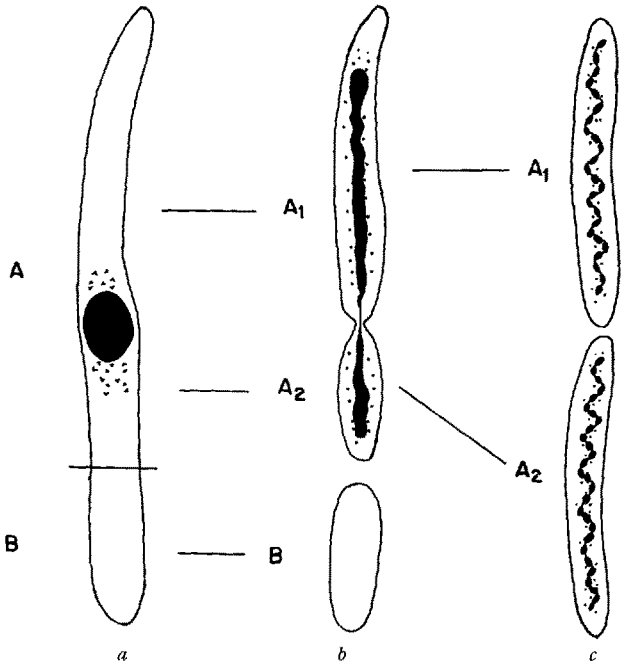
PEEBLES[5], BALAMUTH[6], WEISZ[7]). This would explain the fact, first mentioned by BALBIANI[8], that merotomy does not interrupt the physical cleavage process and may result in the production of unequal sized offspring. On the basis of extensive observations on *Paramecium caudatum*, CALKINS[4] postulated the occurrence of an irreversible division plane in the centre of the cell which was not regulated to suit any given exigency. PEEBLES[5] and CALKINS[4] emphasized that in all cases the outcome of merotomy depended on the physiological condition of the animal.



Division zone in *Spirostomum ambiguum*. *a* Animal in prefission stage with condensed macronucleus. A transverse cut has separated a posterior fragment (*B*) from anterior portion (*A*). *b* 3 h later; fragment *B* dies. Cleavage furrow has appeared in *A* and separated it into two unequal parts, A^1 and A^2. *c* 48 h later; A^1 and A^2 have grown into normal vegetative animals.

STEVENS[9] and WEISZ[1] in *Stentor*, and FAURÉ-FREMIET[10] in *Urostyla*, have shown that binary fission is a reversible process up to a certain stage. Merotomy during later stages showed that the cleavage plane had become irreversible.

Experiments on *Spirostomum ambiguum* provide a further insight into the phenomenon of determination of cleavage furrow in this species. Predivision *Spirostomum* specimens in the progress of binary fission were selected from stock cultures. Animals with condensed macronucleus were picked. The condensation of the macronucleus into a polymorphic body represents an early stage in division (BISHOP[11], PADMAVATHI[12], SESHACHAR and PADMAVATHI[13]). With steel needles, a fourth of the animal, at the posterior end, was cut off (Fig. *a*). This section (*B*) did not contain any macronucleus. The fragment was isolated, but failed to regenerate; it died 2 h later. In 3 h time, a cleavage furrow appeared in (*A*) and separated it into 2 highly unequal parts (A^1 and A^2), the anterior of which was about half the size of the original animal while the posterior was a much smaller body (Fig. *b*).

Both were kept in the nutrient medium and grew into normal animals at the end of 48 h (Fig. *c*). The same result was obtained when the anterior fourth (instead of the posterior) was removed.

The above experiment clearly indicates that (a) a fragment without the macronucleus cannot survive, and (b) the "fission zone" is determined by the time condensation of macronucleus occurs. Regeneration experiments on animals in the vegetative condition (unreported data) show that at this stage the "fission zone" is not yet established. The present study on *Spirostomum ambiguum* leads us to conclude that the "fission zone" is laid down during the process of condensation of the macronucleus and is not subject to change later. According to WEISZ[2], the division process in *Stenor* is found to pass through an early reversible and a later irreversible phase. The fission line itself may be the explicit result of the postulated physiological bisection of the individual. In *Spirostomum ambiguum*, this becomes irreversible after condensation of the macronucleus.

