LAMPBRUSH CHROMOSOMES OF CRESTED NEWTS *TRITURUS CRISTATUS* (LAURENTI)

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Lampbrush chromosomes isolated in saline from oocyte nuclei of newts have been examined by means of a phase-contrast microscope with inverted optical train. The newts which have been studied—four subspecies of *Triturus cristatus*; *cristatus*, *carnifex*, *danubialis* and *karelinii*—have diploid complements of twenty-four chromosomes in somatic cells and twelve lampbrush bivalents in each oocyte nucleus. Though the structural and functional organization common to all lampbrush chromosomes is discussed in some detail, this paper is mainly concerned with descriptions of those morphological characters which serve to identify each particular chromosome.

The axis of a lampbrush chromosome consists of a series of chromomeres connected to one another by short, thin, extensible and elastic filaments. Loops and other objects of a wide range of morphologies are attached laterally to the chromomeres, loops occurring in pairs. When lampbrush chromosomes are broken mechanically, each break occurs transversely across a chromomere, and separates the loop insertions in this chromomere in such a way that a pair of straightened-out lateral loops bridge the gap in the chromosome axis. A thin fibre forms an axis to each lateral loop, and around this axis lies 'matrix'. Chromomeres contain deoxyribonucleic acid (*DNA*), and the interchromomeric filaments and loop axes consist of *DNA* fibres. These *DNA* components of lampbrush chromosomes

are envisaged as the persistent genetic material. Matrix, which consists of ribonucleoprotein (*RNP*), is assumed to be gene product.

Although many lateral loops have similar morphologies, their matrices consisting of very fine fibres projecting radially from loop axis, certain loops are identifiable by peculiarities of matrix texture and quantity. These readily distinguishable structures have been used as 'landmarks' to map the chromosomes, and together with relative axial chromosome lengths they serve for chromosome recognition. The newt chromosomes have been designated I, II, III...XII in diminishing order of relative length.

Fusion is a property of some types of loop matrix, and it can occur within single loops, between sister loops or between homologous loops. It can also occur between non-homologous loops, though such fusions are not haphazard and involve loops of similar matrix texture. Wherever loop form is obliterated by matrix fusion the underlying loop structure can be demonstrated if the loop matrix is partially dissolved in dilute saline.

Certain objects attached to newt lampbrush chromosomes are not organized about a loop basis. Thus telomeres and 'axial granules' each consist of a crescent of *DNA* to which a spherical or roughly spherical mass of *RNP* is closely applied. All conceivable combinations of homologous and non-homologous fusions between these structures can occur, many such fusions involving more than two comparable objects. Chromosomes V and VIII carry 'spheres' attached directly to certain chromomeres, and many of the conceivable types of fusion between these spheres have also been observed.

Non-homologous fusions between comparable objects on one and the same chromosome are described as 'reflected' fusions in contradistinction to non-homologous fusions between different chromosomes. Reflected fusions between certain axial granules on chromosomes II, III, IV and VI occur so frequently that they are useful diagnostic characters.

Homologous lampbrush chromosomes are joined to one another by chiasmata and, as just mentioned, they may also be associated by gene product fusions. All chiasmate associations are junctions between chromosome axes; chiasmata do not occur within the lengths of lateral loops. In all four subspecies chiasmata tend to occur more frequently in regions close to the centromeres than elsewhere, and in subspecies *carnifex* analysis of this chiasma distribution enabled the centromeres to be identified.

In *carnifex* the centromeres are spherical objects, without lateral loops, which lie in the chromosome axes and are slightly larger than the majority of chromomeres. In fixed preparations, in contradistinction to axial granules, they stain Feulgen-positive throughout. *Cristatus* centromeres are similar but smaller, *danubialis* centromeres smaller still. In these three subspecies homologous chromosomes are never associated precisely at their centromeres, and this latter feature was indeed mainly responsible for their identification. In subspecies *karelinii* the centric regions are much more conspicuous, the centromere granule being flanked by thick, Feulgen-positive portions of chromosome axis bare of lateral loops. As *karelinii* oocytes increase in size these thick portions of axis lengthen by the incorporation of neighbouring chromomeres, whose lateral loops meanwhile regress. A comparable process of lateral loop regression and amalgamation of chromomeres occurs throughout lampbrush chromosomes as oocytes reach maturity, and with regard to this phenomenon the centric regions of *karelinii* are thus precocious. Unlike the centromeres of the other three subspecies, homologous centromere granules of *karelinii* are often fused together, and non-homologous and threefold centric fusions have also been observed in this subspecies. Despite these outstanding differences the centromeres of *carnifex* and *cristatus* have without doubt been correctly identified, and they are located at substantially the same places on the chromosomes as those of *karelinii*.

Although the chromosome complements of the four subspecies show overall resemblances, other characteristics apart from the centromeres serve to distinguish between them; and these are detailed. It is significant that loops of certain particular morphologies are present in one or other of the subspecies, but not in the remainder.

As a general rule homologous chromosome sites bear lateral objects of comparable size and texture. However, in all four subspecies the two largest chromosomes which form bivalent I are never associated by chiasmata within regions extending over about half their length, and in these regions the lateral loop patterns do not correspond. Moreover, in certain female newts at specified homologous sites on other bivalents the lateral loops may regularly be of dissimilar morphology. These differences

are conserved throughout life. Several subtle distinctions of this kind are detailed, but there are also more striking individual-specific characters. Thus in *carnifex* there are females with giant loops present on one homologue only of bivalent X, other females with giant loops on both homologues, and yet other females without giant loops on either. Within a population the proportions of these three types of female accord with Hardy–Weinberg expectations, and such individual characteristics are claimed to represent, with respect to gene products, allelic differences at particular genetic loci.

Liberation of synthesized products from the majority of lateral loops has not been observed, but from many of the landmark loops where gene products accumulate in massive quantities it can be inferred that aggregates of these materials are shed from time to time into the nuclear sap. Detached gene products, whose sites of origin have been identified, diminish in size after shedding and they presumably augment the nuclear sap. The trivial variation in morphology of particular lateral loops from one oocyte to another, taken from a single female, is claimed to be due to varying balance between the rates of gene product synthesis and dispersal existing at the time of dissection. The origin and functional significance of the peripheral 'nucleoli', which are so characteristic of amphibian oocyte nuclei, are still uncertain.

Lateral loops are characteristically asymmetric. One of the two portions of loop axis leading from a chromomere is always relatively bare of matrix. Along the lengths of the great majority of lateral loops there is an even transition in matrix quantity and/or texture, but loops of highly irregular outline also regularly show thinner and thicker insertions in chromomeres. Wherever axial breaks occur the polarity of loop asymmetry with respect to the whole chromosome can be determined. Sister loops always show the same polarity, and at those places where axial breaks have been repeatedly observed loop asymmetry consistently displays the same polarity. This fundamental feature of the genetic material is claimed to depend on a polarized mechanism of loop extension from and retraction to its parent chromomere, one end of the loop having therefore been engaged for less time than other parts in synthesis of matrix. If the main and subsidiary arguments supporting this claim are valid, each loop axis must consist of a series of repeats of identical genetic information. To reconcile this theory with the dimensions of mutational sites, so far as these are known, in other organisms, it is suggested that each chromomere consists of a 'master copy' of the genetic code and a store of incompletely specified *DNA*. Further specification of *DNA* from this store occurs whilst the loop axis extends, and it is this secondarily coded material which acts as the template for synthesis of specific *RNP*.

I. Introduction

Lampbrush chromosomes are present in germinal vesicle nuclei of animal oocytes which are developing in ovaries. They were first described by Flemming in 1882 from observations made on the urodele *Amblystoma*. The name 'lampbrush' came into use following Rückert's (1892) description of the oocyte chromosomes of the shark *Pristiurus*, where he compared chromosome structure to that of a much-used 'Lampencylinderputzer'—what one would nowadays call a bottle-brush—which consists of an axis of intertwined fibres bearing fibrous lateral projections. Chromosomes of this appearance have been seen in oocytes of the chaetognath *Sagitta*, the mollusc *Sepia*, the crustacean *Anilocra*, several insects, the echinoderm *Echinaster*, at least two species of shark and many amphibians, reptiles and birds.

Lampbrush chromosomes excited considerable attention from the year of their discovery until about 1910, but from this time until 1937 they were almost totally neglected. In part at least this neglect was due to the inadequacy of the then prevailing cytological techniques involving bulk fixation followed by section cutting and staining. Salivary gland chromosomes of Diptera were similarly neglected. For a survey of the early investigations reference may be made to Callan (1957). Interest in lampbrush chromosomes was

reawakened by Duryee (1937, 1941), who demonstrated how easily they may be isolated in saline from oocytes of frogs and newts and studied in an unfixed state. Duryee drew attention to the great size of the lampbrush chromosomes of the Japanese newt *Triturus pyrrhogaster*; several chromosomes of this species are much longer than dipteran salivary gland chromosomes, which are themselves often referred to as 'giant'.

Duryee's observations were made with an ordinary light microscope; however, the full resolving power of this instrument cannot be brought to bear on unfixed lampbrush chromosomes because of their low refractility and general lack of sharp boundaries. Later observers have been able to use phase-contrast microscopes, which are particularly well suited for the study of these objects. Furthermore, Gall (1954) made an important advance in technique by inverting the optical train of his phase-contrast microscope; as will be explained later, this enabled him to observe for the first time intact and unfixed lampbrush chromosomes at the highest objective magnification.

Following Duryee's pioneering studies, the observations of Dodson (1948), Duryee (1950), Tomlin & Callan (1951), Gall (1952, 1954, 1955, 1956, 1958), Guyénot & Danon (1953), Callan (1955, 1957), Callan & Lloyd (1956, 1960), and Callan & Macgregor (1958) have extended our knowledge of the morphology and physicochemical nature of lampbrush chromosomes. In the light of these observations we may consider the following generalizations to be valid:

(1) Lampbrush chromosomes are bivalent, with homologues attached to each other at one or more places. This was correctly appreciated by Rückert in 1892.

(2) The axis of each lampbrush chromosome consists of a series of chromomeres which stain Feulgen-positive in fixed preparations (Dodson 1948) and which thus contain deoxyribonucleic acid (*DNA*). Chromomeres are connected to one another by a very thin, extensible and elastic chromonema (Tomlin & Callan 1951; Gall 1952; Guyénot & Danon 1953). Rückert and some other early observers were not convinced of the reality of axial chromomeres but in 1941 Duryee's stretching experiments put this question beyond reasonable doubt.

(3) The great majority of chromomeres bear lateral loops. Several of the early workers noticed and drew lateral projections, some of which were reflected to the chromosome axis; and Duryee in 1941 demonstrated that this is a regular feature. These lateral loops are present in pairs, or multiples of pairs (Guyénot & Danon 1953; Callan 1955; Gall 1955). Pairs of loops are of similar size and appearance, but the morphology of loops at different places along the chromosomes is diverse.

(4) Loops at corresponding places on homologous chromosomes may appear similar in size and texture, or they may be consistently dissimilar (Callan & Lloyd 1956, 1960).

(5) Each loop is asymmetric, with a thicker and a thinner insertion at the chromosome axis (Callan 1955; Callan & Lloyd 1960).

(6) When a lampbrush chromosome is stretched and broken, breakage does not occur within a portion of the thin chromonema, but instead transversely across a chromomere; and always in such a way as to separate pairs of loop insertions. Thus axial breaks are regularly spanned by a pair of straightened-out lateral loops (Callan 1955, 1957; Gall 1956).

(7) Loop size and chromomere size are inversely correlated. When the lateral loops are maximally extended the axial chromomeres are minimally small (Callan 1955).

(8) Within each lateral loop there is a very thin loop axis (Callan 1955; Gall 1956). Like the chromonema between chromomeres, this loop axis withstands proteolytic enzymes and ribonuclease (*RNA*ase) but is broken by deoxyribonuclease (*DNA*ase) (Callan & Macgregor 1958). We assume that loop axes and the other *DNA* constituents of lampbrush chromosomes—chromomeres and interchromomeric connexions—represent the persistent genetic material.

