scribed the development of *H. pallida* and illustrated the fertilized egg with only one membrane; he made no reference to a change in the membrane upon fertilization.

Experimental. After dissecting the gonads from an individual, the eggs were pressed from the oviducts into a Syracuse dish where they were washed with one or more changes of sea water. Aliquots of eggs were pipetted to five other Syracuse dishes, each containing 10 ml of sea water. A number of eggs were left in the original dish as a control against accidental cross-insemination. The seminal fluid was drawn from the testes by puncturing them with glass capillaries. The sperm was diluted just prior to use with 1 ml of sea water.

To examine the membrane in relation to self- and cross-fertilization, eight groups (five ascidians per group) were inseminated according to MORGAN'S 5 × 5 test procedure[11]. The eggs from each animal were tested with their own sperm and with the sperm of each of the other four animals in the group. The data from these eight groups were then pooled (Figure 3) to show the correlation between membrane elevation and cross- and self-fertilization.

One dish of cross-sterile eggs was found, but it was omitted from Figure 3, as it contained only 28 eggs.

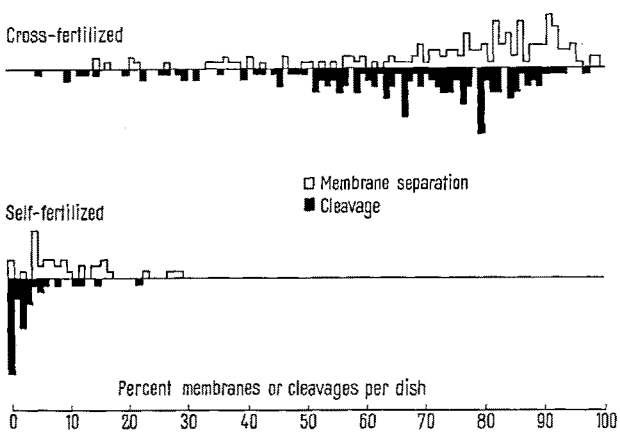

Fig. 3. Pooled data from eight 5 × 5 tests showing cross- and self-fertilization in relation to the percent membrane separation and to the percent eggs cleaved (2 to 4 blastomeres). Each unit of the histogram represents one dish, containing eggs with the indicated percent cleavage or membrane separation. The values are given to the nearest whole percent with values between zero and one shown as 1%. All samples shown had more than 30 eggs and averaged 77 eggs.

However, the appearance of the membranes was no different from that of the self-sterile eggs, as would be expected if the block to fertilization occurs at the chorion in both self- and cross-sterility.

In no case was an egg fertilized (as indicated by cleavage) without showing membrane separation, but membrane separation without cleavage did occur; it was found in an average of 10.3% of the cross-inseminated eggs and 6.8% of the self-inseminated eggs. Following MORGAN[3], who suggested that self-fertilizing sperm may arise by mutations in the sperm tract, one might postulate the occurrence of *incomplete* fertility mutations. These could result in sperm deficient in some factor affecting specificity, such that chorion activation was permitted but nuclear activation was not. However, such a hypothesis does not explain the 3.5% greater ($P < 0.025$) membrane separation without cleavage in the cross- than in the self-inseminated eggs.

The close correlation between membrane separation and cleavage is evidence for a close connection between chorion activation and fertilization; the failure of the egg membrane to elevate in cases of self-sterility correlates with the existence of a block. Care must be taken, however, in interpreting this as evidence for a chorion-located block. While the present findings are in agreement with those of earlier investigators, no time sequence study has been made of membrane separation and cortical response in *H. momus* to exclude the possibility that the observed membrane separation may be a consequence of an antecedent reaction at the egg cortex or elsewhere[12].

Zusammenfassung. Im Ei der Ascidie *Herdmania momus* hebt sich nach der Besamung die Befruchtungsmembran von der äusseren Haut ab. Die Korrelationen, die bestehen zwischen Membranabhebung und Befruchtung, sowie ihrem Unterbleiben und der Sterilität, stützen wiederum die Chorionblocktheorie der Autosterilität.

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Department of Zoology, University of California, Los Angeles (Calif., U.S.A.), August 9, 1962.

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[12] The author wishes to acknowledge the encouragement of the faculty of the University of Hawaii under whose auspices this investigation was made.

Fractionation of the Liver Extract on Sephadex G25

Dextran gels are used for desalting of macromolecular materials[1,2] as well as separation of proteins, peptides and amino acids[3,4] and nucleic acids and nucleotides[5]. Because of the mild conditions during gel filtration and the quantitative separation of low and high molecular compounds, this method is suitable for the isolation of low molecular materials from tissues. This study describes the separation of an extract of rat liver by gel filtration on Sephadex G25. 40 ml of rat liver extract (105.000 g; 60 min) was fractionated on a column of Sephadex G25 (3 × 95 cm) in a buffer (0.01 *M* NH_4HCO_3, pH 8.6) at a speed of 50 ml per hour and at a temperature of 3°C. The fractions were analysed by measuring the absorption at 260 mμ and 280 mμ (Figure a), by conductometric determination of salts, by Bial's reaction for sugars and by a modified method for phosphorus assays[6] (Figure b). The ninhydrin reaction was performed according to MOORE and STEIN[7] (Figure c).

