UNIVALENT MECHANISM AND MISDIVISION

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INTRODUCTION

THE present paper is an attempt to explain the mechanism of the univalents as well as its misdivision at meiosis, as observed in monosomic *Triticum*. Several authors have already published various theories on these problems. We therefore think it appropriate to give first a short review of the modern literature concerning centromere structure and chromosome mechanism.

DARLINGTON (1939) introduced the term misdivision to designate the crosswise division of the centromere of univalent chromosomes at meiosis. According to the same author (1947) the centromere is a compound gene whose division one way or another is forced upon it by orientation in the spindle. Others however suppose that the centromere has a double structure both transversal and longitudinal, the evidence in support of this is structural as well as functional.

The double structure of the proximal heterochromatin has been shown by LEVAN (1946) in *Dipcadi* and *Allium*. He ascribed already to this heterochromatin a special function in connection with the work of the centromere (*l. c.* page 458). The double transverse structure of the proximal heterochromatin has been revealed in the experimental work of STEINEGGER and LEVAN (1947) in *Allium*. The experiments of ÖSTERGREN (1947) on *Hyacinthus* and *Allium show* that the two spindle spherules (longitudinal duplication) are in reality double (transversal duplication).

More conclusive are the observations of LIMA-DE-FARIA (1949) on the pachytene chromosomes of *Secale*. From his observations he assumed that the centromere is composed of three zones: 1) an exterior zone charged with the connection with the arms, 2) a chromomeric zone formed by two pairs of centromeric chromomeres to which are ascribed the property of active mobility, 3) an interior zone responsible for the special cycle of division.

A definite evidence of the centromere having four chromomeric bodies has been given by TJIO and LEVAN (1950). Using a pretreatment with 8-oxyquinoline before squashing root meristems, they obtained exceedingly clear pictures of the four chromomeres forming the centromere in somatic chromosomes of several plant species.

That the centromere functionally is also double, has been shown by LEVAN and EMSWELLER (1938). They observed in *Nothoscordum* that the two telocentric chromosomes resulting from misdivision, behave as if they had a normal centromere. Also indirect but more complete is the evidence given by MÜNTZING (1944) who has been able to identify the two isofragments, as being formed from both arms of the standard fragment of *Secale*.

The clearest functional proof is given by DARLINGTON (1940). He observed that both complementary products of misdivision behave as telocentrics or potential isochromosomes in the pollen grains of *Fritillaria*. Another proof is the observations of DARLINGTON and KOLLER (1947) on the possibility of breaking the centromere in its longitudinal fision at prophase by the action of mustard-gas. This breakage is expressed as a precocious separation of the daughter centromeres.

We can therefore accept that the centromere structure is formed by two pairs of chromomeres or half spindle spherules, each chromatid has a pair of chromomeres and each chromomere is able to form a functional centromere. Thus the centromere has the properties of reproduction (lengthwise division) and misdivision (crosswise division).

As concerning the forces responsible for the movements of the chromosomes we consider ÖSTERGRENS interpretation as the most correct. From his surveys (1949 a, b) we quote the following paragraphs:

«The centromeres are attracted to the spindle poles (α-factor). This attraction only acts towards that pole towards which the centromere is directed. The metaphase centromere at mitosis consists essentially of two anaphase centromeres turned in opposite directions.

There is a tendency of the spindle to extrude bodies out of it (γ-factor), acting, *e. g.* on chromosome arms (in the transverse equilibrium) and on nucleoli. The main factor causing this is the surface tension of the spindle tactoid when the surface around the bodies is in connection with the external surface.

The spindle tends to arrange rod-shaped bodies parallel to the spindle fibres (δ-factor).

In some cases there is a considerable elongation of the spindle during anaphase (stem body). This naturally contributes to increasing the distance between the separating chromosomes.

Chromosome parts lying in the cytoplasm are naturally influenced by cytoplasmic currents».

MATERIAL AND METHODS

The material used in this study consisted of a line of heterozygous speltoid wheat of the B-series (2n=6x-1=41) kindly supplied by Agronom J. MAC KEY, Sveriges Utsädesförening, Svalöv, Sweden.

Meiosis in this material has been described before by SANCHEZ-MONGE and MAC KEY (1948). Misdivision of the univalent chromosome was found in 1,7 % of the first telophases and misdivision of the daughter univalent in 16 % of the second telophases. This rather high frequency of misdivision make the B-speltoid wheat very suitable for studying the mechanism of misdivision.