(9) Around each loop axis is a 'matrix' of ribonucleoprotein (*RNP*). The wide range of loop morphologies is determined by diversity in quantity and texture of this matrix (Callan 1955; Gall 1956), and by the degree to which neighbouring portions may fuse together. Fusion of matrix, which may occur within loops, between sister loops, between homologous loops, and between genetically non-homologous but texturally similar loops, can be so extreme as to obliterate loop form (Callan & Lloyd 1960). We assume that loop matrices and other *RNP* constituents of lampbrush chromosomes, to be described later in this paper, represent gene products.

(10) A variety of objects, which correspond in texture to certain of the lateral structures, may be present free in the nuclear sap (Gall 1954, 1955; Callan 1955). Before loop axes had been discovered Duryee (1941) asserted that these free bodies were lateral loops sloughed off from the chromosomes. In reality, however, the material which sloughs off is loop matrix; the loop axes remain as structural components of the chromosomes.

(11) As oocytes approach maturity the lateral loops regress and ultimately disappear, leaving contracted chromosomes whose chromomeres have amalgamated to form meiotic metaphase bivalents of normal appearance. The metaphase chiasmata holding homologous chromosomes together are directly derived from some, though not necessarily all, of the points where these homologues were associated as lampbrush bivalents.

We may note that Ris (1952, 1955, 1956, 1957) and Lafontaine & Ris (1955, 1958) do not agree with several of the above generalizations. Their views are not in accordance with the available evidence, and have already been criticized in detail by Gall (1958).

Duryee (1950) and Gall (1955) have stressed the immense increases in volume of amphibian oocyte cytoplasm and nucleus which occur whilst the chromosomes are extended in their lampbrush form, and it thus seems reasonable to assume that the morphological metamorphoses of these chromosomes are directly associated with the synthesizing activity of nuclear genes. If this assumption is valid, as we think to be the case, lampbrush chromosomes provide a remarkably favourable opportunity for the study of gene-ordered synthesis, since at least some of the immediate products of gene activity accumulate *in situ* on these chromosomes and thus become directly observable with the light microscope.

If one wishes to proceed with such a study, and beyond the generalizations mentioned above, it is imperative that the various chromosomes and parts of chromosomes forming the complement of at least one animal species be characterized and thus identifiable from one preparation to another. The present account provides such a characterization of the lampbrush chromosome complements of four geographical races of the crested newt, *Triturus cristatus* (Laurenti).

A newt was chosen for this study since newt lampbrush chromosomes are larger and fewer in number than those of their nearest rivals in these respects, the elasmobranchs. This species was chosen for several reasons, particularly because:

(*a*) the females are hardy and survive oft-repeated removal of ovary fragments under non-sterile conditions;

(*b*) the lampbrush chromosomes show a wider range of morphological character than those of the American *T. viridescens*, the only other species for which a comparable study (Gall 1954) has been made;

(*c*) the four races can be distinguished from one another by certain characteristics of their lampbrush chromosomes;

(*d*) the races can be bred in captivity (though with certain difficulties in the case of *Triturus cristatus cristatus*) and can be hybridized to give F_1 females which are fertile when back-crossed to racial males (Callan & Spurway 1951; Spurway 1953), thus permitting genetic analysis of lampbrush characters.

We have attempted only a limited characterization of the lampbrush chromosomes of *T. cristatus* because full characterization of the complement of this or any other newt species is beyond the limits of practicability. There are several thousand chromomeres and lateral loops within the complement, and their morphologies, unlike most of the cross-banding patterns of dipteran salivary gland chromosomes, are not constant from nucleus to nucleus. The morphologies of lampbrush chromosomes vary in accordance with the developmental stages of oocytes, and further variation not associated with stages in development appears to be ascribable to differing degrees of physiological activity of oocytes. Furthermore, the lampbrush chromosome complements of different newts from within a natural interbreeding population show regular individual-specific features, some of which are conspicuous. These characteristics of individuals are not referable to structural rearrangements of the genetic material such as have been so frequently detected in salivary gland chromosomes of the Diptera; rather they are the immediate expression of allelic differences at particular chromosomal sites (Callan & Lloyd 1956, 1960).

For the most part we have confined our attention to chromosomes from oocytes within the size range 0·6 to 1·7 mm diameter. Oocytes smaller than 0·6 mm diameter lack compact accumulations of opaque yolk, whilst oocytes can be mature and ready for ovulation at 1·7 mm diameter; this range therefore includes oocytes throughout the cycle of visible yolk storage. It is not normally possible to isolate undamaged chromosome complements from oocytes smaller in diameter than 0·7 mm by the techniques which we have used in this study. The nuclei of smaller oocytes certainly contain chromosomes of lampbrush form—we have seen them in oocytes of 0·3 mm diameter—but in spite of the painstaking study of stained ovary sections by Guyénot & Danon (1953) we are as yet unable to decide at which developmental stage the lampbrush form is first adopted.

II. Materials and techniques

The four races of newts which we have studied are the following:

Triturus cristatus cristatus (Laurenti)
Triturus cristatus carnifex (Laurenti)
Triturus cristatus danubialis (Wolterstorff)
Triturus cristatus karelinii (Strauch)

The taxonomy of these four subspecies is given by Wolterstorff (1923), and Spurway (1953) provides further information on their characteristics and their geographical distributions.

T. c. cristatus. Our specimens of this race were supplied by the dealers L. Haig and Son. They were collected in the south of England. We have studied the lampbrush chromosomes of seven *cristatus* females in detail, and have made casual observations on a further six in the course of other work.

T. c. carnifex. Our specimens of this race were collected in the vicinity of Naples, Italy, and from 1948 onwards many batches have been sent to us through the courtesy of Professor G. Montalenti and Dr P. Dohrn. We have studied fifteen *carnifex* females in detail, and have fragmentary information from a further seven.

T. c. danubialis. Our specimens of this race were collected near Vienna, Austria, and kindly provided by Professor F. Mainx. We have examined eight *danubialis* females in detail and have fragmentary information from a further two.

T. c. karelinii. Dr H. Spurway gave us six *karelinii* females out of a batch sent to her by Professor C. Kosswig from Istanbul, Turkey. More recently we have obtained further *karelinii* through the courtesy of Dr A. Şengün; these were also collected in the neighbourhood of Istanbul. We have studied five *karelinii* females in detail, and have fragmentary information from a further five.

Several of our 200 and more newts have been living in the laboratory for upwards of 10 years. In St Andrews they have been kept in stagnant water and have been fed twice a week on earthworms or *Tubifex*, with beef liver or spleen provided on occasion. We should like to take this opportunity of expressing our thanks to the late Mr D. G. Brown, Mr J. Mackie and Mr A. Ness, who have most conscientiously cared for the welfare of our stocks.

The newts which we have used in this study have been kept under fairly constant conditions of temperature and illumination. The tank room is permanently lit and its temperature has remained at 16 ± 2 °C except during severe winter weather, when it has occasionally dropped below 10 °C. Under these conditions the males of *carnifex*, *danubialis* and *karelinii* still show reproductive cycles, coming into breeding condition in November and maintaining this state until May or June. Males of *cristatus*, however, rarely reacquire breeding condition if they have been kept over a summer in the laboratory. Females of all four races when they have been kept in captivity for more than a year show less marked cycling, and if well nourished their ovaries generally contain a complete range of oocyte developmental stages throughout the year. However, the oocyte stage which is most convenient for lampbrush chromosome analysis—midway through the laying down of yolk—may be relatively deficient during August and September, as is true of the ovaries of these newts when freshly captured during their breeding seasons.

When ovarian eggs are required a newt is lightly anaesthetized with ether, a short longitudinal ventro-lateral incision is made in the skin and body-wall musculature, and a piece of ovary excised. The piece of ovary is placed in the well of a 'solid watchglass' without saline, a glass square is sealed over the top of the container with a paraffin oil+ Vaseline mixture, and the container placed in a dish containing ice and water if required for immediate use. A piece of ovary 'dry' in a sealed container remains in reasonable

condition for up to a week if stored in a refrigerator at +2 °C, provided that no broken yolky eggs are present. Preparations made from oocytes which have been stored in this way for 2 or 3 days cannot in general be distinguished from preparations made from freshly excised oocytes. Longer storage produces changes which mimic maturation—reduction in lengths of lateral loops and of chromosomes—yet chromosome identification may still be possible. All saline media we have tried have proved deleterious to ovary fragments, which survive best when stored dry.

The wound in the operated newt is sewn up with one or two stitches of plain 3/0 catgut suture, and the newt is then placed in running tap water until it recovers from anaesthesia. Healing is normally complete within 14 days, the stitches disintegrating and being shed with cast skin. Portions of ovary may be repeatedly removed from one newt; it has been our practice to exhaust the animal's left ovary before starting on the right. The possibility of returning at intervals to check on the chromosome characteristics of individual newts has been of great value to us; moreover, we have successfully bred from animals whose chromosome characteristics had earlier been determined from excised pieces of ovary.

For isolation of the germinal vesicle nuclei a tiny fragment is torn from an excised piece of ovary and placed in saline in a 'solid watchglass'. For the past 4 years we have used a saline consisting of 5 parts 0·1 M-potassium chloride and 1 part 0·1 M-sodium chloride. We have not noticed any marked advantage that this mixture has over 0·1 M-potassium chloride alone, which we used prior to 1955, but we have been influenced by the determinations of Dr W. T. W. Potts (personal communication) which show that the nuclei of *carnifex* oocytes contain potassium and sodium in the approximate ratio of 5:1. If the nuclei and chromosomes are to be examined in a life-like state it is essential that the isolating medium should contain no calcium (Duryee 1937, 1941) since this ion, even at low concentration, acts like a fixative.

Nuclei are isolated with the aid of a binocular dissecting microscope giving a magnification of ×40. The diameter of a chosen oocyte is first measured with an eyepiece micrometer, and its attachment to the ovarian wall is then grasped with fine-pointed watchmakers' forceps. The surface of this oocyte is now torn with a sharp needle and the yolk gently pressed out. The nucleus is generally visible as a 'blister' on the ribbon of yolk, and it may be freed from cytoplasm and yolk granules by pumping with a fine-drawn glass pipette of orifice about 0·5 mm. The aperture of the pipette used for this purpose should previously have had its walls rounded off in a flame. It is imperative that saline should flow freely within the pipette: if a stationary air bubble is present in the constricted portion the nucleus will break whilst being cleaned owing to its membrane becoming trapped in the air/water interface.

When a nucleus has been sufficiently cleaned it is transferred by pipette to fluid lying in an observation chamber originally designed by Dr J. G. Gall for use with an inverted microscope. This chamber consists of a 3 in. × 1 in. microscope slide through which a central hole $\frac{1}{4}$ in. in diameter has been bored. Glass strips 1 in. × $\frac{1}{8}$ in. are cemented with natural canada balsam or synthetic resin to the ends of the slide, and on the same side of the slide a $\frac{1}{2}$ in. diameter No. 0 coverslip is sealed across the central hole with paraffin wax. A sectional view of the observation chamber is shown in figure 1. We are

greatly indebted to Mr G. N. Newell of the Institute of Animal Genetics, Edinburgh, who bored several hundred microscope slides for making up in this way.

As soon as the nucleus lies in the cavity of the observation chamber, its membrane is nipped and held by the jaws of a pair of the finest obtainable watchmakers' forceps (Dumont Fils No. 5, ordinary, *not stainless*, steel). A fine-pointed tungsten wire needle, previously sharpened in molten sodium nitrite, is now slipped under the membrane near the part held by the forceps and the membrane torn open. With the membrane still held by forceps the nuclear contents are allowed to spill out near the middle of the cavity of the observation chamber; they fall and spread out over the coverslip forming its floor, the nuclear membrane being meanwhile removed.

