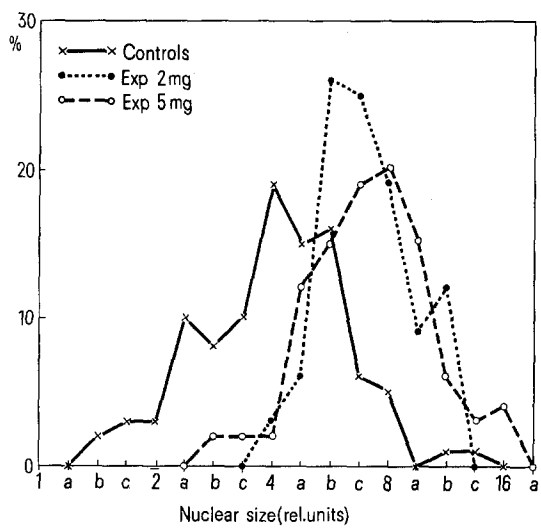
The mean nuclear volumes of these maxima are in the ratio of 1:2:4:4 etc. Deviations from this rule were observed when the normal function of the cells changed. BENNINGHOFF[7] found, in instances of increased cell function, an increase of the nuclear volume, and vice-versa a decrease of the nuclear volume when the cell function decreased ('Functional swelling and shrinking'). JERUSALEM[8] pointed out that an increase of the nuclear volume can be a symptom of a cellular lesion e.g. in the kidneys after administration of alloxan, tryphan blue and in instances of lower nephron nephrosis ('dystrophic' swelling). In both functional and dystrophic changes, the increase and decrease of the nuclear volume is gradual and does not appear stepwise and particularly not in doubling intervals. Moreover, there was no increase in the number of existing 'maxima'.

According to JERUSALEM and ZAKI[9] the occurrence of new cell populations with different nuclear sizes, which manifests in new 'maxima', is a characteristic sign for tissues showing a high rate of cell proliferation. They further added that the nuclei synthetize DNA which does not result in a gradual but stepwise increase of the nuclear volume. They also observed that, on the other hand, the high rate of mitosis leads to a quick reduction of the nuclear volume when the cell has finished the DNA reduplication phase.



Percentage of nuclear size distribution of mesenchymal cells of the periodontal ligament of 28-day-old control and lathyritic rats.

From the distribution of the nuclear sizes in the experimental animals, it can be concluded that the proliferation tendency of the mesenchymal cells is distinctly lower than in normals because the number of 'maxima' was reduced and the mean nuclear volume of these 'maxima' was in the ratio of about 4:8:16. The accumulation of large sized nuclei points to the assumption that the ability of the mesenchymal cells to enter into mitosis and thus reduce the nuclear volume, might have been delayed. However, it is not a conclusive supposition that all large sized cells are of tetra- and octoploid type.

According LEUCHTENBERGER and SCHRADER[10] the nuclear volume can increase even in doubling intervals though a reduplication of DNA fails to appear. It is also impossible to exclude with any degree of certainty regarding a toxic nuclear oedema though it is found only in stages of acute intoxification (JERUSALEM[8]). Lathyritic agents may also cause lasting alteration of membrane functions e.g. of ion transport and thus causing a 'dystrofic' nuclear swelling. All the above mentioned deviations from the normal characteristics point to the fact that in the experimental animals both the normal mitotic cycle and the function of the mesenchymal cells was distinctly altered.

Zusammenfassung. Es wurden biometrisch mesenchymale Zellen des Paradontiums als Parameter der Zellproliferation und -funktion untersucht. Durch Verfütterung von Lathyrogen odoratus wurde ein Lathyrismus erzeugt mit Veränderungen der Membranfunktion und des Ionenaustausches. Die Kerne der Versuchstiere waren wesentlich grösser als bei den Kontrollen (Dosisabhängigkeit).

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18 January 1972.