When microtome slides were used, Müntzings modification of Karpechenko was employed as fixing fluid. The spindle contrasting methods of Östergren and Prakken (1946) gave good results as staining procedure. The anther squash method of La Cour (1947) also proved satisfactory.

To my colleague Joe Hin Tjio, head of the Cytogenetic Department, for his warm friendship and invaluable guidance and critizism, I am privileged to express my deep appreciation. I wish to express also my thanks to Miss Pilar Lardiés and Miss Mercedes Gimeno for excellent technical help.

OBSERVATIONS AND DISCUSSION

UNIVALENT MECHANISM

In the following paragraphs the term *insertion* is used to indicate the attachment of the chromosomal spindle components to the centromeric chromomere, and C'' and C' respectively for the univalent chromosome and daughter univalent.

The C'' usually remains outside the plate at MI and forms only occasionally a trivalent (Sanchez-Monge and Mac Key 1948). At AI the C'' moves to the equator and takes a position with its arm perpendicular to the direction of the spindle fibres (Fig. 1 *a*). This movement is very similar to that of the acentric fragments at mitosis in the neuroblasts of *Chortophaga* (Carlson 1938). This suggests strongly that the centromere has no part in the movement of the chromosome towards the equator but that it is caused by the vortical currents of the protoplasm. During cytokinesis the protoplasm passes from the poles to the equatorial plate near the perifery of the cell, inwards at the equator and polewards along the spindle. If during the metaphase the C'' gets a position near the pole the anaphasic group can drag it to the pole and a disjuntion $20_{\text{I}} + 21_{\text{I}}$ is produced (Fig. 1 *b*).

The contraction of the C'' reaches its maximum at the beginning of telophase. While still at the equator its arms start to diverge till it assumes an X shape (Fig. 1 *c*). This may be explained by several hypotheses, for example: by the electrostatic repulsion (Darlington 1937), elastic repulsion (Östergren 1943), cytoplasmic currents (Carlson 1938) and the δ-factor (Östergren 1949 b). It is however more likely that the elastic repulsion is the cause of the divergence and that later the cytoplasmic current and the δ-factor accentuate it. The centromere then divides and the separation of C' starts. At this moment the centromere becomes active and the C' starts moving towards the poles. The action of the centromere shows itself in the gradual change of the position of the arms. At first its concav-

