Conclusions. Dans les races C57B1, CBA, BALB/C et RF nous avions obtenu[2], après 400 R, 7,5% des cellules présentant des réarrangements chromosomiques. Avec 6% de translocations, les spermatogonies des souris de race AKR/Tr. semblent donc manifester une radiosensibilité assez voisine. S'il est impossible de déterminer avec exactitude la part prise dans ces remaniements par les chromosomes métacentriques, on peut cependant remarquer que les bivalents métacentriques manquent dans 21 cellules, sans qu'il soit possible de conclure si les 2 chromosomes métacentriques ont donné 4 acrocentriques ou s'ils interviennent dans les autres configurations observées dans ces cellules[4].

Summary. A spontaneous translocated strain of mice (AKR/Tr.) with 36 acrocentric and 2 metacentric chromosomes received 400 R of whole-body X-irradiation. Cytological examination of dividing primary spermatocytes at the diakinesis-first metaphase stage of meiosis showed 6% of cells with chromosomal rearrangements.

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Histotopochemistry of Ascorbic Acid during the Formation of Carrageenin Granuloma

It has been proved in many biochemical experiments that ascorbic acid plays an important part not only in preserving collagen but especially in the biosynthesis of collagen in a newly-formed connective tissue. The question of topographic connection of ascorbic acid to the individual components of the connective tissue, however, is not yet quite clear. Thus, for example. ČMUCHALOVÁ and CHVAPIL[1] have found out that there exists a close correlation between the amount of ascorbic acid and the newly-formed collagen during the formation of carrageenin granuloma. They have not found, however, any connection between the number of cells (DNA) and the content of ascorbic acid in the granulation tissue, as proved earlier by WOESSNER and BOUCEK[2] for a granuloma formed around an implanted small polyvinyl sponge.

In the present work an attempt at histotopochemistry of ascorbic acid during the formation of carrageenin granuloma has been made.

Method. In guinea-pigs (males weighing 250–300 g) carrageenin granuloma in the abdominal region was provoked by a subcutaneous injection of 5 ml 1% carrageenin solution in 0.9% NaCl to which penicillin (100 IU/ml) and streptomycin (100 μg/ml) were added. The tissue was withdrawn 2, 4, 7, 9 and 13 days after the injection of carrageenin solution. Very small samples of the granuloma tissue (ca. $2 \cdot 2 \cdot 2$ mm) were treated after GIROUD and LEBLOND (LIPP[3]). The samples were imbedded in paraffin and cut into 5 μ thick slices. Some of the slices were stained with hematoxylin-eosin.

Results. The tissue beneath the skin of the guinea-pig is very edematous 2–4 days after the application of carrageenin; isolated erythrocytes, polynuclear leucocytes and cells of histiocytic character can be observed. The intercellular substance shows fine granules, in some places the original collagen fibres of loose connective tissue are preserved. No black granules of reduced silver can be seen, which signifies that ascorbic acid is not present in the tissues, or, if present, its level is below the critical amount that can be proved by this method (Figure 1).

In preparations of a 7-day-old granuloma we can observe numerous very large cells of fibroblastic character. Their cytoplasm contains small granules of silver. Such granules can also be observed in the close vicinity of fibroblast cytoplasm, in the fibrous collagen structure. Sometimes it is impossible to determine exactly the boundary line between the spindle-shaped cytoplasm and the fibrous structure.

In preparations of 9- and 13-day-old carrageenin granuloma we can also observe numerous spindle-shaped elongated fibroblasts whose cytoplasm contains numerous silver granules, indicating the presence of ascorbic acid. Such granules are also visible in the undulated outline of projections of fibroblasts which gradually change into a fibrous structure (Figure 2).

Discussion and conclusions. Histochemical evidence of ascorbic acid during the formation of carrageenin granuloma practically corresponds to biochemical findings.

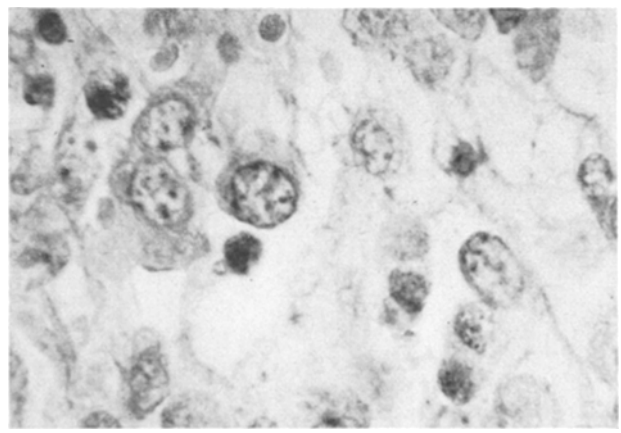

Fig. 1. Carrageenin granuloma 4 days after s.c. injection of carrageenin. The level of ascorbic acid is very low, no metallic granules of reduced silver have formed.