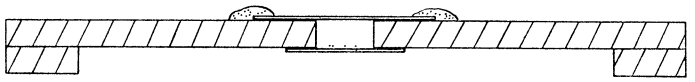

FIGURE 1. Sectional view of the 3 in. × 1 in. microscope slide chamber used for examining lampbrush chromosomes. Explanation in text.

This manipulation of the nucleus within an observation chamber is greatly facilitated if a peculiar form of illumination is employed. A low voltage, high intensity light source provided with a condenser is focused on to the nucleus at right-angles to the optical axis of the binocular, so that the refractile contents of the nucleus are visible by the light which they scatter. Daylight collected by the microscope mirror is projected askew up the optical axis of the binocular to produce further light scatter in the nucleus and its membrane, but sufficient transmitted light is allowed to pass to enable the depth of the nucleus to be appreciated and the instruments to be seen. The optimum setting of the mirror can only be found by trial. The precise amount of fluid in the observation chamber is also of importance. If too much is present and the meniscus too convex, light reflexions from the air/water interface can be disturbing. If too little is present air bubbles cannot be excluded when a top coverslip is later applied.

At various times and for different requirements we have used four saline media in the observation chambers. In these media the expression K/NaCl stands for a mixture of KCl and NaCl solutions giving a molecular ratio K:Na of 5:1.

Medium A

0·05 M-K/NaCl	3 parts
0·001 M-KH_2PO_4	7 parts

This is the most dilute medium which we have employed. It disperses the nuclear sap leaving the lampbrush chromosomes spread out over the lower coverslip within 2 or 3 min. Apart from the 'spheres' (which are defined later) all non-chromosomal objects free in the nuclear sap dissolve, some partially, others completely. The particular value of this medium is that it produces well-spread chromosome preparations virtually free from 'nucleoli' (also defined later) in which the characteristic 'landmarks' are displayed and in which the axial chromomeres (including centromeres) are clearly visible unobscured by the smaller range of laterally attached refractile objects. Most loops are of low optical

contrast. The appearance of the major landmarks may not be lifelike, and cross-reference to preparations in stronger saline is necessary before one can be sure of their natural morphology. However, the appearance of partially disintegrated lateral loops often gives valuable clues as to their hidden internal structure.

Preparations made in medium A deteriorate rapidly. As time passes more and more lateral loop materials dissolve until, after a few hours, only axial structures remain. The constitution of medium A was arrived at empirically some years ago, when it was found that the phosphate constituent, perhaps merely because of the slight buffered acidity which it confers, restrains lateral loop material from at once passing into solution as occurs if chloride alone at this dilution is employed.

Medium B

0·05 M-K/NaCl	7 parts
0·001 M-KH_2PO_4	3 parts

For analytical purposes this is the medium we have found most generally useful. Medium B disperses nuclear sap and dissolves free bodies and loop matrices more slowly than medium A: preparations in medium B can be usefully observed over 2 or 3 days if they are stored at 2 °C when not actually under examination. Medium B is essentially a compromise. It enables sufficient morphological detail to be observed for the rapid identification of individual chromosomes, chromosome axes can be followed with ease, and the preservation of most of the larger laterally attached objects has proved on cross-checking to be life-like for about 2 days.

Medium C

0·1 M-K/NaCl	7 parts
0·001 M-KH_2PO_4	3 parts

In this medium the nuclear sap disperses yet more slowly than in media A or B. If the preparations are stored at 2 °C, most of the free bodies and objects attached to the lampbrush chromosomes' axes and lateral loops remain in life-like preservation for a week or more. These objects may, however, be so densely packed in certain chromosome regions as to obscure chromosome axes. With medium C it is thus often impossible to determine the detailed structural relationships in parts of the lampbrush chromosomes, notably at places where homologues are joined together. Moreover, the centromeres may be difficult to locate. Had medium C alone been employed in this study we might not have noticed the axial peculiarities of chromosome XII, whilst the complex configurations around the centromeres of chromosomes II, III, IV and VI could certainly not have been analyzed. On the other hand, with prior information from preparations made in media A and B, medium C has been of value in establishing the natural appearance of intact lampbrush chromosomes.

Medium D

0·1 M-K/NaCl

This medium is of the same molar strength as that used by Gall in his studies on *T. viridescens*. However, the structural colloid component of the oocyte nuclear sap (Callan 1952) of *T. cristatus* does not disperse in this medium. It is thus not possible to

analyze a complete chromosome complement in medium D, though with prior knowledge the landmarks can be identified and their appearance studied within a few minutes of nuclear isolation. Such observations have demonstrated that a life-like appearance of the chromosomes is preserved by medium C which, as mentioned above, has the advantage over medium D of dispersing nuclear sap colloid. Medium D has therefore only been employed as a final check.

After the nuclear contents have been emptied in an observation chamber, a coverslip is dropped over the top of the preparation, care being taken not to include any air bubbles nor to disturb unduly the fluid within the chamber. The quantity of medium requires to be judged to a nicety in order to achieve this aim. With the top coverslip in place the preparation is left untouched for a few minutes to allow any excess medium to evaporate, and the top cover subsequently sealed to the chamber with Vaseline so as to prevent further evaporation.

Dr J. G. Gall (personal communication) informed us that dissolution of the isolated chromosomes can be delayed if preparations are given a short treatment with formaldehyde vapour before the top coverslip is applied. In our work over the past 4 years we have as a routine placed preparations in a chamber saturated with formaldehyde vapour for 5 min. We can confirm Gall's statement that such treatment does not change the appearance of lampbrush chromosomes, nor does it produce 'fixation' in the ordinary histological sense. If, however, the formaldehyde treatment is prolonged to 20 or 30 min fixation *does* occur, and is immediately evident in the much higher refractility of the chromosomes, with contraction and stiffening of the lateral loops and of the chromosomes as a whole. Such fixed preparations have their uses, but they are much inferior to unfixed preparations if one wishes to study the detailed morphology of the chromosomes. The most serious defect of fixed preparations is that in them the lateral loops clamp down upon chromosome axes to produce a matted felt of fibres whose interrelationships cannot be readily made out.

When a preparation has no further value top and bottom coverslips can be quickly removed in a stream of hot water. After drying, the slide can be used again to make up a fresh observation chamber; this procedure, barring accidents, is indefinitely repeatable.

So far as we are aware Gall (1954) was the first investigator to employ an inverted microscope for studying lampbrush chromosomes. The use of this instrument was a technical advance of the highest importance. Using a conventional microscope it is admittedly possible to examine entire chromosome complements with an oil-immersion lens, provided, however, that the chromosomes be first attached by formaldehyde or osmic fixation to a coverslip which is then inverted over a cavity slide containing oil (Gall 1952). As mentioned already, fixed preparations are much less informative than unfixed preparations. Conventional mounts, with the chromosomes, fixed or unfixed, confined between coverslip and slide, are of relatively little value; it is impossible to lower a coverslip on a group of lampbrush chromosomes lying on a slide in a pool of saline without causing very extensive damage to the material.

We have used an inverted microscope constructed by Cooke, Troughton and Simms, Ltd, of York. It is fitted with a phase contrast condenser unit (of long working distance so that micro-manipulator needles can be used when required) and phase objectives producing

'dark' contrast. A front-silvered mirror reflects light from the objective into the binocular eyepiece assembly: the mirror can alternatively be tripped so as to throw the light into a 35 mm film camera.

It is of advantage when photographing unfixed lampbrush chromosomes to use the primary image produced by the objective lens alone, rather than the image enlarged by the eyepiece, since in this way exposure times can be cut down. Short exposures are necessary because of the violent Brownian movement of the unfixed lampbrush chromosomes when free from nuclear sap. For our first photographs we employed a roller blind shutter placed between the light source and condenser assembly, but with the $\times 95$ oil immersion in use the fastest shutter speed available to us ($\frac{1}{90}$ s) failed to 'stop' Brownian movement. For the past 3 years we have used instead a xenon flash light source, first an instrument kindly lent by Cooke, Troughton and Simms, Ltd. and later one constructed by Dr G. A. Horridge. The flash, nominally $\frac{1}{3000}$ s duration, is more than adequately fast, and it is of sufficient intensity even when very slow film is exposed.

Because of its extremely fine grain and high contrast we have used Ilford Micro-Neg Pan film, a roll of film being cut into short lengths to fit a single-exposure camera. This film is very slow, but its low speed has at no time been a limiting factor.

Even with the practical problem of Brownian movement overcome, phase-contrast photographs record the structure of lampbrush chromosomes at high objective magnification most inadequately. With a length of chromosome axis in focus the lateral loops extend in all directions in and out of focus. This is also their condition in life; they do not lie in single planes convenient for photography. Moreover, light haloes around the larger and more refractile objects seriously interfere with the images of neighbouring structures. Doubtless an interference system would be preferable to phase contrast, but an inverted interference microscope has yet to be designed.

III. Chromosome identification:

Triturus cristatus carnifex (Laurenti)

Since our earliest observations were carried out on *T. c. carnifex* and since we have spent considerably more time working with this subspecies than with the three others, it is appropriate to consider *carnifex* first.

Identification of the individual lampbrush chromosomes depends on a combined knowledge of certain recognition characters and of the relative lengths of the twelve members of the haploid complement. We have built up this knowledge progressively. The chromosomes were first designated arbitrarily by letters of the alphabet but later, when their relative lengths had been established, they were numbered I to XII, chromosome I being the longest and XII the shortest.

(*a*) *Relative lengths*

For *carnifex*, relative lengths were obtained from chromosomes mounted in medium A. Drawings of the axes of the lampbrush chromosomes were made with a camera lucida attached to an inclined monocular tube magnifying $\times 1{\cdot}5$, the paper resting on an inclined platform standing beside the microscope. Objective magnification was $\times 45$ (fluorite, oil

immersion), eyepiece magnification $\times 6$, and the overall magnification $\times 540$ on the drawing paper. The positions of notable 'landmarks' on the chromosomes were also recorded.

At least 30 min were permitted to elapse from the time of removal of the nuclear membrane to the start of drawing in order that the chromosomes should become fully extended in a single plane.

Lampbrush chromosomes are extensible and elastic, but since they do not adhere to one another when first isolated they do not become differentially stretched unless they happen to be badly entangled. Stretched parts of lampbrush chromosomes can be recognized by the restricted Brownian movement of their chromomeres, and the lengths of such stretched regions were naturally not included in the analyzed data. Chromosomes which are stretched particularly violently break across chromomeres, but the broken parts generally remain connected by the pair of lateral loops originating from the broken chromomere; in these circumstances unless the stretching is severe the bridging loops take up the strain, and accurate axial lengths can still be obtained. Moreover, since lampbrush chromosomes are bivalent, the length of one homologue acts as a cross-check on the length of its partner. Normally the lengths of both homologues were determined and averaged. The lengths of the drawn chromosomes were estimated to the nearest millimetre by means of an opisometer (map measurer).

Some time before we were able to recognize all twelve members of the *carnifex* complement we had become familiar with two chromosomes which later turned out to be the fifth and eighth longest, V and VIII. These two chromosomes can be identified at a glance in most preparations. The relative lengths of chromosomes V and VIII were established from drawings of both these chromosomes taken from forty-nine oocytes from three females *A*, *B* and *C*. No systematic individual differences were apparent, and the information was treated as a single body of data. Taking chromosome V as of length 100 units, chromosome VIII is of length $75{\cdot}2 \pm 0{\cdot}8$ units.

The relative lengths of the twelve *carnifex* chromosomes were established from observations made on nine oocytes from female *C* and eleven oocytes from female *D*. The preparations from these twenty oocytes were analysed as fully as possible. Ideally all chromosomes were identified and drawn: this was achieved in seven oocytes. In the remaining thirteen oocytes the complement was only partially analyzed but all chromosomes left undrawn were at least identified.