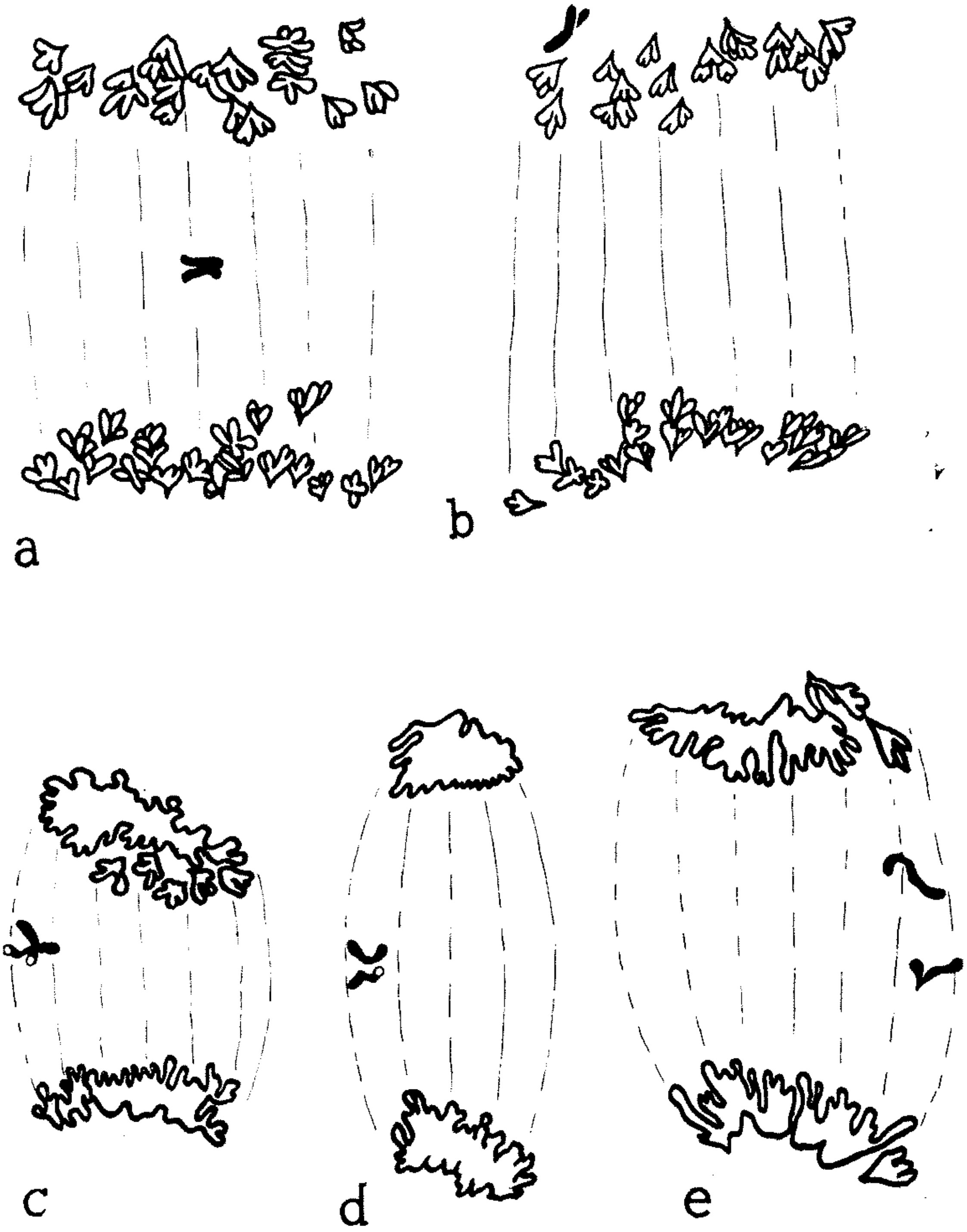

Fig. 1. *a*: first anaphase, and *c* - *e*: first telophase, showing succesive stages of the normal disjunction of the C's. — *b*: the C" pushed by the anaphasic group reaches the pole. — × 1500.

ity is turned towards the pole (Fig. 1 *d*), it then changes gradually its position till it reaches the characteristic shape of the anaphasic chromosomes with its convexity towards the pole (Figs. 1 *e* and 2 *a*). Taking this into account, the structure of the centromere as demonstrated by TJIO and LEVAN (1950) and others and the theories of ÖSTERGREN, it would appear that the centromere is not attached to the traction fibres until the early telophase and that afterwards the C' separate and move to the daughter nuclei. This occurs when the insertion of the fibres in the centromeric chromomeres is normal. If the insertion is abnormal non-disjunction and misdivision take place.