[7] A. BENNINGHOFF, Anat. Ber. *1*, 50 (1949).
[8] C. JERUSALEM, Anat. Anz. *111*, 141 (1962).
[9] C. JERUSALEM and F. G. ZAKI, Anat. Forsch. *38*, 161 (1958).
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[11] Acknowledgments. I wish to thank Prof. A. J. VAN AMERONGEN and Prof. C. JERUSALEM for consistent help and excellent suggestions.
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H-Y Antigens on Mouse Spermatozoa

Using epididymal spermatozoa injected i.p. as a source of Y antigen, KATSH et al.[1] reported that they were able to accelerate or delay the rejection of syngeneic male skin in the C57BL/6 mouse. In addition, it was possible to induce tolerance to such grafts with the appropriate doses of spermatozoa and time interval before grafting. Further, the authors concluded from their data that spermatozoa were a richer source of Y antigen than spleen cells. Because of current interest in histocompatibility antigens and their relation to cell development and differentiation[2,3], the expression of the Y antigen on spermatozoa would appear to provide an extremely interesting example to study, particularly since it should be possible to study any changes in Y antigen expressivity following fertilization, and the subsequent incorporation into a diploid genome. Thus, as a first step in such an investigation, it was considered important to repeat

[1] G. F. KATSH, D. W. TALMAGE and S. KATSH, Science *143*, 41 (1964).
[2] H. E. GOLDBERG, T. AOKI, E. A. BOYSE and D. BENNETT, Nature, Lond. *228*, 570 (1970).
[3] J. PALM, S. HEYNER and R. L. BRINSTER, J. exp. Med. *33*, 1282 (1971).

the work of KATSH et al.[1], and to confirm the findings. Because it was not possible to confirm the results described previously, this report describes my findings, together with some further data.

Materials and methods. Epididymal spermatozoa were obtained as described by KATSH et al.[1]. Washed epididymal spermatozoa, suspended in 1 ml of Hanks BSS as a standard dose were injected before applying the skin grafts. Controls consisted of untreated virgin adult C57BL/6 female mice obtained from a domestically maintained inbred colony which were grafted with syngeneic male skin, using the method described by BILLINGHAM[4]. The grafts were inspected visually to determine the survival end point. Lymphocyte suspensions were obtained by teasing the lymph nodes and suspending the washed cells in Hanks BSS. A standard volume of 1 ml was used.

Results. The results are summarized in Table I. It is clear that I did not find the same results as previously reported, and in order to try and produce graft survivals that would differ significantly from the controls, the dose of spermatozoa was increased up to 16 and 20 million spermatozoa respectively, injected i.p. 14 days before grafting male skin onto female recipients. The results are shown in Table II. The increased doses of spermatozoa resulted not in the production of tolerance, as would have been predicted from the results of KATSH et al., but instead, a rapid rejection of the graft ensued.

One criticism that comes to mind with experiments of this nature is that the results obtained might be due not to the numbers of spermatozoa present, but rather be attributable to the number of other, accompanying cells. Accordingly, cell counts were made on standard suspensions of epididymal spermatozoa. The types of cell found in the suspensions of spermatozoa include the ciliated columnar cells of the epididymal epithelium, a few wandering cells of connective tissue epithelium and some cells of the peripheral blood, introduced during the surgical procedures involved in extirpating the epididymis and collecting the spermatozoa. When differential cell counts were made on such cell suspensions, it was found that the maximal number of contaminating nucleated cells is of the order of 1 to 5%. Of these, approximately 10% are contaminating leucocytes estimated by differential counts as a proportion of red blood cells present, and since these are the important immunogenic contaminating cells, one can estimate that as many as 20 to 100,000 'passenger' cells could have been introduced into the experiments involving 20 million spermatozoa, and may have produced the acclerated graft rejection.

Accordingly, experiments to test the sensitizing ability of syngeneic male lymphocytes were carried out. Doses ranging between 1×10^3 and 1×10^6 were used. The female recipient animals were test grafted 14 days after the injection of lymphocytes, and the survival end point of the grafts determined as before. The results are summarized in Table II, and show beyond any doubt that such cells are a potent source of Y antigen.

In a further attempt to test for expression of Y antigen on the surface of spermatozoa, it was decided to use tubally ligated virgin female C57BL/6 mice and to test the longevity of syngeneic male skin grafts, after the female mice had been in residence with males for periods up to 6 months. The presence of a vaginal plug was taken as evidence of mating, and after finding such a plug, the females were assumed to be mating regularly. The rationale behind these experiments was to minimize contamination by immunogenic cells other than spermatozoa. In this way, it was hoped to show whether or not exposure to spermatozoa, even though in an unquanti-

[4] R. E. BILLINGHAM, in *Transplantation of Tissues and Cells* (Eds. R. E. BILLINGHAM and W. K. SILVERS; The Wistar Institute Press, Philadelphia 1961).

