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Isolation of Poly α-L-Glutamic Acid from the Oviduct of the Domestic Fowl and a Possible Role in Maintenance of Fertility

IT has been known for a considerable time[1] that avian sperm, while in the oviduct, is capable of survival and of fertilizing ova for periods of up to 3 weeks in the domestic fowl and for longer periods in the turkey. *In vitro*, its fertilizing capacity is lost within a few hours. Examination of the oviduct of the domestic fowl after insemination has shown that sperm are congregated at two points, the infundibulum and the utero-vaginal junction[2,3], the sperm being found in invaginations known as "crypts" or "sperm nests"[4], where they are apparently also protected from the action of spermicidal agents[5].

Though the mechanism of storage remains unclear, it must nevertheless include both protective and immobilizing factors. Early observations[6] had suggested that extracts or secretions from the infundibulum were capable of reversibly arresting the motility of avian sperm. Thus at 41° C (the body temperature of the fowl) motility was arrested, and was regained on cooling to 37° C. Such extracts were presumed to contain an immobilizing factor, and attempts were made to isolate materials from the oviduct capable of immobilizing sperm at 41° C *in vitro* for considerable periods of time and yet permit the resumption of motility on cooling to 37° C.

Extracts of the infundibulum and upper magnum were made in 0·13 molar sodium phosphate at *p*H 7·2 and, after centrifuging, were fractionated on columns of carboxymethyl cellulose to yield a material which was extremely effective in arresting motility for several hours at 41° C. The material was purified by way of the silver salt and led finally to the isolation of the sodium salt of poly α-L-glutamic acid. The molecular weight was 73,600 as determined by end group analysis and 81,500 as determined by viscosity. The isolated material gave a positive biuret reaction and on acid hydrolysis yielded glutamic acid only. The material was exclusively in the L-form, as was shown by quantitative decarboxylation with L-glutamic decarboxylase. The polymer was hydrolysed by papain at *p*H 5·0 but not at *p*H 8·0. When further fractionated on columns of DEAE cellulose[7] a single peak was obtained which appeared close to the region at which authentic poly α-L-glutamate with a molecular weight of 90,000 was eluted. Antisera to the isolated material prepared in rabbits by a recent technique[10] cross-reacted with authentic specimens of poly-α-L-glutamate (molecular weight 2,500–90,000) but did not react with specimens of poly-γ-glutamate prepared from culture filtrates of *Bacillus licheniformis*[8]. These properties are taken as establishing that the isolated material is poly α-L-glutamic acid.

The polyglutamate is not uniformly distributed throughout the oviduct, but is found only at the utero-vaginal junction and in the infundibulum and upper magnum. In an average oviduct the quantities isolated represented 0·4 mg and 3·4 mg for these regions respectively. No polyglutamate was isolated from the vagina, the shell gland, the isthmus or the lower magnum. Thus the material appears to be present only in those regions in which sperm are stored. That it is an intrinsic component of the oviduct was shown by isolating it from the oviduct of virgin hens. The material is not therefore introduced by the cock semen. Purified poly α-L-glutamate tested against cock semen arrested motility for 7·5 h at 41° C, although at least half the original motility was regained at cooling to 37° C. This compares with a period of 1–1·5 h for a similar phenomenon with raw semen alone. This effect appears to be specific for poly α-L-glutamate, and no effect is obtained with poly D,L-γ-glutamates, poly α-L-aspartic acid, phosvitin, polygalacturonic acid, polyglucuronic acid, pectins, chondroitin sulphate, various mucopolysaccharides, dextrans of different molecular weights, cerebral gangliosides, RNA and DNA, and heparin. Poly bases such as poly-L-lysine and calf thymus histones were immediately toxic. Furthermore, the effect depends partly on the molecular weight of the polyglutamate, is non-existent with material of molecular weight 2,500, and becomes increasingly marked up to molecular weights of 90,000, the highest molecular weight tested.

Although well known as a synthetic chemical[9], poly α-L-glutamic acid has not apparently previously been isolated from a natural source. Its location in the oviduct of the domestic fowl and its apparent specificity of action as judged by its effects on motility strongly suggest that such material plays a part in the preservation of the viability of sperm *in vivo*. This or similar substances are probably widely distributed in the reproductive organs of other species, where they may play a similar part in fertility, and the distribution of polyglutamic acid and evaluation of its possible role are being investigated.

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Biological Half-life of Mouse Transferrins

MOUSE transferrins, like those of the rat[1], are represented in serum by two prominent β-globulins which bind iron and have slightly different electrophoretic mobilities on starch gel[2]. A mixture of these transferrins can readily be isolated[2] from serum by ion exchange chromatography on DEAE 'Sephadex'. These transferrin preparations contain about 10 per cent 7*S* γ-globulin as a contaminant[2].