Isolation of Nucleic Acids from Gelatine

Evidence has accumulated in recent years that the retarding action exerted by some gelatines in the photographic emulsion system is associated with the presence of small amounts of nucleic acids or their breakdown products.

Steigmann first suggested[1,2], on the basis of the reaction of *p*-dimethylaminobenzaldehyde with gelatine, that pyrimidines and hence nucleic acids were present. However, this reagent is far from specific. The pioneer work in this field is that of Pouradier and Venet[3], who applied several different tests to gelatine. They concluded from their results that gelatines contain 0·3–2 mg/g of RNA + DNA. Wood[4] and Gordon and Swann[5,6] published papers almost simultaneously on relatively specific methods of determining adenine in gelatine (the latter authors also detected guanine). These contributions have not settled the question in what form the nucleic acid fragments occur in the gelatine. Pouradier and Venet believed, since they observed good agreement between phosphorus and ribose or deoxyribose contents, that nucleic acids were present as such. Gordon and Swann were of the opinion that the phosphate is likely to be removed during gelatine manufacture and hence that the impurities of interest were likely to be present as nucleosides. Wood did not express an opinion. Both these latter authors, however, used hydrolytic procedures and hence detected adenine as such. In the present communication we wish to report work establishing that a high proportion of the adenine is present as high molecular weight, relatively undegraded nucleic acid.

We have been interested in studying the distribution of impurities in gelatine effected by various techniques of fractionation. The method mainly used so far is the alcohol coacervation procedure described by Stainsby[7]. For the nucleic acid work a particular gelatine was chosen. This was 'PG 334' (British Glues and Chemicals), a normal alkali-processed hide gelatine. The initial adenine content, as determined by Gordon and Swann's[5] polarographic technique, was quite high—161 p.p.m. The phosphorus content was determined after nitric–perchloric acid oxidation by King's method[8]; the original gelatine contained 292 p.p.m. These two determinations were done on most of the fractions as an index of the kind of fractionation that had occurred. When the conditions of fractionation were adjusted so that about 15 per cent of the gelatine was precipitated in the first fraction, the adenine content of this fraction (designated N1) was 417 p.p.m. and that of all subsequent fractions (four in all) remained fairly constant at about 20 p.p.m. The phosphorus content increased also in the first fraction but to a slightly less extent. Thus, almost 40 per cent of the adenine-containing impurity appeared in the first fraction. This suggested that the impurity might, like the gelatine itself, have a molecular weight distribution, that a proportion of it was relatively high molecular weight (or insoluble) and that it did not seem to be protein-bound.

Conditions were next adjusted to give a very narrow-cut first fraction—about 3 per cent by weight (designated N6). This was found to contain 3,330 p.p.m. adenine and 5,400 p.p.m. phosphorus. Ribose and deoxyribose were also present. In this case, 62 per cent of the adenine content of the gelatine has been concentrated into this narrow-cut fraction. Further concentration was effected by trypsin digestion of this fraction followed by precipitation with ethanol. In this way we have isolated about 200 mg of a fraction containing 6·3 per cent adenine and 7·5 per cent phosphorus. This fraction (designated N18) contained virtually all the adenine content of the N6 from which it was made, but only 70 per cent of the phosphorus. It is believed to be largely a mixture of DNA and RNA. It gives viscous solutions and is readily precipitated by acid. It is probably in the form of a calcium salt and contains 11·1 per cent moisture. From our

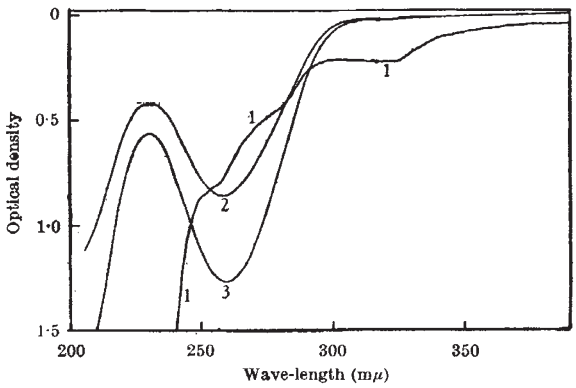

Fig. 1. Ultra-violet absorption spectra in *p*H 7 buffer. Curve 1, gelatin, 5 g/l.; Curve 2, fraction N18, 46·8 mg/l.; Curve 3, DNA (herring sperm), 45·3 mg/l.

results, we conclude that the material as isolated contains at least 90 per cent nucleic acids. The gelatine content, via hydroxyproline[9], was 1·6 per cent. The ribose content was consistent with about 12 per cent RNA, the rest being largely DNA. Ion exchange[10] and paper[11] chromatography established the presence of thymine and cytosine (the first time these pyrimidines have been positively identified in gelatine), but uracil proved more difficult to detect. The identity of the bases was confirmed by their ultra-violet spectra.

Gel filtration on 'Sephadex'[12,13] in phosphate buffer, *p*H 7, gave a single sharp slightly asymmetrical peak, indicating a molecular weight of the order of 400,000. Ultra-violet spectra of the original gelatine, of fraction N18 and of an authentic sample of DNA are given in Fig. 1. The curves show clearly the similarity of the last two and the enormous difference in optical density between N18 and its source gelatine.

Further work is planned, and this, together with photographic assessment, will be described in fuller detail elsewhere. Thanks are due to the Directors of Minnesota 3M Research, Ltd., for permission to publish this communication.

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Peptide Chains of Tropomyosin

This communication reports the determination of the molecular weight of rabbit tropomyosin by equilibrium sedimentation in a solvent consisting of 8 M urea, 0·2 M sodium chloride, 0·025 M sodium dihydrogen phosphate adjusted to *p*H 7·0, and 0·1 M β-mercaptoethanol. Columns 1 mm high were used and the speed was adjusted so that the weight-average molecular weight could be calculated from the refractive index gradient at the mid-point of the column[1,2]. The results are given in Fig. 1 as a plot of $1/M_{app}$ versus concentration, expressed as Δn, the refractive index increment. For the molecular weight