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].

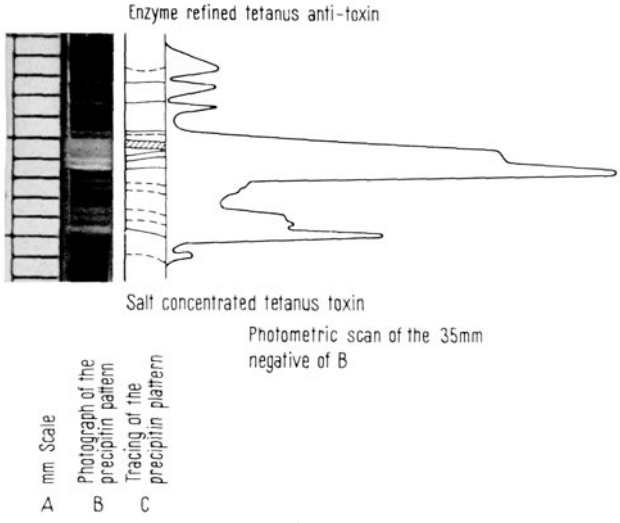

Fig. 2

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.

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[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).

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[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).

previously reported in the literature (BROWN, KELLY, and WATSON[13]; BOWES, ELLIOT, and MOSS[14]; ZAIDES, TOUSTANOVSKII, ORLOVSKAIA, and PAVLIKHINA[15]).

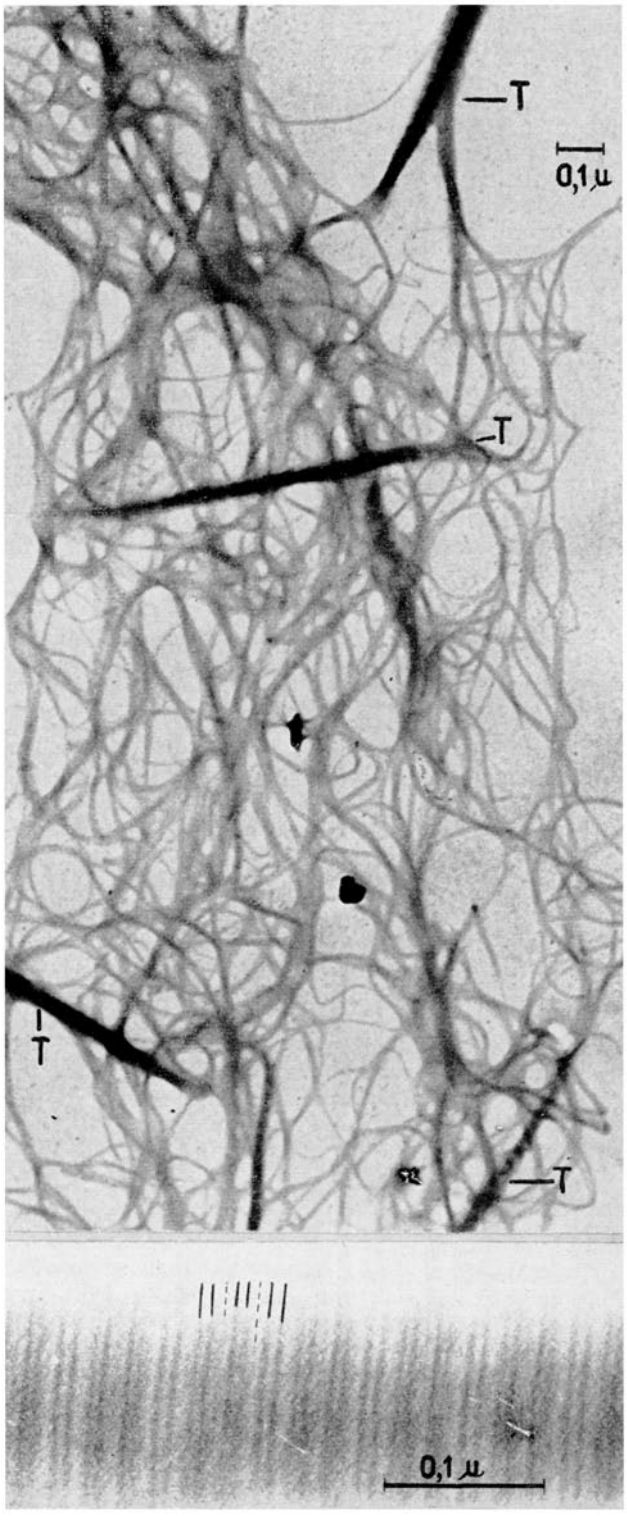

Fig. 1 (above). Electron microscope image of collagen gel (sample α^1). One may see the reticulum of filaments and membranes without periodical structure, with 'tactoides' typically banded. Mag. 60000 ×.
Fig. 2 (below). At a higher magnification the electron microscopic image shows periodical structure of tactoide with eight bands. Mag. 200000 ×. This preparation was stained with PTA.