All partially analyzed complements included drawings of chromosomes V and/or VIII to serve as yardsticks for the lengths of the other chromosomes. In all complements where chromosomes V and VIII were both drawn their length measurements were added together and then divided in the proportions 100:75, giving mutually adjusted lengths to which the lengths of the remaining chromosomes could be related. In other partially analyzed complements lengths were related in direct proportion to the measured lengths of chromosome V or VIII.

The lengths of all twelve chromosomes relative to V of 100 units and/or VIII of 75 units are shown in table 1. The individual means are given separately, together with overall means on the basis of which the chromosome lengths shown on the 'working map' (figure 2) have been drawn. Although the mean relative chromosome lengths established

for the two newts do not tally exactly, the sets of figures are in good agreement and chromosome order based on length is certainly the same in both individuals. Chromosomes X and XI could not be discriminated for length on the basis of these early measurements, but later information has indicated that chromosome X is a trifle longer ($64{\cdot}1 \pm 0{\cdot}4$ units) than chromosome XI ($61{\cdot}7 \pm 0{\cdot}4$ units).

TABLE 1. *T. c. carnifex.* LENGTHS OF THE LAMPBRUSH CHROMOSOMES FROM TWENTY OOCYTES RELATIVE TO CHROMOSOME V OF 100 UNITS AND/OR CHROMOSOME VIII OF 75 UNITS

Corrected relative lengths for chromosomes X and XI are entered in brackets in the column showing overall means.

	female *C*										
oocyte no. ...	2	3	4	5	6	7	8	9	10	mean	S.E. ±
oocyte diameter (mm) ...	1·14	1·08	1·26	1·38	1·20	1·14	1·08	0·90	1·14	—	—
chromosome											
I	148	137	130	136	140	138	140	125	145	137·7	2·3
II	128	123	122	126	127	134	136	—	127	127·9	1·7
III	123	—	—	—	—	124	127	117	124	123·0	1·6
IV	115	—	—	—	116	106	109	114	106	111·0	1·9
V	100	100	—	—	100	100	100	100	100	100	—
actual length of V (μ)	*610*	*558*	—	—	*651*	*574*	*532*	*552*	*528*	—	—
VI	96	91	—	—	89	92	90	89	93	91·4	1·0
VII	95	95	—	83	87	83	92	87	96	89·7	1·9
VIII	75	—	75	75	75	75	75	75	75	75	—
actual length of VIII (μ)	*430*	—	*503*	*433*	*480*	*418*	*446*	*440*	*369*	—	—
IX	70	74	70	—	69	72	64	67	69	69·4	1·1
X	70	69	64	67	63	65	62	61	67	65·3	1·1
XI	65	63	—	64	63	62	60	57	65	62·4	0·9
XII	49	50	43	47	46	45	42	42	44	45·3	1·0

	female *D*													overall	
oocyte no. ...	1	2	3	4	5	6	7	8	10	11	13	mean	S.E. ±	mean	S.E. ±
oocyte diameter (mm) ...	1·26	1·56	1·44	1·56	1·44	1·38	1·62	1·56	1·62	1·62	1·44	—	—	—	—
chromosome															
I	141	144	—	140	128	124	128	122	125	129	131	131·2	2·4	134·4	1·8
II	—	119	135	—	115	—	118	117	—	118	—	120·3	3·0	124·6	1·9
III	—	118	112	—	113	—	119	110	122	112	111	114·6	1·5	117·8	1·6
IV	—	111	—	111	105	—	104	100	117	113	115	109·5	2·1	110·1	1·4
V	100	100	100	100	100	—	100	100	100	100	100	100	—	100	—
actual length of V (μ)	*537*	*565*	*578*	*583*	*534*	—	*466*	*507*	*474*	*492*	*464*	—	—	—	—
VI	—	97	—	—	87	—	94	—	86	95	90	91·5	1·8	91·4	0·9
VII	—	90	82	—	85	78	82	89	88	80	88	84·7	1·5	87·1	1·3
VIII	—	75	75	75	75	75	75	75	75	75	75	75	—	75	—
actual length of VIII (μ)	—	*381*	*448*	*407*	*437*	*482*	*368*	*403*	*302*	*332*	*344*	—	—	—	—
IX	—	73	68	68	63	—	69	64	—	63	68	67·0	1·3	68·2	0·8
X	59	60	67	60	65	60	59	60	—	57	60	60·7	0·9	62·9 (64·1	0·9 0·4)
XI	—	64	—	66	64	62	63	58	62	65	66	63·3	0·8	62·9 (61·7	0·6 0·4)
XII	36	52	49	—	47	—	43	45	48	40	47	45·2	1·6	45·3	0·9

(*b*) *Absolute lengths*

Although a knowledge of the absolute lengths of the lampbrush chromosomes does not bear directly on the problem of chromosome identification, it is convenient to deal with this topic now. Our information is derived from freshly excised oocytes of diameters ranging from 0·6 to 1·7 mm. Two or more identified chromosomes lying in medium C

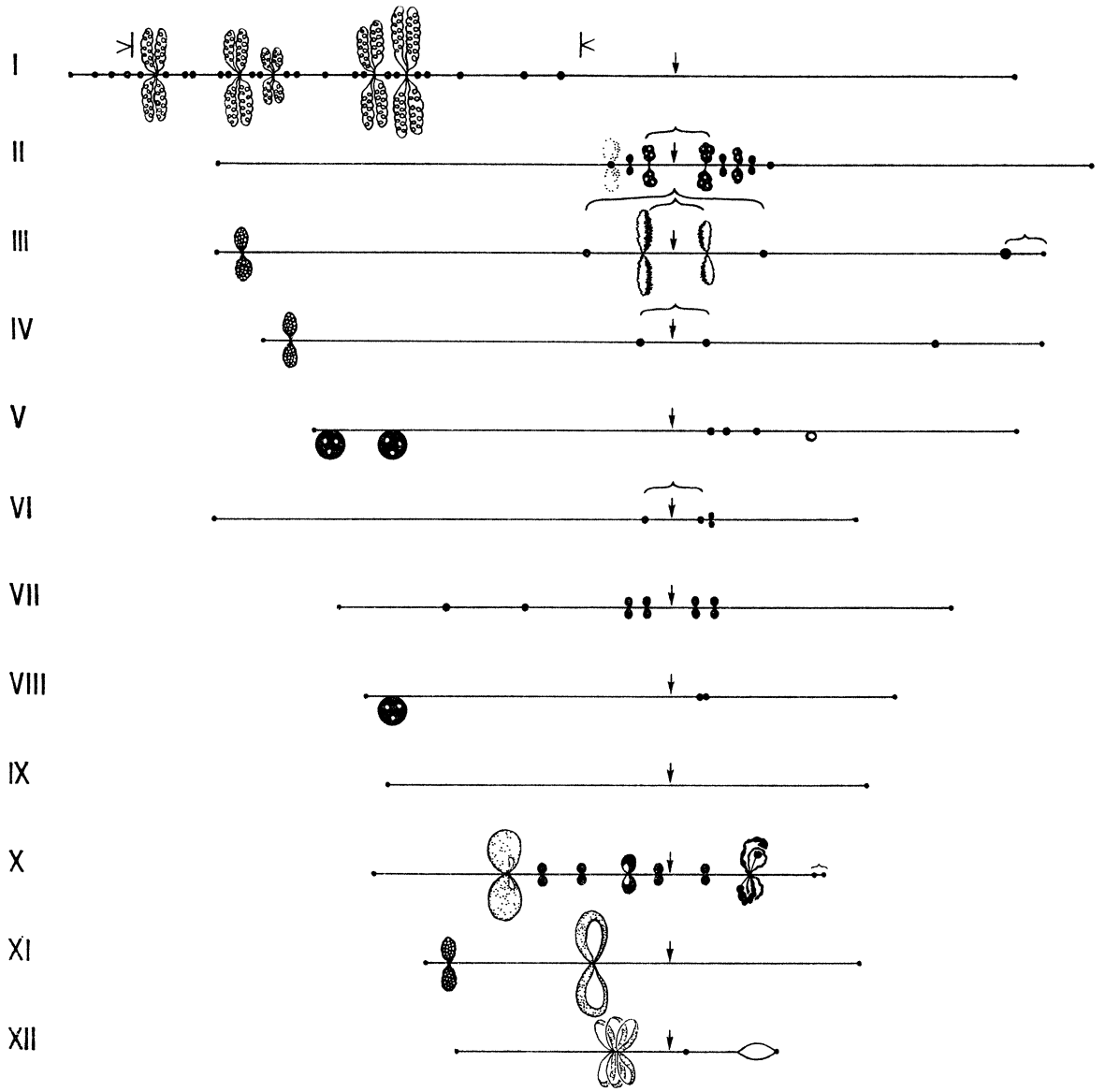

FIGURE 2. Working map of the twelve lampbrush chromosomes of *T. c. carnifex*. Centromere positions are indicated by the vertically alined arrows. Structures which often show 'reflected fusion' are linked together by brackets. The limits of the heteromorphic region of chromosome I are marked by ⩾ and ⩽. Further explanation in text.

were drawn from each of sixty-four oocytes from twelve females, and their lengths measured. From these measurements mean estimated lengths for chromosome V were calculated on the basis of the known relative lengths of other chromosomes to V.

The results are shown in table 2, where the oocytes are classified by size. Each entry is an estimated length of chromosome V from one oocyte, the letter in brackets following

each entry signifying the newt from which the oocyte was taken. We had anticipated finding a trend towards shorter chromosomes in larger oocytes, since at ovulation chromosome V measures only a few tens of microns in length. No such trend is apparent within the size range of the oocytes studied, a range which covers the entire phase of compact yolk accumulation. Chromosome contraction must therefore occur suddenly at the very end of yolk accumulation, and since we have not included oocytes on the verge of ovulation our data do not record this contraction.

A striking feature of table 2 is the extreme variability of chromosome length within oocyte size classes. We cannot associate this variability with any known factor.

TABLE 2. *T. c. carnifex*

oocyte diameter (mm)	estimated lengths of chromosome V (μ)				
0·60	495 (*D*)	560 (*E*)	645 (*D*)	681 (*D*)	
0·66	545 (*E*)	562 (*E*)	826 (*C*)		
0·72	807 (*C*)	824 (*M*)			
0·78	545 (*Q*)	572 (*P*)	632 (*W*)	690 (*P*)	699 (*P*)
0·84	440 (*R*)	443 (*R*)	503 (*P*)	528 (*E*)	565 (*W*)
0·90	369 (*R*)	384 (*R*)	538 (*P*)	704 (*M*)	778 (*L*)
0·96	411 (*R*)	518 (*F*)	587 (*E*)	663 (*J*)	715 (*J*)
1·02	443 (*F*)	470 (*W*)	458 (*F*)	596 (*N*)	760 (*E*)
1·08	508 (*J*)	578 (*M*)	582 (*C*)	617 (*N*)	716 (*J*)
1·14	571 (*N*)	623 (*C*)	641 (*L*)	643 (*M*)	
1·20	585 (*E*)	591 (*G*)	667 (*N*)		
1·26	536 (*D*)	600 (*M*)	638 (*M*)	660 (*C*)	
1·32	588 (*L*)	674 (*M*)			
1·38	474 (*E*)	530 (*N*)	582 (*C*)	582 (*D*)	
1·44	465 (*D*)	535 (*D*)			
1·50	489 (*D*)				
1·56	560 (*D*)	568 (*D*)			
1·62	446 (*E*)	468 (*D*)			
1·68	472 (*E*)				

(*c*) *Recognition characters*

Figure 2 is a working map of the lampbrush chromosomes of *T. c. carnifex*, showing the relative lengths of the twelve members of the complement, the positions of their centromeres (as discussed in §III *d*), and the locations of useful recognition characters or 'landmarks'. In the working map the longer arm of each chromosome is drawn projecting to the left; this convention defines the 'left-hand end' of each chromosome. In the following account the positions of the landmarks will be given in terms of 'units' from the left-hand end, a 'unit' being defined as one-hundredth part of the length of chromosome V, or its equivalent in length on another chromosome.

