; hydroxyproline was analyzed chemically by the method of Troll and Cannan[12].

The protein nitrogen contained in A and B is 18.2% and 16.5% respectively. The amino acid composition of each sample is shown in the Table; our results agree with those

[1] J. H. Highberger, J. Gross, and F. O. Schmitt, Proc. Nat. Acad. Sci. U.S. *37*, 286 (1951).

[2] F. O. Schmitt, Rev. modern Physics *31*, 349 (1959).

[3] S. Fitton Jackson and J. T. Randall, in *Nature and Structure of Collagen* (Butterworths Scientific Publications, London 1953), p. 181.

[4] F. O. Schmitt, J. Gross, and J. H. Highberger, Symposia Soc. exp. Biol. *9*, 148 (1955).

[5] J. Gross, Metab. Interrelat. *4*, 32 (1952).

[6] M. J. Glimcher, in *Calcification in Biological Systems* (Amer. Ass. for Advancement of Science, Washington D.C. 1960), p. 421.

[7] S. Schiller, M. B. Mathews, H. Jefferson, J. Ludowig, and A. Dorfman, J. biol. Chem. *211*, 717 (1954).

[8] A. Bairati, E. Clerici, and G. Esposito, Med. Lav. *52*, 338 (1961).

[9] J. P. Dustin, E. Schram, S. Moore, and E. J. Bigwood, in *Cromatografia* (Il pensiero scientifico Ed., Roma 1955), p. 94.

[10] S. Moore and W. A. Stein, J. biol. Chem. *211*, 907 (1954).

[11] B. Pernis and Ch. Wunderly, Biochem. biophys. Acta *11*, 209 (1953).

[12] W. Troll and R. K. Cannan, J. biol. Chem. *200*, 803 (1953).