Amino acid composition of several collagen samples[a]

	A	B	α_1	α_2	β_1	β_2
Aspartic acid	5.27	4.49	6.54	10.47	4.05	5.87
Glutamic acid	7.63	9.26	10.41	7.67	7.87	13.67
Serine	3.57	5.79	3.88	9.58	5.17	4.36
Histidine	1.75	2.35	0.00	0.00	3.56	5.19
Arginine	1.04	7.69	10.11	6.97	6.36	7.54
Hydroxyproline	9.43	5.47	9.31	9.99	9.47	8.28
Glycine	28.27	23.22	26.81	20.76	27.48	24.25
Threonine	4.03	1.34	6.56	5.95	3.37	2.27
Alanine	14.16	19.42	20.38	11.29	16.58	14.36
Proline	3.73	5.85	4.50	5.36	5.64	3.88
Valine	3.75	4.98	4.46	4.97	4.57	4.63
Leucine + Isoleucine	5.28	6.70	5.95	5.39	5.66	4.34
Tyrosine	0.00	3.44	0.00	0.00	0.00	0.00
Lysine	1.08	0.00	0.00	0.00	0.00	1.41
Methionine	1.01	0.00	0.00	0.00	0.00	0.00

[a] N as % of Protein N.
[b] Hydroxyproline determined by the chemical method (TROLL and CANNAN[12])

In particular, it must be noted that sample A contains about 14.12% hydroxyproline expressed as g of amino acid/100 g of protein, and lacks tyrosine, while sample B contains 8.19% hydroxyproline expressed as above and an increased amount of thyrosine; it is therefore likely that the treatment undergone by sample B was not sufficient to isolate the collagen protein in pure form.

Sample A and B were solubilized with 0.01 *M* acetic acid and labelled A^1 and B^1, solid sodium acetate was added in an amount to give a final concentration 0.125 *M*, pH 5.8 (FITTON JACKSON and RANDALL[3]) and the samples were dialyzed for 24 h against distilled water.

Two different precipitates were obtained, namely α^1 and β^1; their amino acid compositions determined after hydrolysis and paper chromatography, as above specified, are practically identical (Table) and correspond closely to that of the purer sample A.

It is therefore evident that in any case collagen precipitates from its solutions in a pure form, as shown both by the increase of hydroxyproline from 5.47 in B to 9.47 in β^1 (or from 8.19% to 14.18% if expressed as g of hydroxyproline/g of protein) and by the disappearance in β^1 of tyrosine which, in the less purified sample B, is as high as 3.44% of the total protein nitrogen; the amino acid composition of α^1, being unchanged as compared to that of A, confirms the purity of this last collagen sample.

The addition to separate aliquots of samples A^1 and B^1 of sodium acetate, as specified previously, plus mucopolysaccharides from silicotic hyaline masses to give a final concentration of 0.02%, gives origin to two precipitates labelled α^2 and β^2. Their amino acid composition is practically superimposable upon that of α^1 and β^1; thus showing that the chemical composition of collagen is the same when precipitated either by sodium acetate or by the salt plus mucopolysaccharides (Table).

[13] G. L. BROWN, F. C. KELLY, and M. WATSON, in *Nature and Structure of Collagen* (Butterworths Scientific Publications, London 1953), p. 199.
[14] J. H. BOWES, R. G. ELLIOT, and J. A. MOSS, in *Nature and Structure of Collagen* (Butterworths Scientific Publications, London 1953), p. 117.
[15] A. L. ZAIDES, A. A. TOUSTANOVSKII, G. V. ORLOVSKAIA, and L. V. PAVLIKHINA, Biophysics *4*, 29 (1959).

From a gross morphological point of view, the precipitates α^1 appears as a gelatinous, thick and milky mass, the greater in bulk the more complete has been the dispersion of the fibrils in A. Optical microscopic observation does not reveal any elementary fibrils; in phase contrast the image is practically irresolvable; in dark field a milky diffraction is shown. In a thick drop the gel shows the characteristic birefringent trajectories of collagen.

Under the electron microscope the gel appears formed by a reticulum varying in composition: some zones show a delicate cotton-like appearence consisting of a structure so thin that the single constituents hardly reach the limits of resolution; some other zones show systems of membranes so interconnected that they form a relatively coarse reticulum. Furthermore, some filaments appear clearly spiralized (thickness between 150 and 300 Å). In any case, no structure is normally banded. This gel, however, includes also short, typically banded tactoidal fibers several microns long and as thick as 150–400 Å, with a period ranging from 620 to 690 Å, which show six intraperiodal bands after uranil acetate staining and seven to eight bands after phosphowolframic acid (PTA) staining.