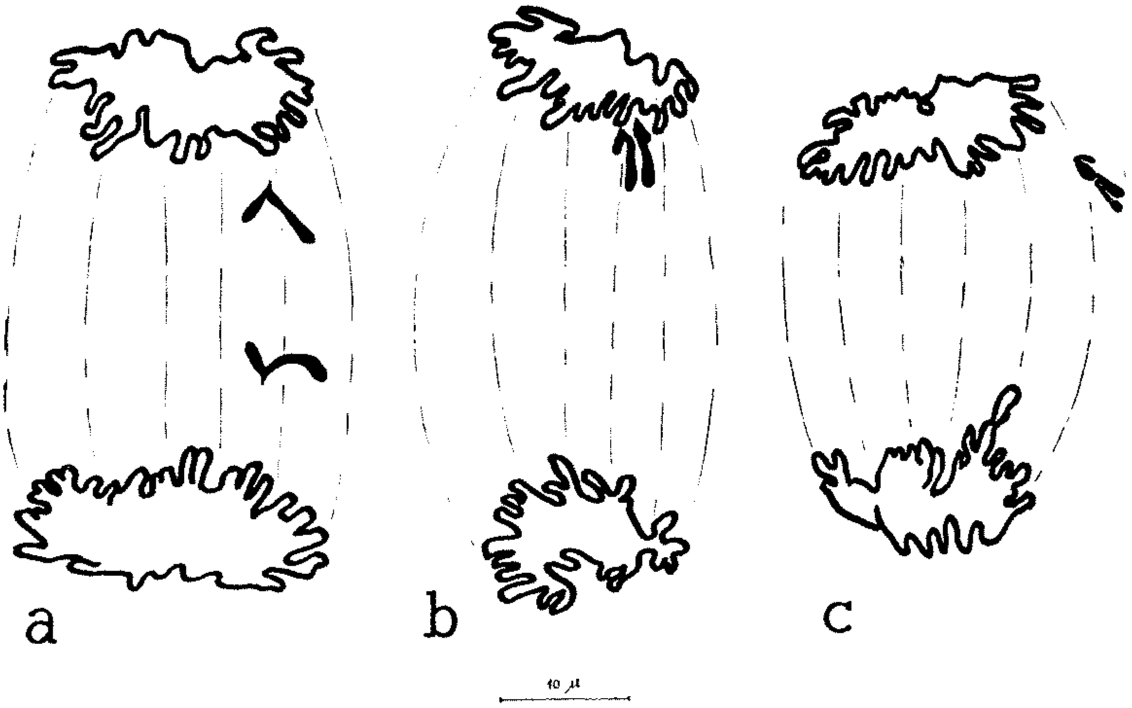

Fig. 2. First telophase. — *a*: normal disjunction. — *b* — *c*: non-disjunction. — × 1400.

NON-DISJUNCTION

Sometimes non-disjunction occurs; both C' move to the same daughter nucleus (Fig. 2 *b* and *c*). In these cases both pairs of centromeric chromomeres are attracted towards the same pole by an erroneous monopolar insertion of their traction fibres.

MISDIVISION

Misdivision of the centromere can be produced by an erroneous insertion of the traction fibres and also by non-reaction of the centromeric chromomeres to the spindle (SANCHEZ-MONGE 1950). When the centromeric chromomeres of one arm are normally inserted in the spindle but those of the other arm lack insertion a double attraction-misdivision or *a-misdivision* (SANCHEZ-MONGE 1950) is produced. It starts with a divergence of the proximal ends of the chromatids of the arm inserted in the spindle .This divergence and the position of the resulting telocentrics demonstrate clearly that there is an attraction exerted by the poles and that it acts on the centromeric chromomeres (Fig. 3 *a*).

The misdivision observed by Winge (1924) in a monosomic *Triticum* is still another kind of *a*-misdivision. It happens when the pairs of centromeric chromomeres belonging to each arm are inserted in traction fibres acting in opposite directions (Fig. 3 *b* and Fig. 5). In this case two' potential isochromosomes are formed (Darlington 1940).

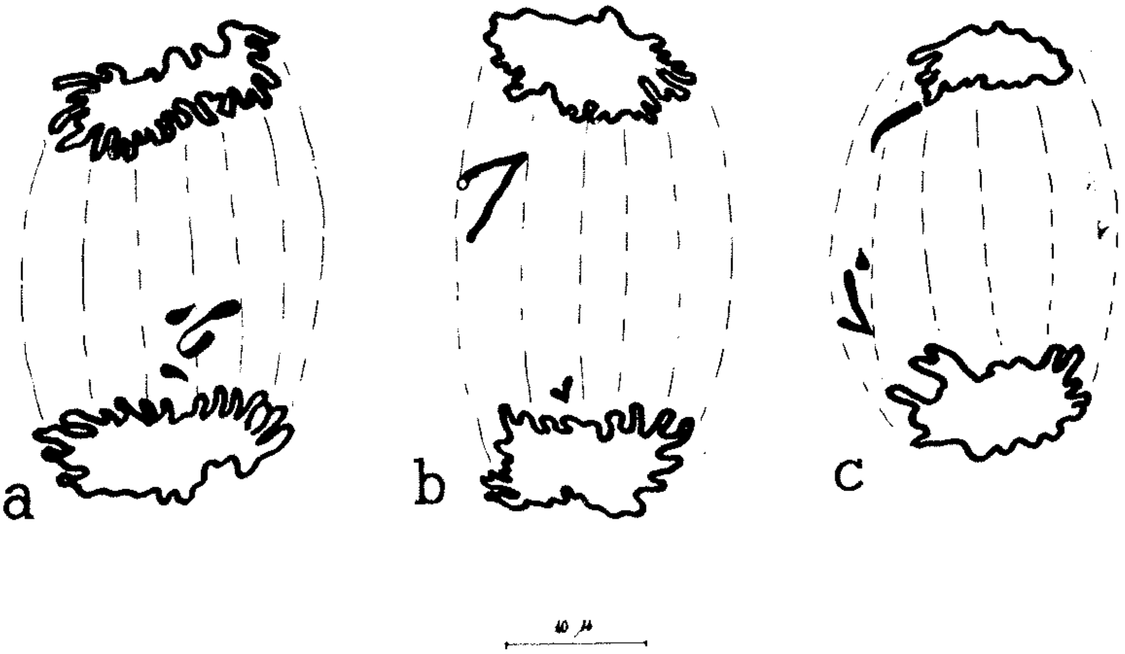

Fig. 3. First telophase. — *a*: double *a*-misdivision. — b: other form of double *a*-misdivision. — c: *p*-misdivision of one of the C's. — × 1500.

