tative manner, would induce an accelerated graft rejection.

Two groups of animals were used in the experiment; a pilot group of 4, and a subsequent panel of 14. In the first group, there was a uniform and rapid rejection of all grafts by day 8, however, in the larger group, such a rapid rejection was only found in 1 animal. It is clear that these results will require further investigation in order to reconcile these findings. However, several factors are important to consider in such experiments: first is that the number of spermatozoa deposited in the vagina of a regularly mating animal could be extremely high, since it has been estimated that in the rat, a single ejaculation contains in the order of 60×10^6 spermatozoa. Further, there may have been considerable variation in the actual mating patterns of the mice, which was not detected in the experimental design. Another consideration may be that if there were small numbers of accompanying cells, the uterine route may be a more effective route for immunization than the intraperitoneal one.

Discussion. The results indicated that using the intraperitoneal route, the immunogenicity of epididymal spermatozoa is questionable, and certainly cannot be considered comparable to that of lymphocytes, where the Y antigen is considered. Further, because of the problem of contaminating cells, a different source of spermatozoa, possibly cells obtained from ejaculates should yield more definitive information. Whether there is any difference between epididymal spermatozoa, and those in ejaculates is another question, which is currently under investigation.

Zusammenfassung. Nachweis, dass jungfräuliche weibliche C5 7BL/6 Mäuse weder beschleunigte noch verzögerte Ablehnung von syngeneischer männlicher Hautübertragung nach einer einzigen i.p. Einspritzung von 1 bis 8 Millionen syngeneischen Spermien zeigen.

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Phylogenesis of the Azurophil Leucocyte Granules in Vertebrates

Stressing the difficulties of homologizing, by the usual light microscopic methods, the leucocytes in lower vertebrates, HOLMES[1] states: 'It may well be that the internal ultrastructure of leucocyte granules will provide a basis for a more widely generalized homology than is possible with the Romanowsky dyes.' In a previous study[2] of the leucocyte granules of lower vertebrates, however, we had difficulty in classifying, even by electron microscopy, the leucocytes seen in teleost renal tissue. Similarly, conflicting opinions can be found in the work of ANDREW[3] and that of FEY[4]. Nevertheless, when we extended our studies to other fishes, we observed a granule type whose undifferentiated form is seemingly common to all vertebrates and analogous with the azurophil or primary granules of neutrophil leucocytes of higher vertebrates including mammals.

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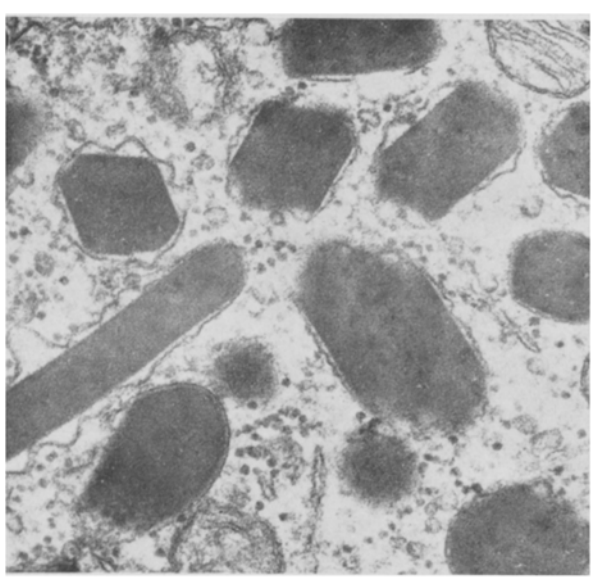

Fig. 1. Neutrophil granulocyte, spleen, *Scyliorrhinus canicula*. The mature granules consist of inclusions which are polygonal or square, others are more elongated. The inclusions develop from fibrillar-tubular elements of the immature granules. Approx. ×40,000.