Similar data are obtained for sample β^1, which precipitates on addition of sodium acetate to the B^1 collagen solution. In this case, however, together with a greater amount of fibers of tactoidal shape, many long needle-like filaments, with all the characteristics of native collagen, are observed under the electron microscope. The amount of these fibrils varies from sample to sample, probably in connection with its degree of purity.

The macroscopic appearance of precipitates α^2 and β^2 is closely related to that of samples α^1 and β^1; the sediments, however, are usually contaminated by the presence of small amounts of coarse impurities which settle down easily. Under phase contrast and dark field examination, the presence of filamentous formations, often longer than 20 μ, very similar to the native collagen fibrils, is usually observed in both cases. Polaroscopic and electron microscope investigations confirm the presence of both long fibrils with the characteristic banded structure of native collagen and of a remarkable number of tactoidal fibrils.

Results show that, in spite of the chemical identity of collagen A and of the α^1 precipitate, the morphology of the latter is far from that of typical collagen, whose organization is reproduced in sample α^2 precipitated by means of sodium acetate and by adding mucopolysaccharides. On the contrary, the precipitate β^1 obtained from B^1, whose lesser degree of purity as compared to A^1 is probably due to contamination by polysaccharides from the ground substance of the connective tissue, shows the presence of typically banded collagen fibers. No difference is seen between the electron microscope appearance of the β^1 and β^2 precipitates.

Our data suggest that, on addition of sodium acetate to a pure collagen solution (A^1), a peculiar dispersed gel with a partial molecular arrangement rather close to that of the native collagen precipitates. In this gel one may observe all the stages in the organization of the protein, from unbanded structures to tactoidal fibers showing periodicity, in spite of the lack of regulator mucopolysaccharides.

Therefore, it may be confirmed that collagen protein may aggregate either in the presence or in absence of regulator colloids in several ways, and that the word 'collagen' should define a protein and not a filamentous structure, since the elementary banded fibrils found in the connective tissues are only one of the many para-crystalline states which collagen molecules may assume.

Zusammenfassung. Es wird gezeigt, dass die gelösten Eiweisskörper bei Abwesenheit von Regulierungssubstanzen ein netzförmiges Gel bilden. Dieses enthält Fibrillen ohne periodische Struktur und nadelartige «Tactoïde» mit typischer Periodizität.

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Istituto di Anatomia e Patologia Generale dell'Università di Milano (Italy), February 14, 1962.

[16] This investigation was partly supported by a research grant from the High Authority of the European Community for Coal and Steel.

Juxtaglomerular Cells, Renal Pressor Substances and Nephrosclerosis[1]

Partial constriction of the renal artery, perinephritis or partial renal infarction causes hypertension. Normal kidneys contain large amounts of the enzyme renin which is pressor through its effector agent, angiotensin; acute renal ischemia elicits an immediate rise in blood pressure associated with an increased release of renin; many forms of experimental and clinical hypertension are associated with renal lesions. These observations have led to the acceptance by some of the hypothesis that the renal pressor system has a primary role in the pathogenesis of hypertension. However, repeated attempts during the last 20 years to demonstrate greater amounts of renin or angiotensin in renal vein or peripheral blood have been mostly unsuccessful or, at best, equivocal. Many explanations have been presented to account for this failure. Among them, the two extremes are: renin plays only an accessory and incidental role, if any, so that hypertension, including renal hypertension, can exist without excess circulating renin; hypertension is due to hyperfunction of the renal pressor system but the present analytical methods are too crude to detect small but crucial increases in blood renin. Whatever the merits of these arguments, the fact remains that renin is present in kidneys and possesses various activities other than pressor. Hence, one could assume that it intervenes in physiologic or pathologic situations other than hypertension, as already indicated by the recent demonstration of its participation in the regulation of aldosterone secretion[2].

Possibly because of the narrow and unfruitful approach of the past, investigations have lately become more basic and diversified in attempting to test the endocrine function of kidneys during hypertension as well as during conditions associated with salt imbalance. Three methods have been extensively used: determination of granularity of the juxtaglomerular cells, determination of pressor activity in kidney extracts, and determination of pressor activity in renal and peripheral blood. We are aware that they all

[1] Whenever used, the term renin has the same meaning as renal pressor substances and refers to a biological activity, not to a definite chemical entity.

[2] C. C. J. Carpenter, J. O. Davis, and C. R. Ayers, J. clin. Invest. *40*, 2026 (1961).