P. B. PADMAVATHI

Department of Zoology, University of Mysore, Central College, Bangalore (India), July 10, 1956.

Zusammenfassung

Regenerationsexperimente haben gezeigt, dass bei *Spirostomum ambiguum* die Lage der Einschnürungsstelle schon vor der Teilung festgelegt wird. Die Determination der Furche erfolgt zur Zeit der Kondensation des Makronukleus.

High Angle X-Ray Diffraction and Chemical Studies on the Nature of Fibrous Glia [1]

With a view to the limited amount of knowledge so far available on the nature of fibrous glia, we have performed biochemical and biophysical investigations on the so-called "corneal laminae" which may be found in the lining of the human cerebral ventricular cavities. These laminae are thick in cases of hydrocephalus and are composed of pure fibrous glia (BAIRATI, PANNESE[2]).

Chemical Investigations.– Total lipids have been determined by the method of FOLCH *et al.*[3]. The amino-acid composition of the proteins of fibrous glia has been

[5] F. PEEBLES, Biol. Bull. *23*, 154 (1912).
[6] W. BALAMUTH, Anat. Rec. *75*, 86 (1939).
[7] P. B. WEISZ, Quart. Rev. Biol. *29*, 207 (1954).
[8] E. G. BALBIANI, Ann. Micrograph. *4*, 369 (1882).
[9] N. M. STEVENS, Arch. Entwmech. Org. *16*, 461 (1903).
[10] E. FAURÉ-FREMIET, Bull. sci. Fr. Belg. *44*, 215 (1900).
[11] A. BISHOP, Quart. J. micr. Sci. *67*, 391 (1923).
[12] P. B. PADMAVATHI, J. zool. Soc. India *7*, 91 (1955).
[13] B. R. SESHACHAR and P. B. PADMAVATHI, J. Protozool. (in press).

[1] From the Institute of normal Anatomy, University of Milan, Italy (Director Prof. A. BAIRATI), and the Clinic for Occupational Diseases, University of Milan (Director Prof. E. C. VIGLIANI). The Siemens Kristalloflex III X-ray apparatus, which has been used in this research, is part of the equipment of the Industrial Hygiene Laboratory of the Montecatini Co., attached to the Clinic for Occupational Diseases.

[2] A. BAIRATI, Boll. Soc. Ital. Biol. sper. *25*, 931 (1949). – E. PANNESE, Z. Zellforsch. (in press).

[3] J. FOLCH, I. ASCOLI, M. LEES, J. A. MEATH, and F. N. LE BARON, J. biol. Chem. *191*, 833 (1951).

determined by the method of PERNIS and WUNDERLY[4] and that of MC FARREN and MILLS[5], and the UV. absorption spectrum of the same proteins has been established in 0·1 *N* NaOH solution, according to GOODWIN[6]. The results of the UV. analysis are shown in Figure 1; total lipids were found to be about 15% of dry tissue.

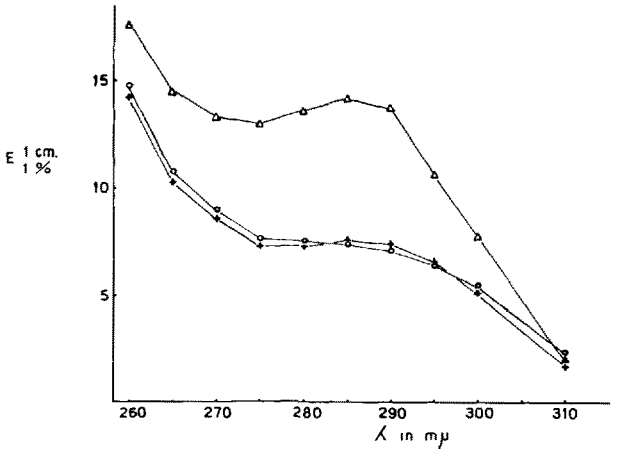


Fig. 1. – Spectrophotometric UV. absorption curve of: o–o–o fibrous glia; +–+–+ human hair keratin; △–△–△ fibrinogen.