The centromeric chromomeres of each sister chromatid react independently to the spindle. This can be seen from Fig. 3 *c* where a C' shows a normal anaphasic movement while the other one has misdivided. Here the cytoplasmic currents (Carlson 1938) and the γ and δ factors of Östergren (1949 b) are the cause. These forces provoke at first the separation of the distal ends of the C' arms which come to lie in a straight line and orientated in the direction of the spindle, then the two arms are pushed away in opposite directions stretching the centromeric region till it breaks and the misdivision is achieved. This type of misdivision is called *p-misdivision* (Sanchez-Monge 1950).

The orientation of the daughter univalent in the direction of the spindle fibres during *p*-misdivision is not the cause of misdivision itself but a stage of it. The mechanism of *p*-misdivision is somewhat like the lobation, fragmentation and transport to the poles of the persistent nucleolus of *Ceiba pentandra* (Tjio 1948).

In 1938, Upcott, illustrating the misdivision in *Tulipa* published pictures which are similar to the two types of misdivision described here. Some of her figures suggest that the forces causing *p*- and *a*-misdivision may act together. The distinction Upcott made between the *a* and *b univalent* types (*l. c.* Fig. 6) is not in agreement with our observations. We suggest that they are just two successive stages in the division of the univalent.

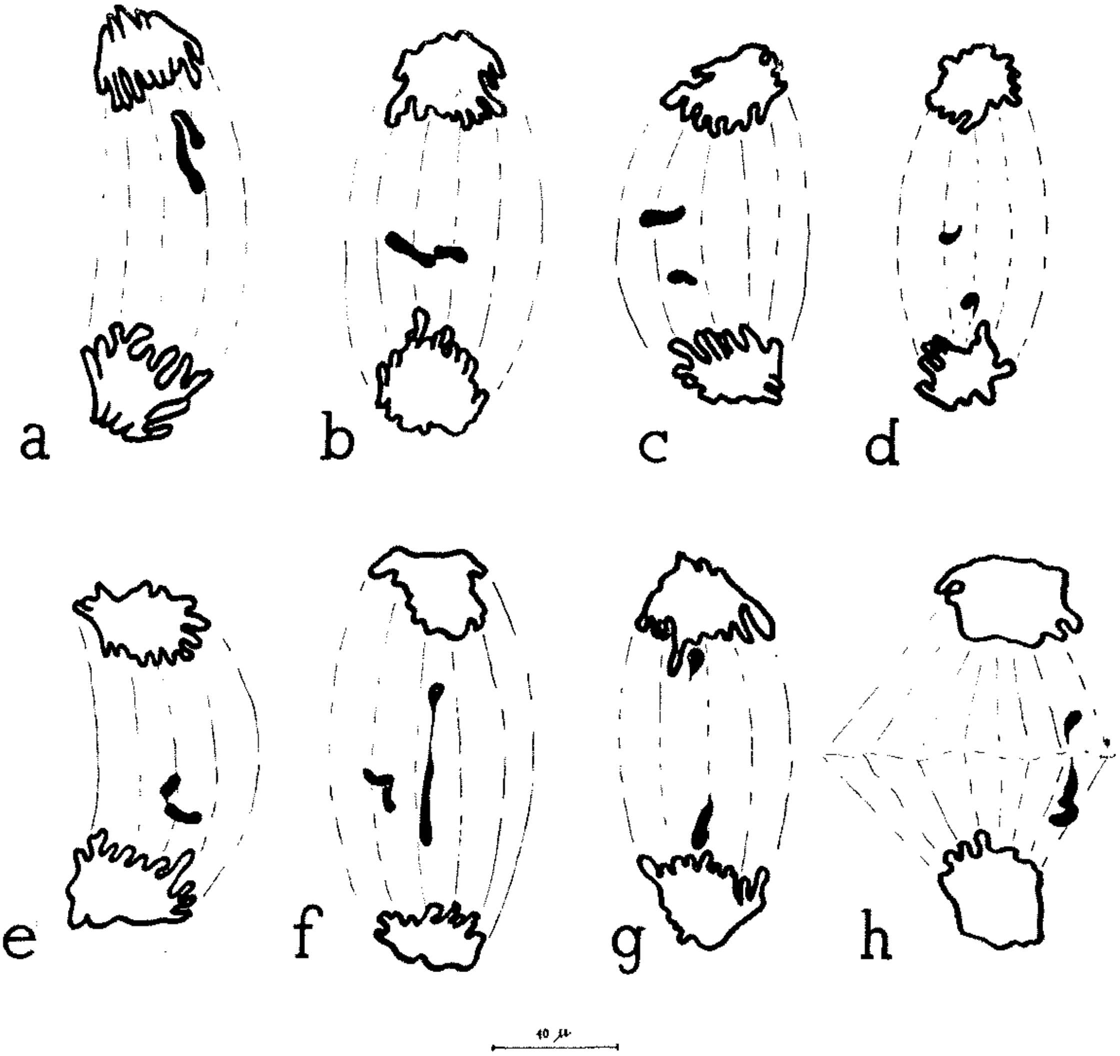

Fig. 4. Half dyad cells at second telophase. — *a*: normal reaction of the C' to the spindle. — *b* — *d*: succesive stages during *a*-misdivision. — *e* — *g*: succesive stages during *p*-misdivision. — *h*: *p*-misdivision completed by cell wall formation. — × 1350.

As in the first meiotic mitosis the reaction of the centromeric chromomeres to the spindle fibres can be normal, erroneous or completely lacking during the second division.

Usually each half dyad has a C'. If the insertion of the centromeric chromomeres is normal (monopolar) the C' moves to one of the