Before discussing the recognition characters some general remarks are necessary. Reference will frequently be made to 'normal' lampbrush loops in contradistinction to landmarks. The adjective 'normal' is intended to signify the appearance of the overwhelming majority of lateral loops in a typical preparation. Normal lateral loops are attached in pairs or multiples of pairs (clusters) to chromomeres. They are but slightly contorted and it is usually possible to follow through their entire lengths without difficulty. In large examples a loop axis is generally visible (e.g. figures 8 *a* and *b*; and 35, 36, plate 18), though its thickness verges on the resolving limit. Around this axis lies a less refractile

'matrix', which on casual inspection appears to be made up of minute granules, and which for purposes of illustration has been drawn in this way. However, when large normal loops are carefully observed under critical illumination the material around the loop axis frequently gives the appearance of being made up of exceedingly fine radially projecting

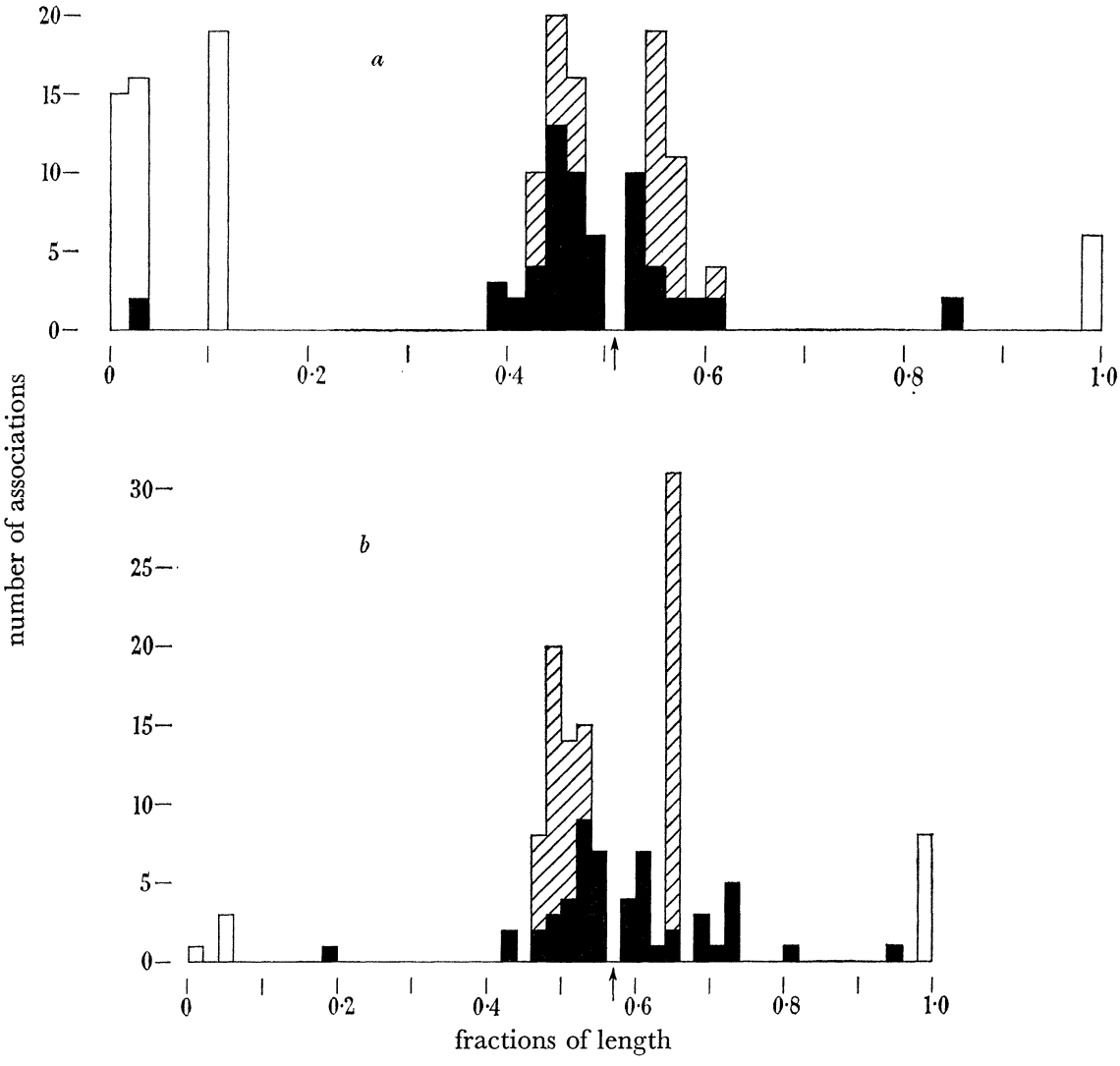

FIGURE 3. *T. c. carnifex* ♀*B*. Histograms showing the distributions of associations between homologous chromosomes (*a*) within forty-three examples of bivalent V and (*b*) within thirty-seven examples of bivalent VIII. Sphere-bearing arms of both chromosomes lie to the left of the centromeres, whose positions are indicated by arrows. The relative lengths of the histograms correspond to the length relationship between chromosomes V and VIII. Outlined white areas represent gene product fusions (of telomeres and of spheres), black areas represent chiasmata, and cross-hatched areas represent axial granule fusions.

threads. Smaller normal loops are of similar appearance but they may not display loop axes as clearly or at all. Each normal loop is asymmetric, one end being wider and more dispersed, the other thinner and more dense. There is a gradual transition from one condition to the other along the length of the loop. Loop axes are more evident in the wider and more dispersed portions.

Reference will also be made to 'granular' loops. The adjective 'granular' is intended to signify the appearance of lateral loops within whose matrix of less refractile material highly refractile spherical granules or globules 0·5 μ or more in diameter are embedded. The granules show Brownian movement but none the less remain attached to lateral loops. Similar granules also occur free in the nuclear sap. Granular loops are seen in preparations made in media B or C but not in preparations dispersed in medium A; in

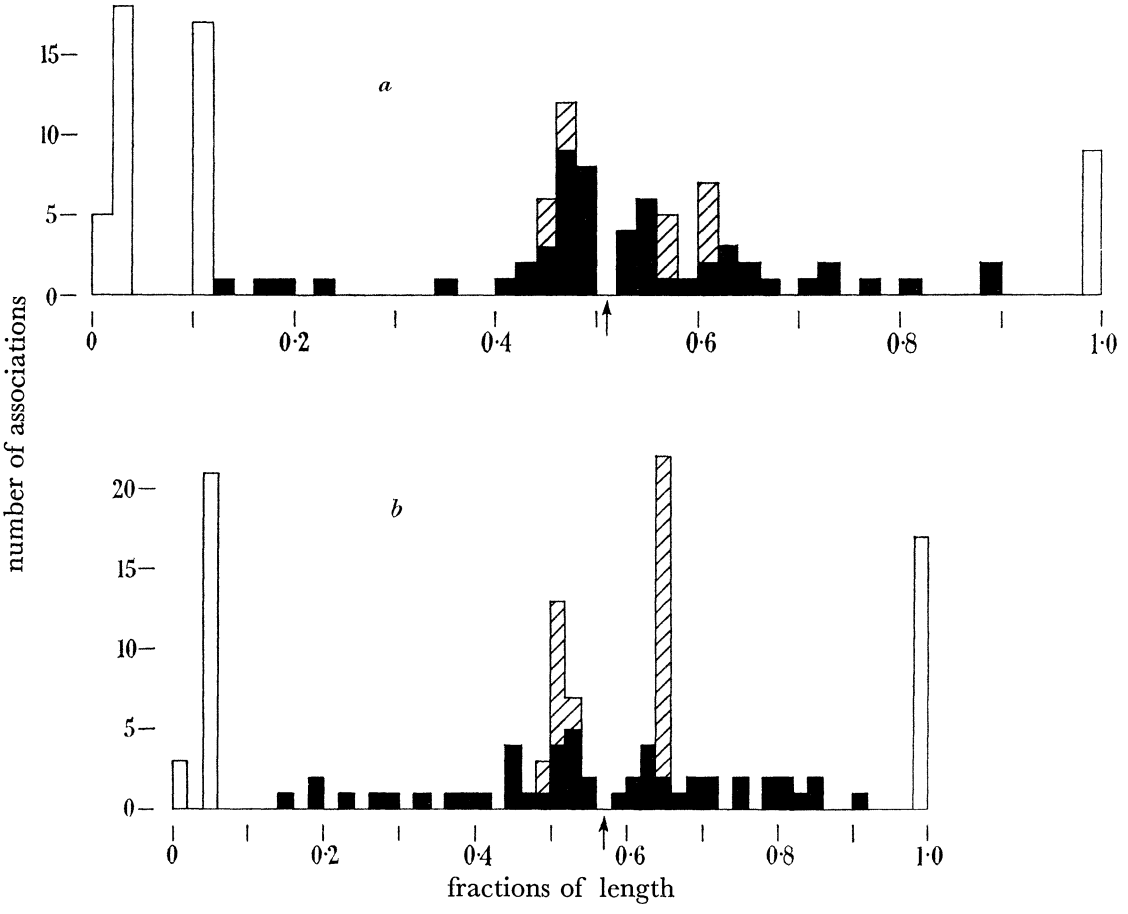

FIGURE 4. *T. c. carnifex* ♀*A*. Histograms showing the distributions of associations between homologous chromosomes (*a*) within twenty-nine examples of bivalent V and (*b*) within thirty-five examples of bivalent VIII. Sphere-bearing arms of both chromosomes lie to the left of the centromeres, whose positions are indicated by arrows. The relative lengths of the histograms correspond to the length relationships between chromosomes V and VIII. Outlined white areas represent gene product fusions (of telomeres and of spheres), black areas represent chiasmata, and cross-hatched areas represent axial granule fusions.

medium A the granules pass into solution leaving loops of 'normal' appearance. In media which conserve the granules there is great variation between oocytes as to the numbers of lateral loops carrying granules (and also the numbers of free granules in the nuclear sap); at many sites loops may be granular in one preparation but without granules in another. In any one newt smaller oocytes tend to contain more granular loops than do larger oocytes. Moreover, we have the distinct impression that some batches of oocytes give preparations with more, others with fewer, granular loops (and free granules). It is possible that the proportion of granular to non-granular (normal) lateral loops varies

according to an oocyte's physiological state at the time of its removal from a newt ovary; however, this is merely surmise. Apart from the variable granular loops there are also, at certain sites, loops which are consistently granular in all appropriately mounted preparations. Some of these have recognition value.

Each lampbrush chromosome ends in what appears at first sight to be a particularly large chromomere. In fixed and stained preparations these telomeres each consist of a small crescent of Feulgen-positive material to which a smooth round mass of Feulgen-negative material is attached. There are intercalary objects of similar nature at certain places on the chromosomes and these we term 'axial granules'. When of relatively small size, 1 or 2 μ in diameter, these axial granules appear to lie symmetrically within the chromosome axis, though as with the telomeres each contains only a small superficial crescent of Feulgen-positive material to one side of the main mass. In the case of larger axial granules the Feulgen-positive crescent lies more nearly in the chromosome axis, with the main mass of Feulgen-negative material displaced laterally.