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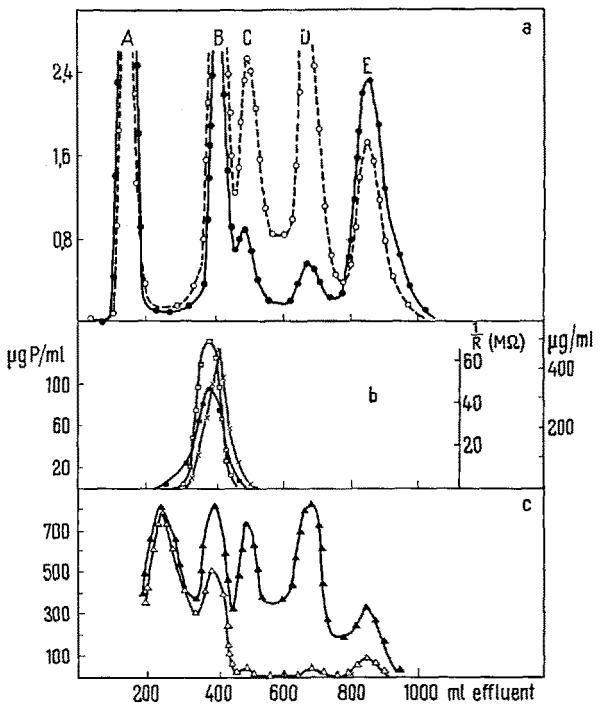
Fraction A contains high molecular materials, mainly proteins and s-RNA. The other fractions (B, C, D, E) are of low molecular nature; all pass through a dialysation membrane. The chemical analysis and the maximum of the UV-absorption of these fractions are shown in the Table. Fraction B includes substances containing sugars, aminosugars, sugar phosphates and some of the amino acids and low peptides. Amino acids also eluate between the peak A and B. Materials containing sugar are eluted from the column in the ascending part of peak B. The fractions C, D, E contain mainly peptides, nucleotides and nucleopeptides.



Elution diagram of rat liver extract on the column Sephadex G25. o——o O.D. at 260 mµ; •——• O.D. at 280 mµ; x——x conductivity; □——□ carbohydrate (Bial) as µg/ml; ■——■ phosphorus µg/ml; △——△ ninhydrin colour; ▲——▲ ninhydrin colour after hydrolysis expressed as µg of leucin/ml.

Analysis of low molecular fractions of rat liver

Fraction		B	C	D	E
Ninhydrin colour[a]		157.5	14.9	42.5	117.0
Ninhydrin colour after hydrolysis		202.5	390.0	960.0	860.0
Phosphorus	%	12.45	0.184	0.914	0.38
Carbohydrate	%	33.7	0.768	0.557	0.526
Hexosamine after hydrolysis	%	0.586	—	—	—
Maximum UV-absorption	mµ	265	260	255	275

[a] Ninhydrin colour expressed as µg of leucin/mg

All low molecular fractions are heterogeneous in the electric field. Lyophilized fractions were separated by preparative electrophoresis on paper in 0.1 *M* ammonium acetate buffer pH 5.9. All chromatographic fractions contain compounds with UV-absorption and are ninhydrin positive[8].

Zusammenfassung. Es werden einige niedrigmolekulare Stoffe aus Rattenleberextrakt nach der Sephadex G25-Fraktionierung beschrieben. Die Lokalisierung von Zukker, Aminosäuren, Peptiden, Nukleopeptiden und verwandter Stoffe wurde im Elutionsdiagramm vorgenommen.

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[8] The author wishes to thank Miss E. BENKOVIČOVÁ for technical assistance.

Spontaneous Leukaemia in a Sprague-Dawley Rat

Spontaneous leukaemia in rats is remarkably rare. Few reports are available since 1936 when WILENS and SPROUL observed the first cases[1–6]. A spontaneous myeloid chloroleukaemia is described here since (1) no incidence has been reported yet in Sprague–Dawley rats, and (2) the disease was accompanied by some intriguing renal tubular changes. The nature of these changes and their relation to the leukaemic process is not clear.

The rat was a 13-month untreated female of the above mentioned strain.

Hematology: WBC, 77000; RBC, 5.2 millions; Hgb, 13.3 g. The peripheral blood smear (Wright's stain) showed evidence of leukaemic invasion by immature cells of the granulocyte series (Figure 1). Two types were prominent: (1) large cells with large rounded nuclei and very little basophilic cytoplasm containing a small amount of azurophil granulation, and (2) large cells with rounded or polygonal nuclei and a greater amount of polychromatophil or acidophil cytoplasm containing numerous neutrophil granules. The differential count revealed that 70% of the white blood cells were Type 1 (promyelocyte) and Type 2 (myelocyte) cells with a number of atypical forms.

Significant autopsy findings included: *spleen*—enlarged to ten times normal size, solid, greyish coloured; *lymph nodes*—markedly enlarged, characteristic light green colour; and femoral *bone marrow*—similar green colour.

Microscopically the cellular infiltration was most conspicuous in the R.E. organs. The liver showed an extreme degree of infiltration of the periportal, perivascular spaces and sinusoids by immature myeloid elements together with considerable liver cell damage.

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