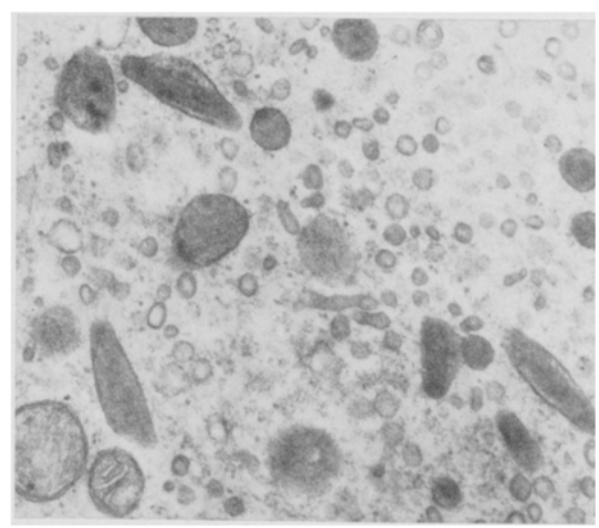

Fig. 2. Neutrophil leucocyte, spleen, *Acipenser ruthenus*. Granules with fibrillar-tubular elements; in some more mature granules they are closely packed, indicating a tendency to form inclusions. Approx. ×31,000.

Material and method. Blood and haematopoietic tissues (spleen, kidney) of the following fishes were studied: Elasmobranchiata, Selachidea: *Scyliorrhinus canicula*, Chondrostein, Ganoidei: *Acipenser ruthenus*, Teleostei, Anguilliformes: *Anguilla anguilla*, Clupeiformes: *Esox lucius*, Cypriniformes: *Abramis brama, Ctenopharyngodon idella, Hypophthalmychtis molitrix, Scardinius erythrophthalamus*, Cyprinodontiformes: *Xyphophorus helleri*, Perciformes: *Lucioperca lucioperca, Pterpohyllum scalare, Lepomis gibbosus* and *Gobius niger*. The leucocyte granules were examined with the usual light microscopic methods and with electron microscopy of osmium tetroxide-, or glutaraldehyde-osmium tetroxide-fixed material.

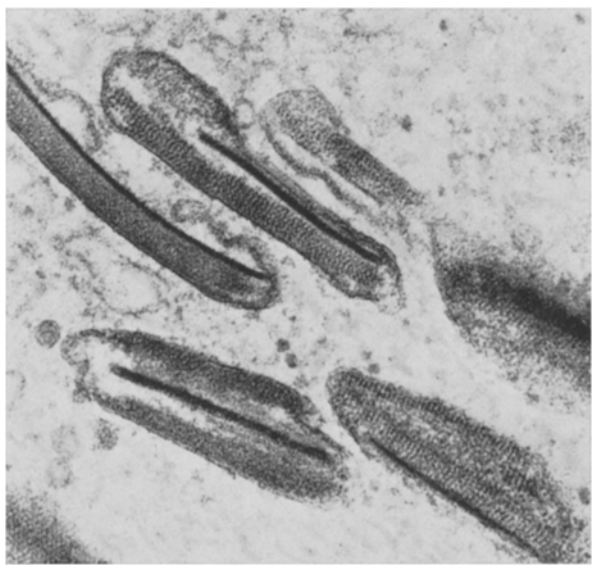

Fig. 3. Figures 3–5 neutrophil leucocytes, kidney. Mature granules of *Tinca tinca* with thin electron dense inclusions. The granule matrix shows a periodicity perpendicular to the inclusions. Approx. ×45,000.

Result. Besides the eosinophil leucocytes characterized by round, large and usually homogeneously electron dense granules in all fishes studied, the other leucocyte type, believed to represent mature neutrophils, contained granules of quite a different appearance in *Scyliorrhinus canicula* (Figure 1), in *Acipenser ruthenus* (Figure 2), *Esox lucius* and in the *Cypriniformes* (Figure 3), in *Anguilla anguilla* and the Perciformes (Figure 4), and in *Xyphophorus helleri* (Figure 5). When, however, immature cells were studied, it became evident that all these granules derived from the same granule form dominantly present in the mature leucocytes of *Anguilla anguilla* and the Perciformes, i.e. an elongated granule containing fibrillar-tubular structures of about 80–100 Å in diameter. With the condensation of these structures and, presumably by incorporating other material, the granules of the different fishes may develop inclusions (crystalloids) of different sizes and shapes. In some species transitory forms of granules with fibrillar-tubular elements and inclusions may be seen in 1 granule (Figures 2 and 5).