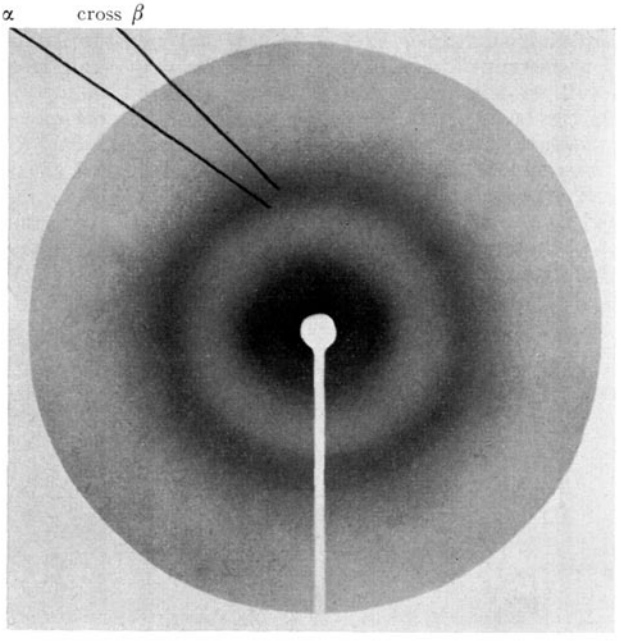


Fig. 2. – X-ray diagram given by native fibrous glia.

The discussion of chemical data may be summarized as follows: the hypothesis (WILKE and KIRCHER[7]) that fibrous glia is identical to fibrin has not been confirmed. As a matter of fact, the amino-acid composition and UV. absorption of glial proteins are very different from those of fibrinogen; moreover, fibrinogen and fibrin quickly dissolve in boiling 0·1 *N* NaOH, while fibrous glia withstands this treatment.

On the contrary, the chemical investigation brings out some similarities between fibrous glia and keratins. In this respect, it appears that we must classify fibrous glia among soft keratins with low cystine content (MATOLSTY[8]), and moreover we must point out that lipophilic amino-acids are present in larger amounts in fibrous glia than in most keratins, while the reverse holds true for hydrophilic amino acids.

Physical Investigation. – High angle X-ray analysis of stretched glial laminae has been performed with a Siemens Kristalloflex III Apparatus.

We have examined native glia at room humidity (Fig. 2) or air dried after treatment with fat solvents; moreover lipid-free glia was stretched in steam (whereby an increase in length of about 80% took place) and then subjected to X-ray analysis (Fig. 3). The Figures give evidence, for untreated glia, of an equatorial reflexion at 10·3 Å and of three meridional reflections at 5·1 Å (moderately strong), at 4·6 Å (very weak) and at 4·2 Å (strong). The last reflection disappears from figures of lipid-free glia. In the Figures of lipid-free, steam-treated glia the main feature is the presence of a second equatorial reflection at 4·65 Å. The interpretation of these findings may be summarized as follows: in the native state fibrous glia has an α-configuration (with some evidence of the supercontracted cross-β-phase) and, after stretching in steam, it undergoes a typical α-β-transformation.

Conclusions. – While high angle X-ray analysis shows that the proteins of fibrous glia must be considered as a member of the k-e-m-f group, chemical data show that it can not be identified with any known member of the class, but that it bears some resemblance to proteins of the keratin group.