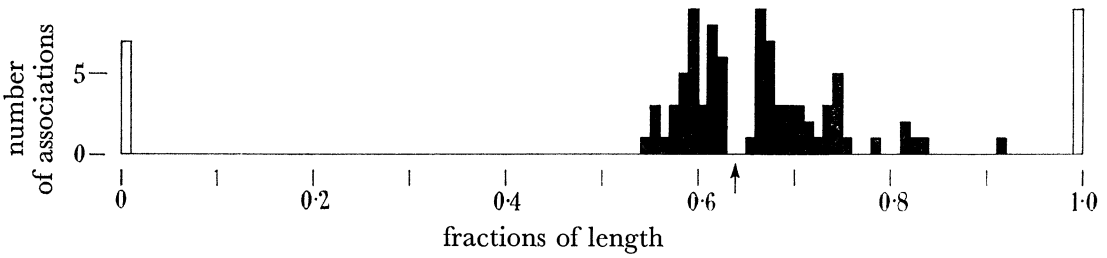

FIGURE 5. *T. c. carnifex* ♀*P.* Histogram showing the distribution of associations within forty-two examples of bivalent I. The heteromorphic arm of chromosome I lies to the left of the centromere, whose position is indicated by an arrow. Outlined white areas represent telomere fusions, black areas represent chiasmata.

A description of the more useful recognition characters for each chromosome now follows.

Chromosome I: relative length 134 units.

In oocytes ranging in diameter from 0·9 to 1·6 mm, conspicuous axial granules and much contorted long loops are the most evident peculiarities of this chromosome. There are some twenty-five of these axial granules, smooth, round and considerably larger (1 to 2 μ diameter) than the generality of chromomeres of this and other chromosomes. The axial granules, which give part of this chromosome a beaded appearance, are restricted to the region lying between 0 and 70 units. Scattered along this same region are several long contorted multiple loops (figures 8*d*; and 37, 73, plates 18 and 21) of differing sizes and degrees of refractility, which stand out in particularly strong contrast to normal loops when the chromosomes are isolated in medium A; this is because their matrix passes into solution less readily than the matrix of normal loops. The contorted loops are interspersed with the large axial granules but themselves arise from very tiny chromomeres. When fully developed they form tangled masses of filaments of uniform thickness which hang in clusters from the chromosome axis. No other chromosome bears such lateral loops: they are peculiar to chromosome I.

The regions of bivalent I which bear large axial granules and contorted loops never show precise correspondence homologue with homologue. For this reason we have termed them heteromorphic. One chromosome always carries a greater number of contorted loops than its partner. The interhomologue differences are constant from oocyte to oocyte within individual newts, but are not constant as between individuals (figure 7*a* to *d*).

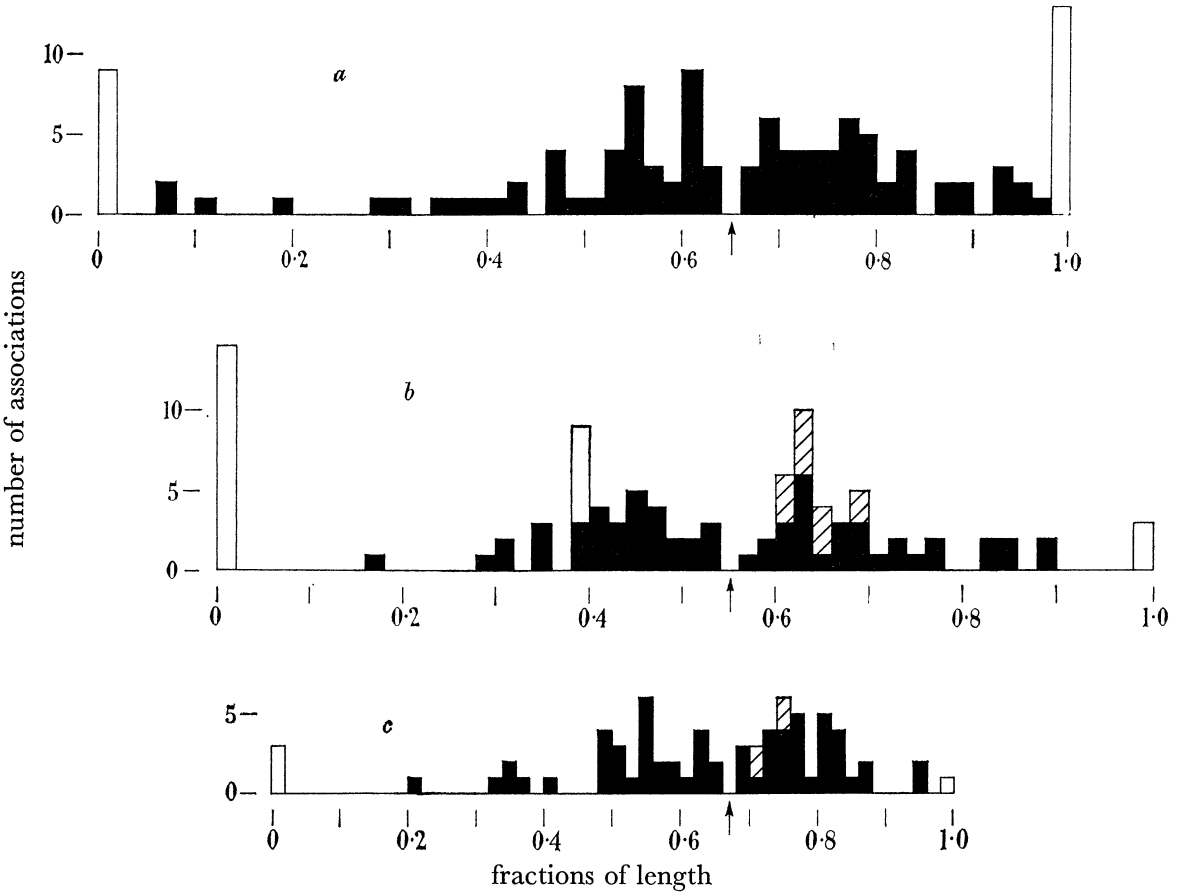

FIGURE 6. *T. c. carnifex*, several ♀♀. Histograms showing the distributions of associations between homologous chromosomes (*a*) within thirty-seven examples of bivalent *X*, (*b*) within forty-five examples of bivalent XI, and (*c*) within thirty-five examples of bivalent XII. The longer arms lie to the left of the centromeres, whose positions are indicated by arrows. The relative lengths of the histograms correspond to the length relationships between chromosomes X, XI and XII. Outlined white areas represent gene-product fusions, of telomeres, and of giant loops in the case of bivalent XI, black areas represent chiasmata, and cross-hatched areas represent axial granule fusions.

In the working map we have indicated those sites (at 12, 24, 29, 43 and 48 units) where conspicuous contorted loops are most frequently present on that homologue which bears the greater number of such loops. In certain newts, and notably ♀*J*, the contorted loops at one site (24 units) are very much more refractile than all others (figures 7*b* and 8*e*; and 31, 38, plate 18) and serve for the immediate recognition of chromosome I.

The axial granules and contorted loops characteristic of chromosome I are less conspicuous in smaller oocytes, though they can be clearly identified in oocytes ranging in size down to 0·7 mm diameter. In such small oocytes the contorted loops are smaller and

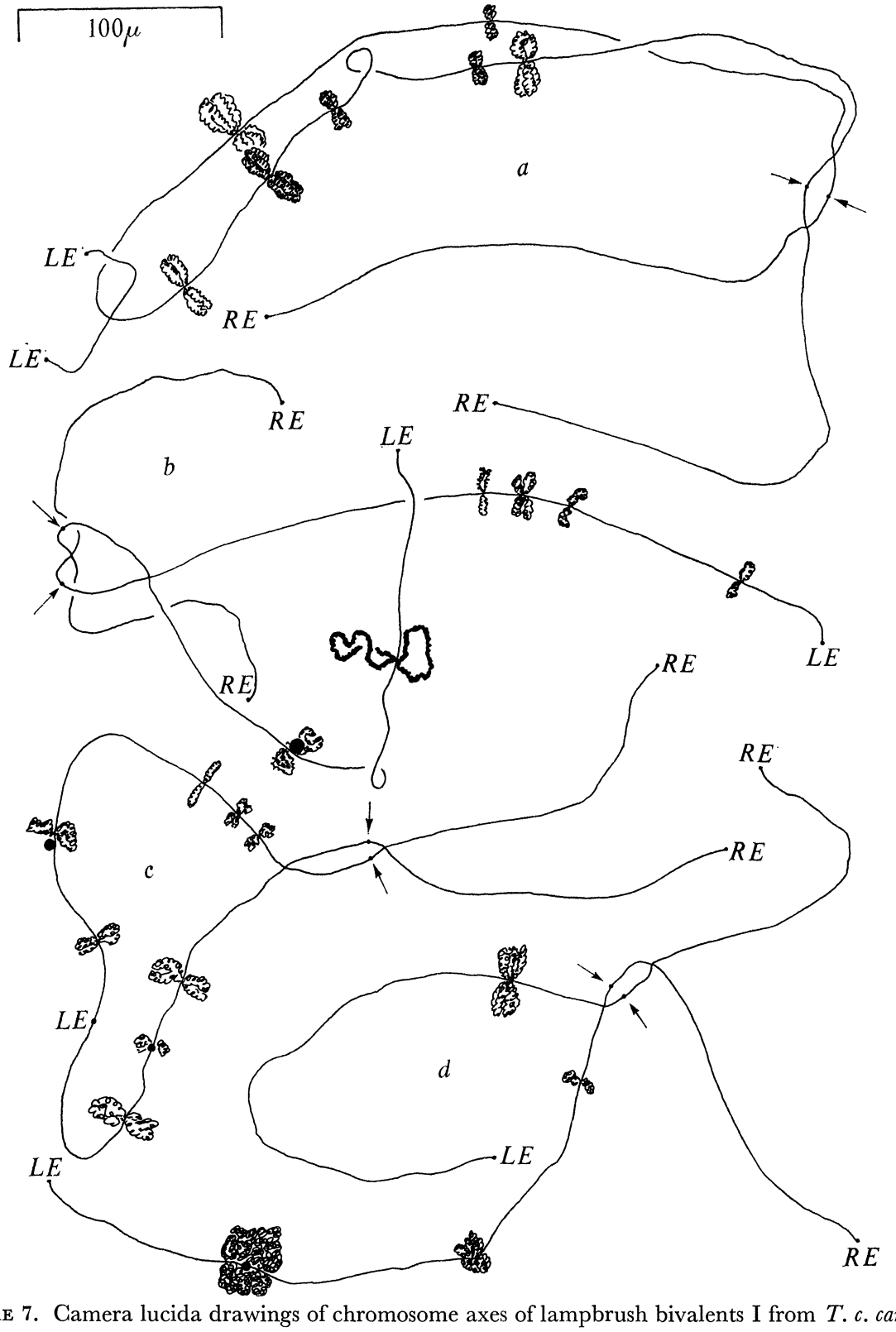

FIGURE 7. Camera lucida drawings of chromosome axes of lampbrush bivalents I from *T. c. carnifex* oocytes in the size range 0·8 to 1·2 mm diameter showing the more conspicuous 'heteromorphic' loops which characterize four individual females: (*a*) is from ♀*C*; (*b*) is from ♀*J*; (*c*) is from ♀*P*; and (*d*) is from ♀*R*. The centromeres are marked by arrows; the ends of left and right arms are indicated by *LE* and *RE*.

less refractile, and they give the appearance of having originated from normal, though particularly long, lateral loops (figures 8*a* and *b*; and 35, 36, plate 18).

In oocytes 1 mm and less in diameter there are several large granular loops (figure 8*c*) within the heteromorphic regions of chromosome I, but we have not paid much attention to their distribution. Similar loops are present on other chromosomes.