Discussion. In addition to the specific (secondary) ones, granules very similar in appearance to those seen in fishes, have been described, in mammalian neutrophil leucocytes[5–9]. These granules may differ in size, shape, electron density and may contain inclusions of various appearances. That may be one of the reasons why 2 to 5 granule types have been described in the mature neutrophil leucocyte of mammals[5,6,10,11]. The Auer bodies seen in immature myeloid or monocytomyeloid cells and believed to originate form azurophil granules[12–14] are very similar to those seen in immature fish neutrophil leucocytes.

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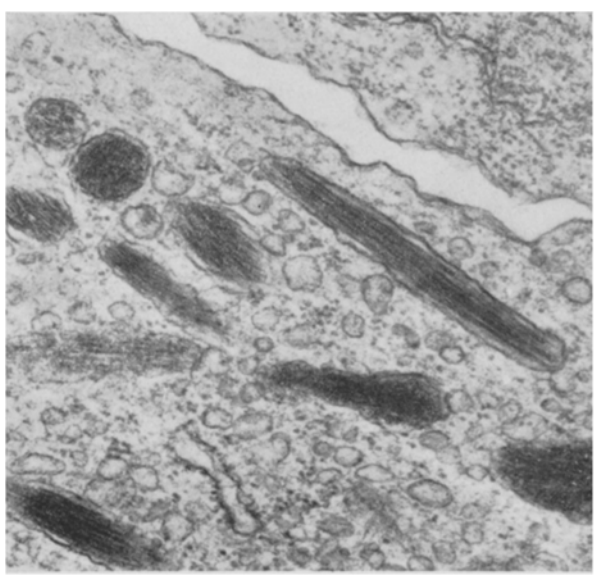

Fig. 4. Mature granules of *Anguilla anguilla* with fibrillar-tubular structures about 80–100 Å in diameter. Approx. ×38,000.

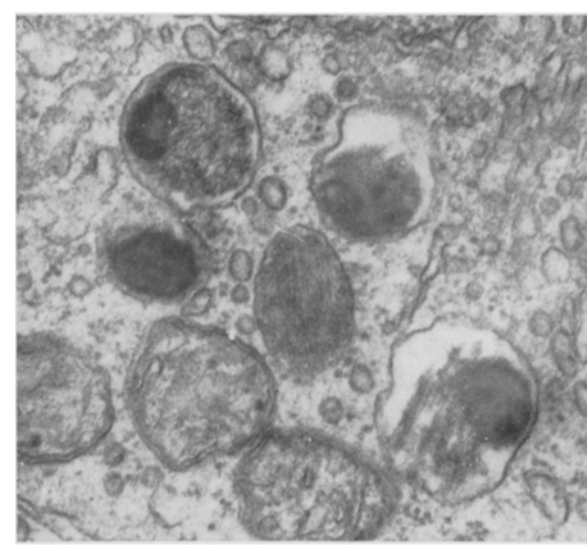

Fig. 5. Immature and mature granules of *Xylophorus helleri*, some only with fibrillar-tubular structures and others also with inclusions. Approx. ×52,000.

Thus we suggest that a common type of neutrophil leucocyte granule may be present in all vertebrates. It might have been on the basis of a certain similarity between the granules of mammalian monocytes and the neutrophil leucocytes of teleosts that the latter were named azurophil leucocytes by PAPPENHEIM[15]. Since the cells of fishes homologous with the neutrophil leucocytes of mammals dominantly contain granules corresponding to the primary or azurophil granules, the term azurophil leucocytes applied to these cells seems to be logical.