This is, by the way, consistent with the fact that both gliocytes and keratin-producing cells are of ectodermal origin, and supported by recent electron microscopic observations which we have performed on fibrous glia showing that glial fibers are composed of protofibrils

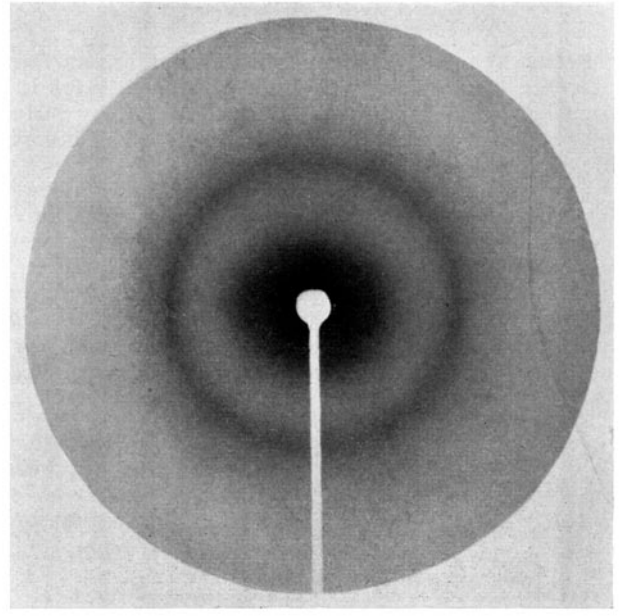


Fig. 3. – X-ray diagram given by fibrous glia previously treated with fat solvents and then stretched in steam.

[4] B. PERNIS and CH. WUNDERLY, Biochim. biophys. Acta *11*, 209 (1953).

[5] E. F. MC FARREN and C. A. MILLS, Analyt. Chem. *24*, 650 (1952).

[6] T. W. GOODWIN and R. A. MORTON, Biochem. J. *40*, 628 (1946).

[7] G. WILKE and H. KIRCHER, Dtsch. Z. Nervenheilk. *167*, 529 (1952).

[8] A. G. MATOLSTY and C. A. BALSAMO, J. biophys. biochem. Cytol. *1*, 339 (1955).

having an axial periodicity of 200 Å. This is in excellent agreement with the acial periodicity of 198 Å found by low-angle X-ray investigation (Mc Arthur, Bear[9]) of α-keratin.

A. Bairati, B. Pernis, and G. Frigerio

Institute of Anatomy, University of Milan, Italy, July 22, 1956.

Zusammenfassung

Gliafasern wurden chemisch und röntgenographisch untersucht. Aminosäureanalyse und Ultraviolett-Absorption erlauben folgende Feststellungen: Die von Wilke und Kircher angenommene Identität von Gliafasern und Fibrin kann nicht bestätigt werden; die Glia widersteht einer 0,1 *n* NaOH Lösung bei 100° C, während Fibrin unter den gleichen Bedingungen rasch gelöst wird; ausserdem sind Glia und Fibrinogen hinsichtlich Aminosäurekomponenten und UV.-Absorption vollständig verschieden. Es bestehen dagegen interessante Ähnlichkeiten zwischen Glia und Keratinen: UV.-Absorption der Glia und des menschlichen Haarkeratins sind fast identisch. Gliafasern enthalten verschiedene Aminosäuren (Glutaminsäure, Asparaginsäure, Valin, Phenylalanin, Glycin) in ähnlichen Mengen wie die Keratine.

Ferner finden sich im Gliagewebe Lipoide zu etwa 15% des Trockengewichtes. Die röntgenographische Untersuchung bestätigt die Gegenwart von Lipoiden und weist den Proteinen der Glia die α-Konfiguration der k-e-m-f-Gruppe der faserigen Proteine zu. Gliafasern erleiden durch Streckung im Wasserdampf eine typische α-β-Umwandlung.

[9] I. Mc Arthur, Nature *152*, 38 (1943). – R. S. Bear, J. Amer. chem. Soc. *65*, 1784 (1943).

Paper Electrophoresis of Cytoplasmic Proteins From Normal and Pathological Liver Cells[1]

This report deals with the electrophoretic separation on paper of cytoplasmic soluble proteins from normal liver cells and from cells affected by vacuolation or fatty degeneration. Cell vacuolation of the liver was produced according to Pichotka[2]. Fatty liver was induced by carbon tetrachloride. The technique of Adjutantis[3] for the extraction of the cytoplasmic proteins was improved by perfusing the liver in order to remove completely the blood proteins, and by using ultrafiltration under a pressure of 6–8 atm of nitrogen instead of the dialysis to minimize proteins denaturation.