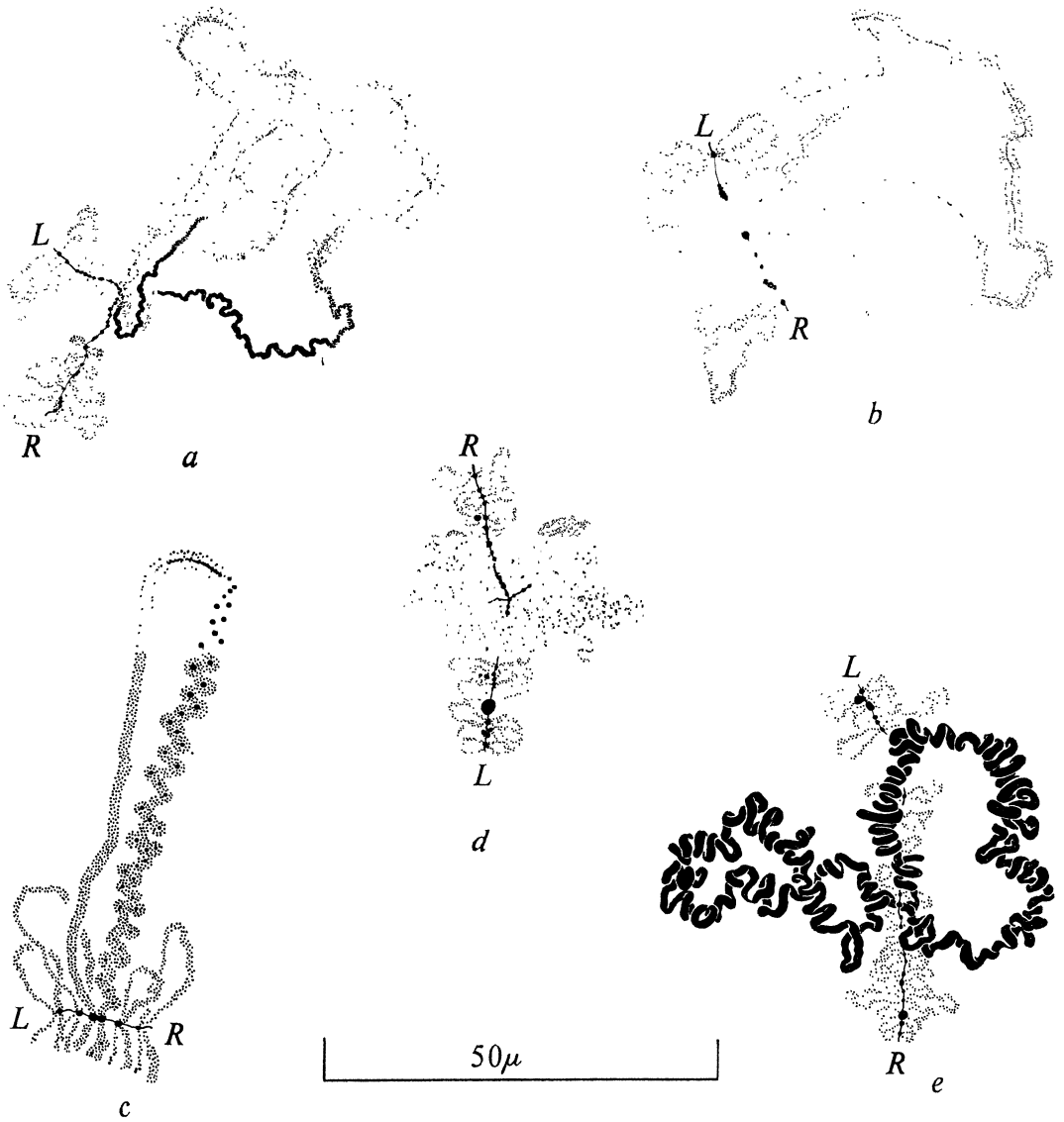

FIGURE 8. Camera lucida drawings of distinctive lateral loops at various sites along the heteromorphic arms of bivalent I of *T. c. carnifex*: (*a*) is from ♀*D*, 0·6 mm oocyte, at 6 units; (*b*) is from ♀*C*, 0·7 mm oocyte, at 12 units; (*c*) is from ♀*G*, 0·9 mm oocyte, at 66 units on the arm *not* drawn on the working map; (*d*) is from ♀*CA*, 1·2 mm oocyte, at 12 units; (*e*) is from ♀*J*, 1·1 mm oocyte, at 24 units. Orientation of the chromosome axes in relation to the working map is indicated by *L* (left) and *R* (right).

Chiasmate association between the two chromosomes forming bivalent I never occurs in the region between 9 and 72 units (figure 5). Generally the very long heteromorphic arms are free to their extremities—and these great lengths free from chiasmata are distinctive. Occasionally the heteromorphic arms are associated in the region between 0 and 9 units (figure 7*a*).

Chromosome II: relative length 125 units.

In the mid-region of this chromosome, and extending from 59 to 79 units of its length, are a series of lateral 'lumpy' objects which are more or less spherical in form but with irregular outlines (figures 9*a* and *b*, 10*a*, *b*, and *c*; and 42, 43, 44, 45, plate 19). These objects are in general dense and uniform in texture, though the largest, some 10 μ in size, may contain a few vacuoles. The lumpy objects are of similar appearance in small and in large oocytes, and for the most part they give no indication of having originated from structures of loop form. In small oocytes of 0·6 to 0·9 mm diameter they are by far the most striking recognition character within the entire chromosome complement. Although similar lumpy objects are also found near the middle of chromosome VII, those of chromosome II are considerably and consistently larger, and more numerous.

These lateral lumpy structures may be fused together in various ways: within the length of one and the same chromosome, producing reflexions of its axis; or between partner chromosomes, not necessarily at homologous places (figures 9*b*, 10*b* and *c*). The lumpy objects are not symmetrically distributed about the mid-point of chromosome II and it is therefore possible by extension to distinguish between the two chromosome arms on either side of this central region. Unlike several recognition characters for other chromosomes, the appearance and serial distribution of the lumpy objects of chromosome II varies little from newt to newt.

We have paid particular attention to a 'marker' loop which lies just to the left of the series of lumpy objects at 56 units (*M* in figures 9*a* and *b*). This loop, which in small oocytes is of great size, projecting 50 μ or more from the chromosome axis, originates at or very close to a large axial granule. The marker loop is strikingly asymmetric, the thicker portion being stiff and granular, its granules variable in size. The matrix of the marker loop is rigid, as demonstrated by the absence of Brownian movement amongst its granules. There is another large axial granule, not accompanied by conspicuous loops, which lies just to the right of the series of lumpy objects at 78·5 units.

In oocytes of less than 1 mm diameter there are a few conspicuous granular loops which lie well away from the middle region of chromosome II, but we have not paid great attention to them.

Chromosome III: relative length 118 units.

In oocytes ranging in size from 0·6 to 1·2 mm diameter the most striking feature of chromosome III is a median group of stiff, highly refractile loops which are usually amalgamated to form a spiky irregular mass. On either side of this group of stiff loops are two axial granules 2 to 3 μ in diameter at 53 and 77 units, which in most examples of this chromosome are fused together giving rise to an axial reflexion (figure 50, plate 19). Since chiasmata are formed within this reflected region or nearby, and since the large axial granules of partner chromosomes may also be fused to one another, the mid-region of chromosome III from small oocytes generally appears as a complex knot (figure 11*b* and *c*). In oocytes larger than 1·2 mm diameter the stiff loops of chromosome III are less conspicuous or absent altogether, but the reflected axial region is still diagnostic.

In oocytes ranging in size from 0·6 to 0·8 mm and from 1·1 to 1·6 mm, the left arm of chromosome III bears subterminally at 3·5 units conspicuous vacuolated objects up to

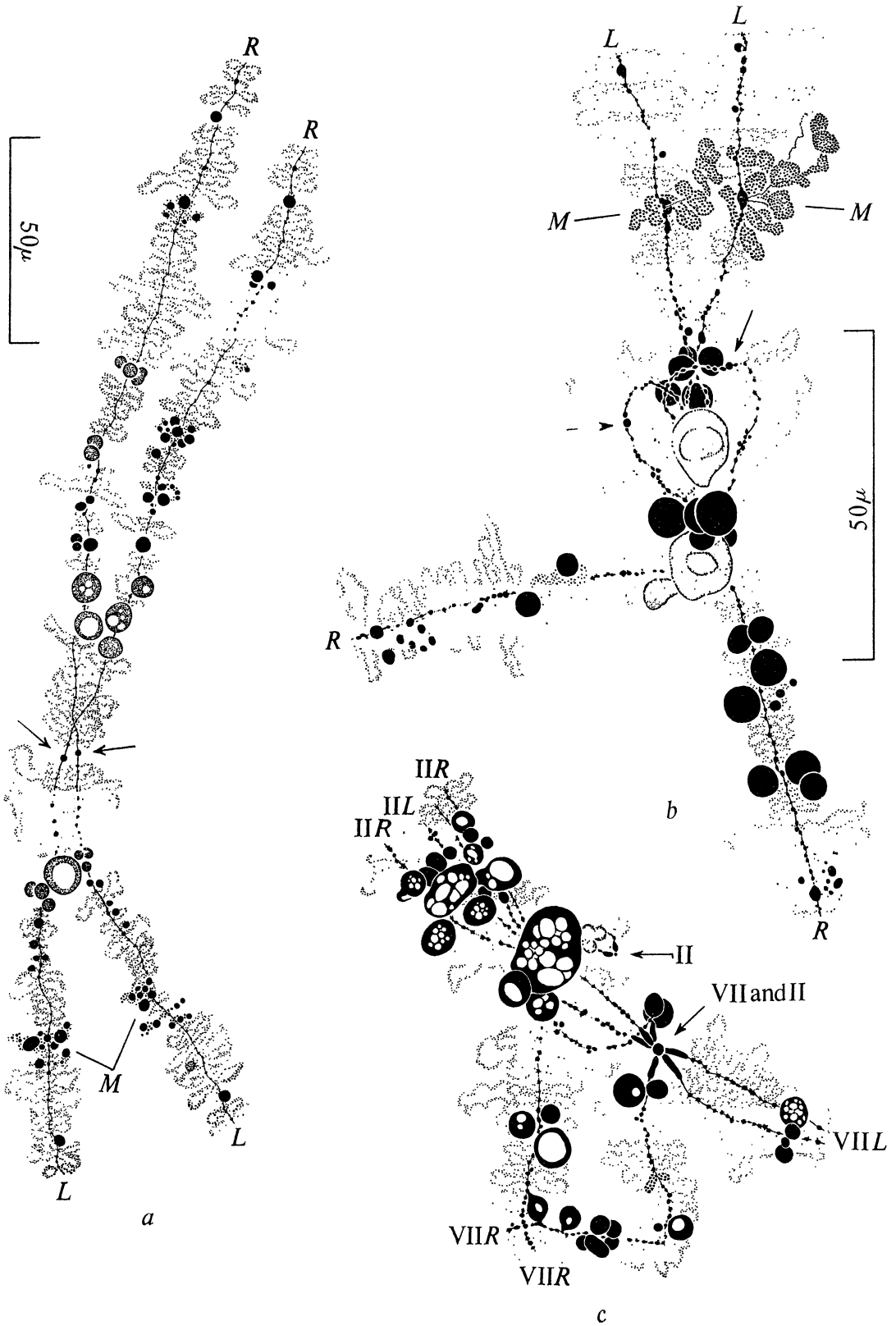

FIGURE 9. (*a*) and (*b*) are camera lucida drawings of the middle regions, from about 55 to 80 units, of bivalents II of *T. c. carnifex*: (*a*) is from ♀*O*, 1·3 mm oocyte, displaying a simple extended configuration and 'marker' loops (*M*) inconspicuous (magn. × 660); (*b*) is from ♀*W*, 0·7 mm oocyte, displaying an axial configuration complicated by fusion, and marker loops conspicuous (magn. × 1060). (*c*) is from *T. c. karelinii* ♀*G*, 1·1 mm oocyte, displaying complex interconnexions between the middle regions of bivalents II and VII, and notably a fusion between both centromeres of VII with one centromere of II (magn. × 1060). Orientation of the chromosome axes in relation to the working maps is indicated by *L* (left) and *R* (right). The centromeres are marked by arrows.