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Action de deux polybases sur la charge et l'agrégation des plaquettes[1]

Différents agents sont susceptibles d'induire l'agrégation plaquettaire aussi bien in vivo qu'in vitro. Mais les mécanismes de cette agrégation restent mal connus. Parmi les hypothèses possibles, il faut envisager des modifications physico-chimiques de la surface plaquettaire et, en particulier, les variations éventuelles de la charge électrocinétique. Une telle hypothèse a d'ailleurs été proposée et vérifiée partiellement[2–5].

Le but de ce travail est de vérifier la validité de la corrélation charge-agrégation à l'aide de deux polybases, le DEAE Dextran (diethyl-aminoéthyldextran hydrochlorure) de poids moléculaire 2×10^6 et le polybrène 1,5 dimethyl-1,5 diazaundecamethylene polymethobromide.

Matériel et méthode. La mobilité électrophorétique des plaquettes a été mesurée à l'aide d'un appareil d'électrophorèse en phase liquide, décrit par ailleurs et appelé cytosphéromètre[6,7]. La valeur moyenne de la mobilité est le résultat du comptage de 50 cellules par mesure et les résultats sont exprimés sous forme de mobilité relative par rapport à la mobilité en plasma autologue.

En ce qui concerne l'agrégation des plaquettes, celle-ci a été réalisée photométriquement par enregistrement de la déflection d'un galvanomètre. Les suspensions de mesure contenant de 150000 à 250000 éléments ont été obtenues par centrifugation lente d'un sang prélevé sur citrate depuis moins de 2 h. Toutes les expériences ont été réalisées à 25 °C.

Résultats. A) Action sur la mobilité électrophorétique. Nous avons cherché, en premier lieu, à préciser si ces produits avaient une action sur la mobilité en fonction du temps. C'est pourquoi nous avons étudié, à dose fixe, les variations éventuelles de mobilité pour des temps d'incubation allant de 2 min à 2 h. Nous n'avons trouvé aucune variation.

Pour ces concentrations allant de 10^{-3} à 1 mg/l de DEAE-Dextran ou de polybrène, nous avons tracé (Figure 1) la variation de mobilité relative. On constate alors une diminution continue de la charge avec des doses croissantes de deux polybases.

B) Etude de l'agrégation des plaquettes. L'agrégation des plaquettes est observée dans la cellule d'électrophorèse lorsque la mobilité électrophorétique est réduite de 15 à 20% environ. Nous avons également étudié le phénomène, photométriquement, pour des doses identiques à celles utilisées précédemment.

Les résultats (Figures 2, 3 et 4) montrent pour les deux substances une action parallèle. On observe en effet une absence d'agrégation aux concentrations faibles (10^{-4} mg/l); puis aux doses plus importantes l'agrégation s'effectue progressivement. Elle est totale à la concentration de

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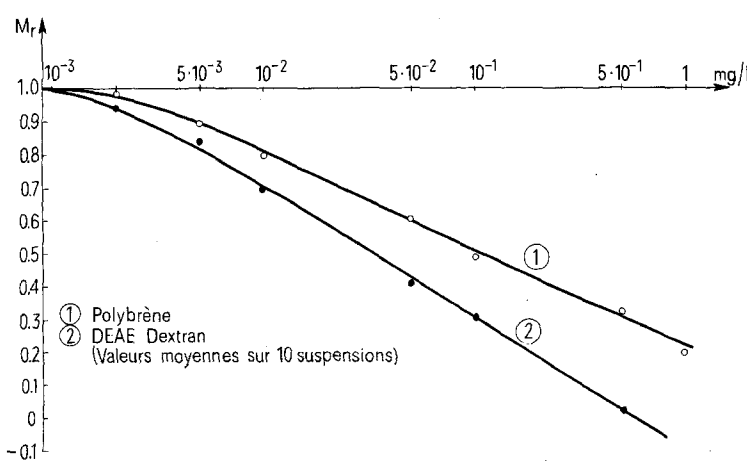

Fig. 1. Action de 2 polybases sur la mobilité electrophorétique relative des plaquettes humaines.