Figure 1 shows the electrophoretic pattern of soluble proteins from normal liver cells. The four peaks have been numbered in order of mobility. Sometimes the pattern shows a new moderate peak, which is to be related to an albumin-like protein.

Pattern of cytoplasmic proteins extracted from vacuolated liver cells is shown in Figure 2. The pattern contains a very marked amount of albumin-like proteins (peak 5). The pattern of soluble cytoplasmic proteins from fatty livers looks simpler than those from normal cells and it only exhibits three peaks (Fig. 3).

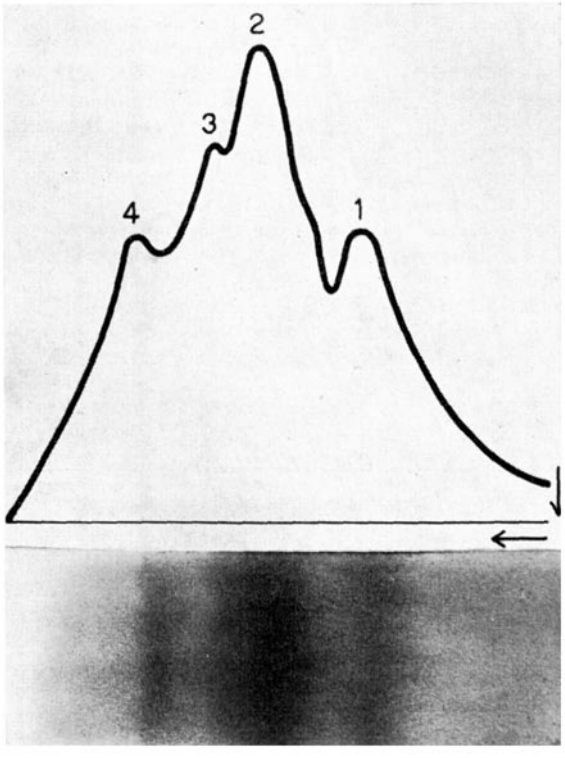


Figure 1.

The results obtained with normal livers, particularly the very low amount of albumin-like proteins, are in agreement with the findings of previous authors concerning rat liver (Gigante *et al.*[4], Demling[5], Demling[6]),

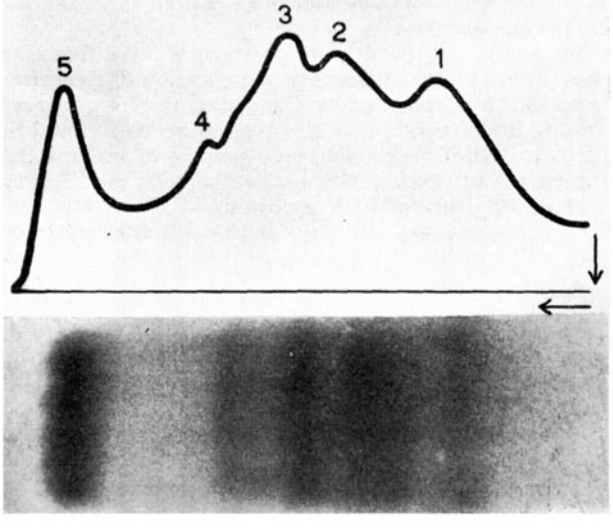


Figure 2.

[1] This investigation was aided by a grant from the "Consiglio Nazionale delle Ricerche".

[2] J. Pichotka, Beitr. path. Anat. *107*, 117 (1942).

[3] G. Adjutantis, Nature *173*, 539 (1954); *174*, 1054 (1954).

[4] D. Gigante, M. Capone, and A. Rossi-Espagnet, Riv. Infort. Mal. prof. *2*, 3 (1954).

[5] L. Demling, Klin. Wschr. *30*, 74 (1952).

[6] L. Demling, Z. ges. exp. Med. *122*, 416 (1951).