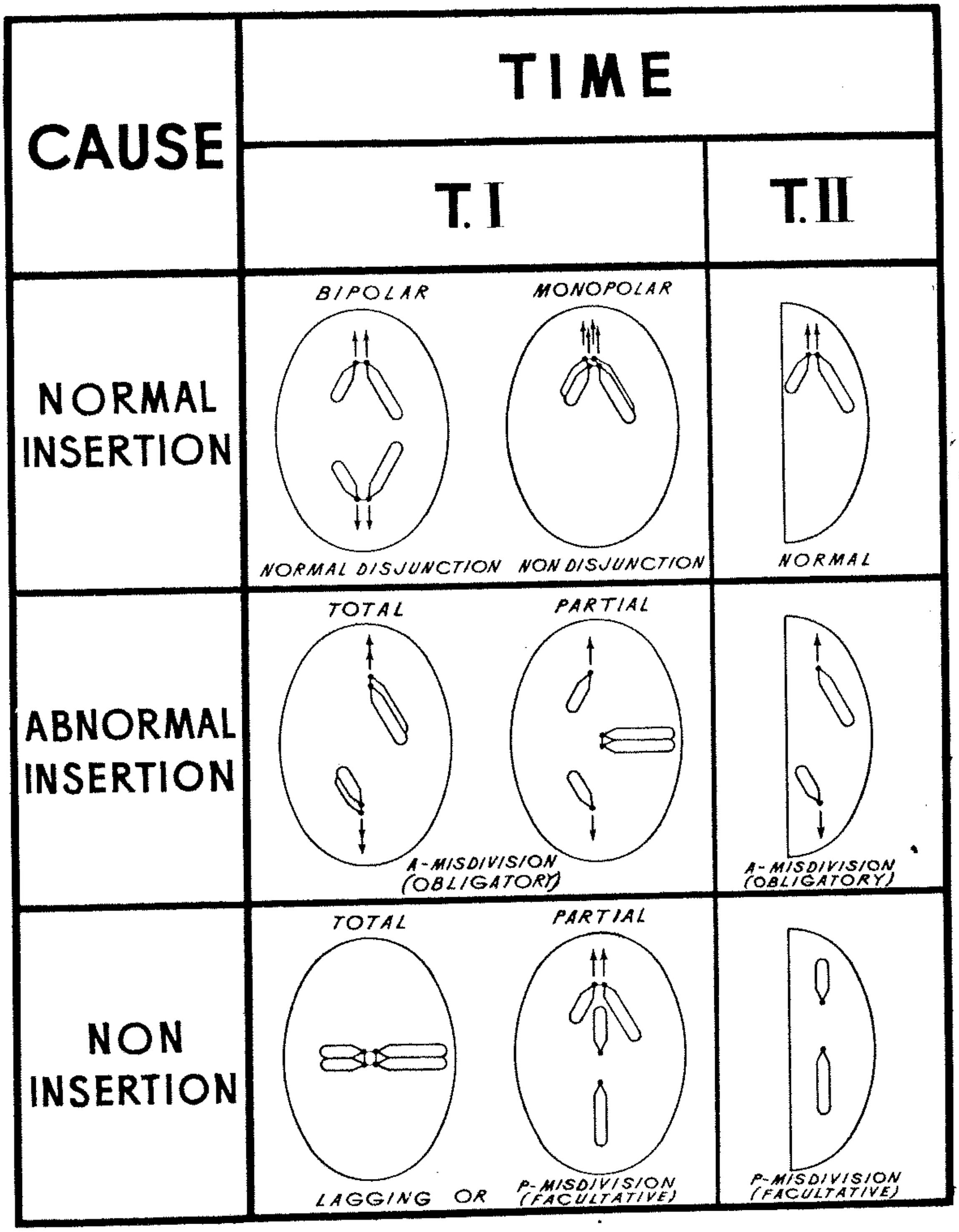

Fig 5. — Scheme of the different types of behaviour of the univalents, in relation to the reaction of its centromeric chromomeres towards the spindle.

poles (Fig. 4 *a*). In some cases two C' are seen moving to different poles or to the same pole. If the insertion of the centromeric chromomeres is erroneous *a*-misdivision occurs, both arms are attracted to the poles in an opposite direction. Figures 4 *b*, *c* and *d* depict successive stages during *a*-misdivision of the C'. Thus misdivision of the C' is obviously due to an error of insertion of the spindle fibres in the centromeric chromomeres and not to an error of insertion of the chromosome arms in the spindle spherules as MÜNTZING (1946) suggested. His explanation can hardly be applied to single chromatids (daughter univalents).

In cases where the centromeric apparatus fails to react to the spindle fibres the C' remains as a laggard at AII and *p*-misdivision may occur at TII. Figures 4 *e*, *f* and *g* show the characteristic sequence of it, separation of the distal ends, orientation in the direction of the spindle fibres, stretching of the arms and misdivision.

In some cases the spindle factor does not act further than the stretching of the arms. The misdivision is then completed by the formation of the cell wall (Fig. 4 *h*). Similar *p*-misdivision has been observed in *Avena* by NISHIYAMA with cell wall formation (1929) as well as without (1934).

Figure 5 is a scheme of the stages leading to normal disjunction, non-disjunction and misdivision of C'' and C'.