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This means that a considerable increase in the amount of ascorbic acid can only be proved when the formation of collagen starts. Conversely, ascorbic acid has not been demonstrated histochemically in the early stages of development of carrageenin granuloma, although this does not mean that it has not been present at all. Some small error may be due to insufficient sensitivity of the technique. Only with an accumulation of fibroblasts has the level of ascorbic acid considerably increased.

The findings have confirmed not only our earlier observation (BARTOŠ[4]) but also the observation of KASABJAN[5] showing that ascorbic acid is found mainly in the cytoplasm of fibroblasts and/or epitheloid cells. Examination of slices under an optic microscope has also revealed that ascorbic acid is present in the peripheral portions of fibroblasts which, in some places, show a gradual transition to the fibrous structures of collagen.

The findings lead to the conclusion that during the formation of new connective tissue ascorbic acid is dependent on the presence of fibroblasts and/or on the young newly-formed fibrous structure in the closest vicinity of these cells. If we accept the opinion that ascorbic acid takes part in the formation of hydroxyproline or hydroxylysine, we may believe that it is in the fibroblasts that this process takes its course most intensively.

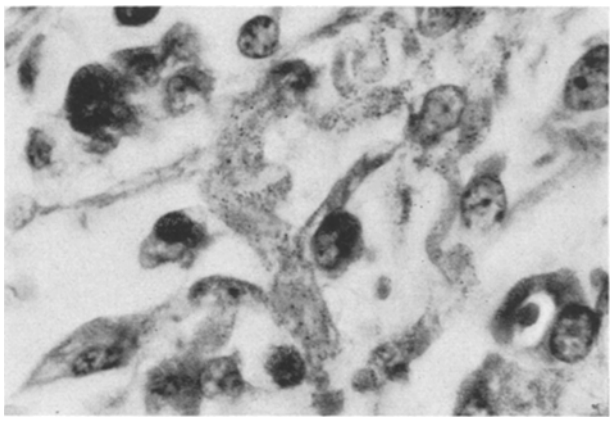

Fig. 2. Carrageenin granuloma 9 days after s.c. injection of carrageenin. The black granules in the cytoplasm of the cells or in the collagenous structure which is being formed are not granules of ascorbic acid but granules of reduced silver.

Zusammenfassung. Es wird die Histotopochemie der Askorbinsäure im Laufe der Karagenin-Granulombildung untersucht. Die Askorbinsäure ist an die Fibroblasten, eventuell an die junge Faserstruktur gebunden, die sich in einer unmittelbaren Nähe des Fibroblasten-Cytoplasmas befindet. Es wird angenommen, dass sich die Askorbinsäure an der Hydroxylation von Prolin oder Lysin beteiligt, und dass dieser Prozess am intensivsten in den Fibroblastenzellen vor sich geht.

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[6] Acknowledgment: I should like to thank Ing. B. ČMUCHALOVÁ of the laboratories of Dr. M. CHVAPIL for supplying the experimental material.

Isolation of Bacteriophages from the Bovine Rumen

Although rumen microorganisms have been the object of intensive study because of their role in rumen physiology[1,2], the isolation of bacteriophages from rumen sources seems not to have been described. It is the purpose of this communication to point out that bacteriophages are prevalent in the rumina of cattle.

While investigating the survival of bacteria supposedly not native to the rumen[3], bacteriophages active against several *Serratia* spp. indigenous to the rumen were isolated. Almost every sample of rumen fluid examined contained active bacteriophages. Host range studies[4] of these phages showed that rumen *Serratia* spp. were not susceptible to phage isolated from soil, water and sewage. The phages isolated from the rumen would not lyse a number of *Serratia* spp. from a variety of other sources.

Whether the *Serratia* spp. and phages were indigenous to the rumen or came from exogenous sources (drinking water, soil or feed) could not be ascertained. The maximum number of *Serratia* spp. found in the drinking water was 20 viable cells/ml, and feed and soil were negative. Survival curves, obtained when *Serratia* spp. and phages were introduced into the rumen or inoculated into nutrient broth, indicated that the phages adsorbed to the cells, but no increase in plaque-forming units occurred at 39 °C. This inhibition of replication by incubation at elevated temperatures was probably similar to that described by POLLARD and WOODYATT[5], which might indicate that neither the *Serratia* spp. nor phages were normal rumen inhabitants. An alternative explanation is that the phages active against *Serratia* spp. were propagated in the rumen by infecting bacteria closely related to *Serratia* spp. (see reference [6]). Nevertheless, these findings stimulated a search for phages of bacteria more commonly associated with rumen populations.

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