Table I. Survival times of syngeneic male skin on virgin female C57BL/6 recipients after i.p. injection of epididymal sperm

Sperm dose ($\times10^6$ cells)	Interval before grafting (days)	No. of recipients	Distribution of graft survival times 10	12–20	21–29	30–38	39–60	MST (days)
0	0	24	24	22	15	5	0	27.8±3.0
1	5, 14 or 21[a]	31	31	31	28	10	0	31 ±2.8
4	5 or 14	9	9	9	5	1	0	26.8±2.6
8	5 or 14	15	15	15	8	2	0	24.6±2.9
16	14	30	30	10	5	4	0	14.4±3.1
20	14	9	9	4	2	0	0	14.8±3.7

[a] There was no statistical difference between groups grafted at 5, 14 or 21 days.

Table II. Survival times of syngeneic male skin on virgin female C57BL/6 recipients, after i.p. injection of male lymphocytes

Lymphocyte dose ($\times10^3$ cells)	Interval before grafting (days)	No. of recipients	Distribution of graft survival times 8	9–15	16–22	23–29	30–39	40	MST (days)
0	0	24	24	24	22	15	1	0	27.6±2.4
10	14	8	10	8	2	0	0	0	16.8±2.6
25	14	8	8	2	0	0	0	0	10.4±2.7
50	14	10	10	7	1	0	0	0	15.9±2.7
100	14	12	12	9	4	0	0	0	16.2±3.0
500	14	11	11	4	2	0	0	0	10.8±3.1
1000	14	10	10	7	4	0	0	0	19 ±2.5

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.

[1] W. HOLMES, in *Functions of the Blood* (Eds. R. C. MCFARLANE and A. H. T. ROB-SMITH; Blackwell Scientific Publications, Oxford 1961), p. 271.
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[3] W. ANDREW, *Comparative Hematology* (Grune and Stratton, New York, London 1965).
[4] F. FEY, Fol. haemat. *86*, 1 (1966).

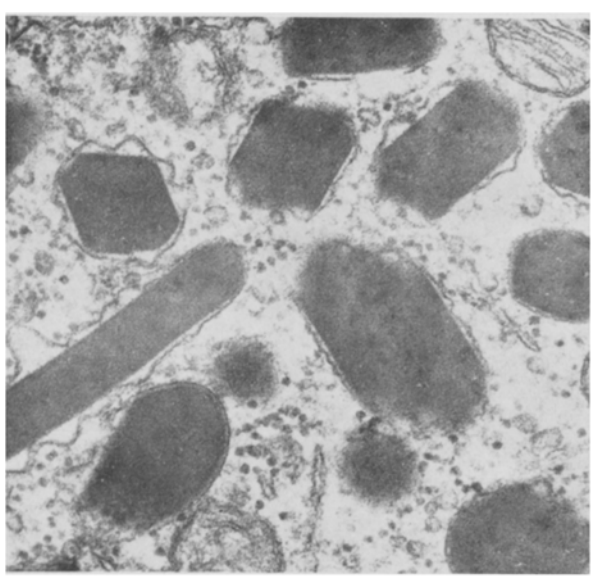

Fig. 1. Neutrophil granulocyte, spleen, *Scyliorrhinus canicula*. The mature granules consist of inclusions which are polygonal or square, others are more elongated. The inclusions develop from fibrillar-tubular elements of the immature granules. Approx. ×40,000.

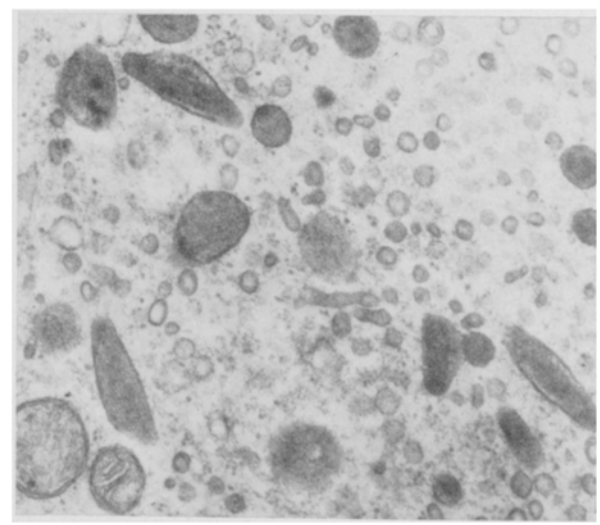

Fig. 2. Neutrophil leucocyte, spleen, *Acipenser ruthenus*. Granules with fibrillar-tubular elements; in some more mature granules they are closely packed, indicating a tendency to form inclusions. Approx. ×31,000.