SUMMARY AND CONCLUSIONS

The behaviour at meiosis of the univalent chromosomes in monosomic *Triticum*, is explained according to the quadruple structure of the centromere and the theories of ÖSTERGREN on the mechanism of chromosome movements.

The following conclusions are arrived at:

1. The movement of the univalent at AI towards the equator is independent of the centromeric forces.
2. A normal insertion of the spindle fibres in the centromeric chromomeres at TI and TII produces the normal disjunction of the daughter univalents.
3. An erroneous insertion leads to *a-misdivision* (obligatory) and a lack of insertion causes *p-misdivision* (facultative).

RESUMEN

(MECANISMO DE LOS UNIVALENTES Y MISDIVISION)

Se intenta explicar el comportamiento meiótico del cromosoma univalente en plantas monosómicas de *Triticum*, basándose en la cuádruple estructura del centrómero y en las teorías de ÖSTERGREN sobre el mecanismo de los movimientos cromosómicos.

Se llega a las siguientes conclusiones:

1. El movimiento hacia el ecuador que realizan los univalentes en la primera anafase meiótica es independiente de las fuerzas centroméricas.

2. La disyunción normal de los univalentes hijos se produce por una inserción bipolar normal de las fibras del huso en los pares de cromómeros centroméricos.

3. Cuando se da una inserción errónea monopolar se produce la no-disyunción de los univalentes hijos. Si la inserción errónea es bipolar se produce obligatoriamente una *a-misdivisión*. El fallo de la inserción puede conducir a una *p-midivisión*.

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