phorus by the sorption procedure from a filtrate of a mixed marine algal culture. The filtrate was used as an experimental medium in order to test the method at higher phosphorus-levels than were normally encountered in the natural sea-water samples used for the development of the method (10–30 μgm. phosphorus/litre). The algal culture was filtered through a 'Millipore *HA*' membrane, and sub-samples of the filtrate taken either for direct digestion by the procedure of Burton and Riley[1] or for treatment by the recommended sorption technique with subsequent digestion of the precipitate. The inorganic orthophosphate content of the filtrate was estimated by a routine acid molybdate–stannous chloride technique[2] and found to be 26 μgm. phosphate-phosphorus/l. Therefore more than half the phosphorus in the filtrate was organic.

The technique has the advantage that considerable concentration of low-phosphate water can be obtained, since the precipitate will dissolve in as little as 5 ml. of dilute acid. Digestion is simple and rapid, for volumes to be evaporated are small and there is no decrepitation. Reagent blanks are low and there is no 'salt error'. The technique might also be useful for the preliminary isolation of organic phosphorus compounds in sea-water prior to further examination.

Recovery of phosphorus is low and erratic when the precipitation is carried out in wide or shallow vessels, when the precipitate is recovered by filtration or when the precipitate is agitated while in contact with the supernatant. Similarly, some phosphorus remains in the supernatant if the sample is centrifuged too soon. A minimum of 4 hr. standing is required for the transition from the initially formed unstable colloidal solution of aluminium oxyhydroxide through the α-hydroxide to the insoluble but stable γ-hydroxide. Time of standing is not critical after this initial 4-hr. period.

B. NEWELL

Commonwealth Scientific and Industrial Research Organization,
Division of Fisheries and Oceanography,
Cronulla, New South Wales.

[1] Burton, J. D., and Riley, J. P., *Mikrochim. Acta*, **9**, 1350 (1956).
[2] *C.S.I.R.O. Australian Oceanogr. Stn. List*, **47** (1961).

BIOPHYSICS

Protein in the Nucleus Pulposus of the Intervertebral Disk

THE nucleus pulposus of the normal human inter vertebral disk consists of collagen fibrils dispersed in a gel of polysaccharide and protein complexes. During ageing collagen fibrils are deposited as a matrix showing increasing orientation with age as seen from X-ray diffraction investigations[1]. In the case of specimens of more than 70 years of age, the normal photograph shows with the diagram of oriented collagen a clearly defined ring of spacing 4·7 Å., which is similar to that associated with a denatured β-protein[1] The presence of other components in the disk, for example, calcium phosphate, etc., will be discussed in a later publication. It was postulated from consideration of earlier work[1] that the soluble protein molecules normally found in a folded form in the nucleus had unfolded and formed a hydrogen-bonded lattice which gave rise to this spacing.

From this it was thought possible that in certain cases the typical denatured β-protein ring might be found in younger specimens; in consequence an extensive survey was made of disks from all age groups. The results of this survey were based entirely on X-ray diffraction observations (using copper K_α radiation at 40 kVp. and 40 m.amp.). The specimens were freeze-dried and exposures were of only 0·5 hr. duration in order that the swollen matrix could be preserved. About four samples from each nucleus were examined. The results are summarized in Table 1.

Table 1

Age range (years)	Without β-protein reflexion	With β-protein reflexion	Percentage with β-protein
0–15	15	0	0
16–30	13	3	19
31–45	8	1	11
46–60	2	5	71
61–75	11	15	58
76 and over	0	4	100

Ten per cent below 45 years of age showed the β-protein reflexion, while 65 per cent above 45 years of age showed the β-protein reflexion. About 20 per cent between 16 and 45 years showed the β-protein reflexion; this is of some significance since the age range 0–15 years included a large number of very young disks (less than 3 weeks old). In general the reflexion when it appears in the photograph of the younger disks is weaker and more diffuse, but in aged specimens is sharp and strong. There is a high coefficient of correlation 0·92 between the appearance of the 4·7 Å. reflexion and the age of the disk, which suggests that the occurrence of the β-protein ring may be taken as an indication of what has been referred to by other writers[2] as "pathological ageing".

Further preliminary investigations[3] indicate that the incidence of the β-protein reflexion in the nuclei pulposi of prolapsed disks is higher than in normal disks. If further investigations, which are being carried out, confirm this then the prolapsed disks can be regarded as having undergone some form of "pathological ageing". It might be possible, therefore, that the younger disks of Table 1 showing the β-protein ring would have been more susceptible to prolapse than those which did not. On the other hand, the presence of the β-protein phase may be consistent with a greater degree of fibrosis in the nucleus, and if this were so then the disk may be less susceptible to prolapse than one with a lower degree of fibrosis. If, however, some of the increased β-content of the nucleus is associated with the process of prolapse and is not solely inherent in the nucleus before this occurs then it could be that the physical disturbance caused by prolapse may initiate a spontaneous transformation of some of the soluble nuclear protein to an insoluble β-form; a condition somewhat similar to that obtaining in the transformation of soluble to insoluble protein in denaturation (for example, soluble → insoluble silk fibroin).

P. R. BLAKEY
F. HAPPEY

Department of Textile Industries,
Bradford Institute of Technology,
Bradford, 7.

A. NAYLOR
R. L. TURNER

Bradford Royal Infirmary.

[1] Naylor, A., Happey, F., and MacRae, T. P., *Brit. Med. J.*, ii, 570 (1954).
[2] Mitchell, P. E. G., Henry, N. G. C., and Billewicz, W. Z., *J. Bone Joint Surg.*, **43**, B, 141 (1961).
[3] Naylor, A., 1962 Arris and Gale Lecture (Royal College of Surgeons, London, January 1962).