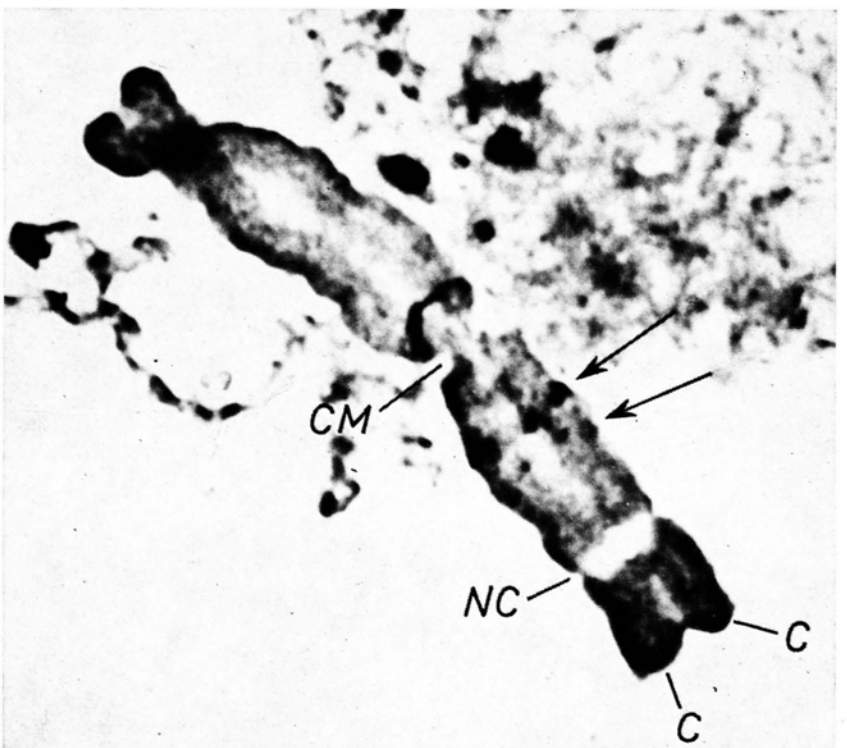

Fig. 1. Isolated M chromosome of *Vicia faba* treated with trypsin and stained with Giemsa. The arrows mark two *longitudinally* subdivided bands. In the sister-chromatid this "interrupted banding" is less clearly visible. In the non-nucleolar arm the interruption of the band is lacking completely, obviously because here the digestion was not sufficiently strong. C. chromatid; CM, centromere; NC, nucleolar constriction. Size of the chromosome: 40 μm.

Banding in Trypsin-treated Chromosomes of *Vicia faba*

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Banding, chromosome structure, Giemsa, quinacrine, trypsin-digestion

The existence of specific banding patterns allows the identification of individual human chromosomes and helps to elucidate the molecular organization of the chromosome. These patterns are obtained by staining the chromosomes either with fluorochromes or – after pretreatment in specific ways – with Giemsa. We wondered whether pretreatment with trypsin would alter the bands observed in chromosomes of the broad bean *Vicia faba*. This question seems legitimate because digestion with trypsin reveals a sub-division of the *Vicia faba* chromatid into two length structures [1, 2].

Chromosomes of *Vicia faba* were treated with trypsin and then stained with quinacrine, quinacrine mustard and Giemsa. In the Giemsa series the trypsin-treatment had the additional purpose to induce the bands [3, 4]. If in *Vicia faba* the bands are produced by staining with fluorochromes (without trypsin-pretreatment) [5–8], their appearance does not reflect the existence of length structures. Photographs of Giemsa stained *Vicia faba* chromosomes do not seem to have been published so far although studies of that kind (without trypsin-pretreatment) have been carried out [9].

Seeds of the broad been *Vicia faba* (var. Suttons' Prolific Longpod) were grown and treated with colchicine as in former experiments [10]. Metaphase chromosomes of cells of primary root tips were isolated as described elsewhere [11]. To 1 ml of the phosphate buffered (0.066 M, pH 7.0) chromosome suspension, 0.5 ml of a trypsin [12] solution (0.35 mg trypsin per ml 0.066 M neutral phosphate buffer) were added. Then a drop of the chromosome suspension was given on a slide. The interval between the addition of trypsin and the dropping on the slide had to be chosen not too short because then no subdivision of the chromatid would be achieved and not too long because then no bands would appear. The optimum interval depended mainly on the activity of the trypsin preparation and was on the average about $2^1/_2$ min at room temperature. The preparations were air dried, mounted in Giemsa solution and sealed with Eukitt. The Giemsa solution was prepared as described in ref. [4]. Staining of air dried trypsin-treated preparations with quinacrine and quinacrine mustard was performed as described elsewhere [6, 8].

In the Giemsa-stained M chromosome (the metacentric chromosome of *Vicia faba*) which we were mainly studying, we found one band in the non-nucleolar arm and two bands in the nucleolar arm. This agrees with the fluorescence results of other authors [5–8]. Furthermore we observed in several cases *a longitudinal interruption of the bands* of the M chromosome (and also of other chromosomes), as shown in Fig. 1 *. In the quinacrine and quinacrine mustard series this longitudinal subdivision was also found, however, the bands were stained less clearly than in the Giemsa experiments, obviously because of the trypsin-treatment. The bearing of these results on current hypothesis of the structure of the eukaryotic chromosome will be discussed elsewhere.

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* Fig. 1 s Table on page 356 a.

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[12] The results described below were obtained not only by using once-crystallized trypsin but also with highly purified, namely TPCK treated trypsin (both from Serva, Heidelberg). Therefore it seems that it is the action of trypsin itself and not that of biochemical impurities which is responsible for the effects observed.