LETTERS TO THE EDITORS

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Non-antigenicity of Gelatin

INTEREST in the inability of gelatin to act as a full antigen, that is, to produce specific anti-bodies when injected into an animal, has been revived by the observations of Haurowitz, Tunca and Schwerin[1], and also by the suggested use of isinglass for blood transfusion[2]. Haurowitz *et al.* find that whereas the intravenous injection of arsanil-azo-globulin into rabbits leads to the deposition of the bulk of the arsenic in the liver and bone-marrow[3], the injection of arsanil-azo-gelatin is followed by rapid excretion of the arsenic in the urine with very little deposition in the liver. The conclusions reached by these authors is that arsanil-azo-gelatin does not act as a full antigen because it is insufficiently deposited in the reticulo-endothelial cells of the body.

The non-antigenicity of gelatin may, however, be due to more than one factor, and at least four possible explanations have been or can be offered for the *in vivo* immunological inertness of this protein (cf. Wormall[4]), namely: (1) gelatin is relatively deficient in aromatic groupings; (2) it is deficient in carbohydrate groupings; (3) it may be rapidly excreted in the urine after intravenous injection into an animal; (4) it is usually prepared from collagen by prolonged treatment with boiling water or steam, and this treatment may well be sufficient to destroy any antigenic power of the preparation.

The introduction of aromatic groupings, including tyrosine, into gelatin does not produce a compound which has antigenic powers comparable with those of the majority of other proteins[5]. Thus, although some of these conjugated gelatin derivatives produce antibodies which react with the corresponding conjugates prepared from globulin and certain other proteins, these antibodies react very feebly or not at all with the gelatin derivatives; the results suggest that the non-antigenicity of gelatin is not due solely to lack of aromatic groupings. Similarly, the failure does not appear to be due solely to lack of tyrosine plus carbohydrate[6].

The third explanation given above receives strong support from the observations of Haurowitz and his colleagues, and it seems possible that the major part of injected gelatin or gelatin derivative is so rapidly excreted in the urine that little remains in the body to incite antibody formation. Gelatin and several other proteins with molecular weights less than 70,000 are rapidly excreted in the urine by anæsthetized cats and rabbits and by isolated perfused kidneys of dogs[7]. On the other hand, proteins such as egg albumin and the Bence-Jones protein, both of which are excreted by the kidney, are known to be fully antigenic; thus loss by excretion in the urine is probably not the only factor which determines the non-antigenicity of gelatin.

The fourth possible explanation can be excluded if it is shown conclusively that native collagen is non-antigenic, but unequivocal proof of this does not appear to be available. Glue is devoid of antigenic activity[8], and so is isinglass, "which is a collagen rather than a gelatin"[2]. Apart from these observations, however, it appears that little attention has been given to the parent protein as distinct from the partially hydrolysed product, gelatin. It would seem desirable that a more complete immunological study should be made of the antigenic properties of the natural unhydrolized collagens. Such an investigation might certainly help to throw some light on the vexed problem of the antigenicity of proteins.

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[1] Haurowitz, F., Tunca, M., and Schwerin, P., *Biochem. J.*, **37**, 249 (1943).

[2] See Taylor, N. B., and Moorhouse, M. S., with Stonyer, A. J., *Canad. Med. Assoc. J.*, **49**, 251 (1943). Pugsley, H. E., and Farquharson, R. F., *Canad. Med. Assoc. J.*, **49**, 262 (1943). Cited from *Bull. War Med.*, **4**, 464 and 465 (1944).

[3] Haurowitz, F., and Kraus, F., *Hoppe-Seyl. Z.*, **239**, 76 (1936). Haurowitz, F., *Hoppe-Seyl. Z.*, **245**, 23 (1937).

[4] Wormall, A., *St. Bartholomew's Hosp. Reports*, **70**, 199 (1937).

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[6] Clutton, R. F., Harington, C. R., and Yuill, M. E., *loc. cit.*

[7] Bayliss, L. E., Kerridge, P. M. T., and Russell, D. S., *J. Physiol.*, **77**, 386 (1933).

[8] Ramsdell, S. G., and Walzer M., *J. Immunol.*, **14**, 207 (1927).

Capacity of Hyaluronidase to Increase the Fertilizing Power of Sperm

THE capacity of hyaluronidase to liquefy the highly viscous gel which cements the cumulus cells around the unfertilized tubal egg of the rat was described by McClean and Rowlands[1], and confirmed in the mouse by Fekete and Duran-Reynals[2] shortly afterwards. We suggested that the gel is hyaluronic acid similar to that in synovial fluid and, to enable its disintegration to occur as a preliminary to fertilization, a certain unspecified concentration of hyaluronidase must be established by the sperm in the vicinity of the egg. The fact that intromission of such a large number of sperm is necessary to ensure fertilization, therefore, may well be related not only to the safe passage of one or more sperm into the Fallopian tube but also, and possibly more especially, to the establishment of this requisite concentration of enzyme. Some preliminary experiments, which are described below, have now been carried out in rabbits to investigate the capacity of hyaluronidase to increase the fertilizing power of dilute sperm suspensions.

Ovulation was induced in Dutch and Himalayan rabbits by intravenous injection of 50 I.U. of chorionic gonadotrophin. A mixed sample of semen from 8–12 rabbits was collected in an artificial vagina and its sperm-count estimated using a Zeiss hæmocytometer. The semen was then diluted in Baker's[3] solution so as to contain 2×10^7 sperm per c.c., and from this were then prepared (1) further dilutions to give suspensions of sperm varying between 2×10^6 to 2×10^4 per c.c., and (2) a hyaluronidase-containing filtrate of the sperm, the latter having been inactivated by heating at 50° C. for 5 min. and separated by vigorous centrifugation. This sperm-free filtrate was then mixed in equal proportions with the various sperm suspensions and 2 c.c. of the mixtures inseminated artificially into each rabbit at approximately 7 hr. after the injection of gonadotrophin, that is, 4 hr. before the expected time of ovulation. Sperm counts were again made wherever practicable on the inseminates. The amount of hyaluronidase in the semen, and when possible also in the inseminates, was estimated by its capacity to prevent the appear-