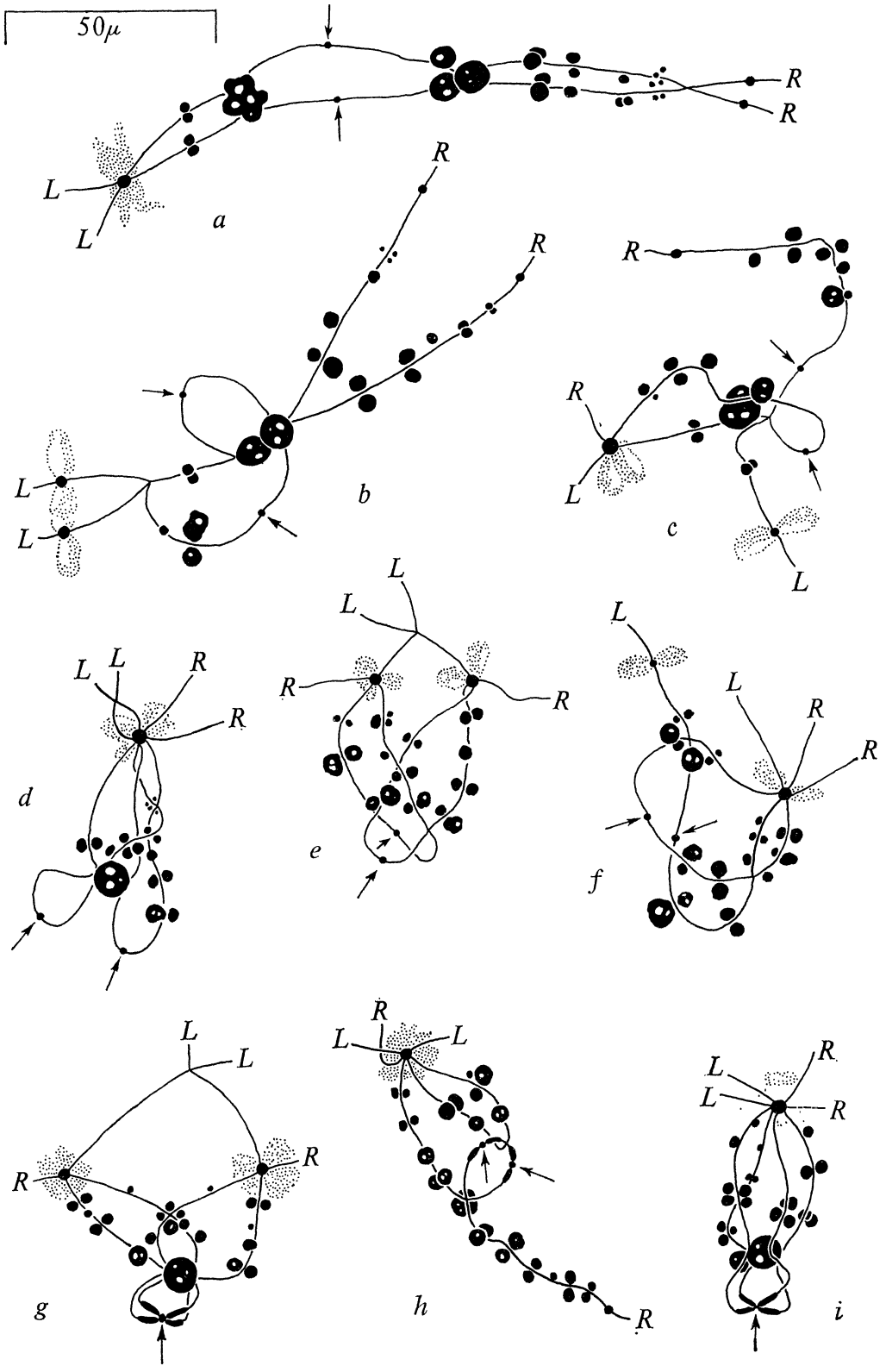

FIGURE 10. Camera lucida drawings showing the courses of chromosome axes in regions neighbouring the centromeres of lampbrush bivalents II from oocytes in the size range 0·9 to 1·3 mm diameter: (*a*), (*b*) and (*c*) from *T. c. carnifex*; (*d*), (*e*) and (*f*) from *T. c. cristatus*; (*g*), (*h*) and (*i*) from *T. c. karelinii*. Different axial relationships established by a variety of axial granule and 'lumpy loop' fusions and by chiasmata are demonstrated. The 'marker' loops are shown dotted, the centromeres are indicated by arrows, and the left and right arms of the chromosomes are marked *L* and *R* respectively.

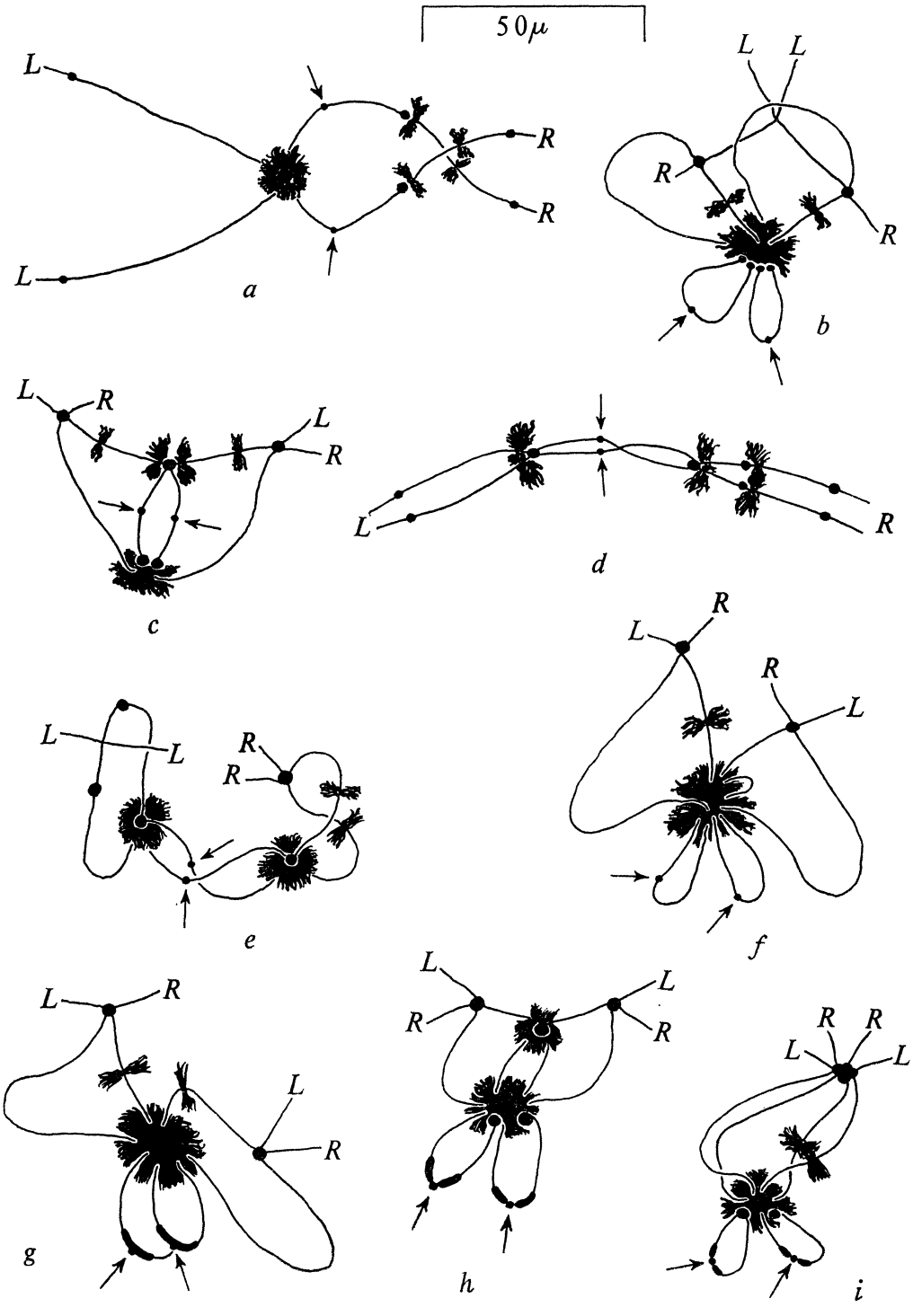

FIGURE 11. Camera lucida drawings showing the courses of chromosome axes in regions neighbouring the centromeres of lampbrush bivalents III from oocytes in the size range 0·8 to 1·4 mm diameter: (*a*), (*b*) and (*c*) from *T. c. carnifex*; (*d*), (*e*) and (*f*) from *T. c. cristatus*; and (*g*), (*h*) and (*i*) from *T. c. karelinii*. Different axial relationships established by a variety of axial granule and 'stiff loop' fusions and by chiasmata [in (*b*), (*d*) and (*e*)] are demonstrated. The centromeres are marked by arrows, and the longer (left) and shorter (right) arms of the chromosomes are marked *L* and *R* respectively.

8 μ or so in size and with irregular outlines (figures 17*a*; and 49, plate 19). These objects, generally found in pairs though on occasion singly or in clusters of three or four, are different in texture from the lumpy objects present on chromosome II; for want of a more descriptive title we have termed them 'currant buns'. Oddly enough, in oocytes of about 0·9 to 1·0 mm diameter, these subterminal objects are inconspicuous or absent, their places being occupied by normal loops. Very short lateral loops characterize the chromosome region lying to the right of the 'currant buns'. The right arm of chromosome III at 112 units bears a subterminal axial granule of 3 to 4 μ diameter, by means of which, in the absence of 'currant buns', the two ends of this chromosome may still be distinguished from one another. The subterminal axial granules of bivalent III may be separate from one another or fused together. Fusion between this axial granule and the neighbouring telomere is also often encountered, giving rise to a terminal axial reflexion forming a ring (figure 50, plate 19).

Chromosome IV: relative length 110 units.

This chromosome does not show very striking or distinctive landmarks. Its left arm may possess subterminal 'currant buns' identical in appearance to those of chromosome III (figure 17*b*), and just as in the case of chromosome III the 'currant buns' of chromosome IV are well developed in oocytes ranging from 0·6 to 0·8 mm and from 1·1 to 1·6 mm diameter, inconspicuous or absent in oocytes of 0·9 to 1·0 mm diameter. This circumstance might lead to confusion of chromosome IV with chromosome III. Moreover, as in the case of chromosome III, there are in the mid-region of chromosome IV at 53·5 and 62·5 units two conspicuous axial granules which are frequently fused together giving rise to an axial reflexion (figure 52, plate 20). Fortunately, however, for purposes of identification, chromosome IV does not bear a median group of stiff loops at any stage, and furthermore the mid-region axial granules of chromosome IV are less than half as far apart (9 units) as the similar mid-region granules on chromosome III (24 units). The right arm of chromosome IV bears a 2 to 3 μ axial granule about one-third of the distance from the end to the middle of the chromosome, at 96 units; this landmark serves to distinguish the two arms of chromosome IV when dealing with examples lacking 'currant buns'.

Chromosome V: relative length 100 units.

This chromosome may be identified by two similar spherical objects, smooth in outline and reaching some 15 μ in diameter, one lying very close to the chromosome end at 2 units and the other a little further in at 11 units along the left arm (figures 12*b* to *h*; and 29, 30, 57, plates 17 and 20). Though directly attached to the chromosome axis these 'spheres' lie to one side: it is often possible to follow the axis running around part of the circumference of the sphere. Small examples of the spheres are homogeneous; larger examples contain vacuoles, whilst extremely large spheres contain vacuoles and single very dense granules within each of these vacuoles.

Homologous arms of chromosome V may each carry two separate spheres (figures 12*b* and *c*; and 29, plate 17) or they may be jointly associated with spheres, association occurring in the distal (figures 12*d*; and 57, plate 20 , proximal (figure 12*e*), or much more

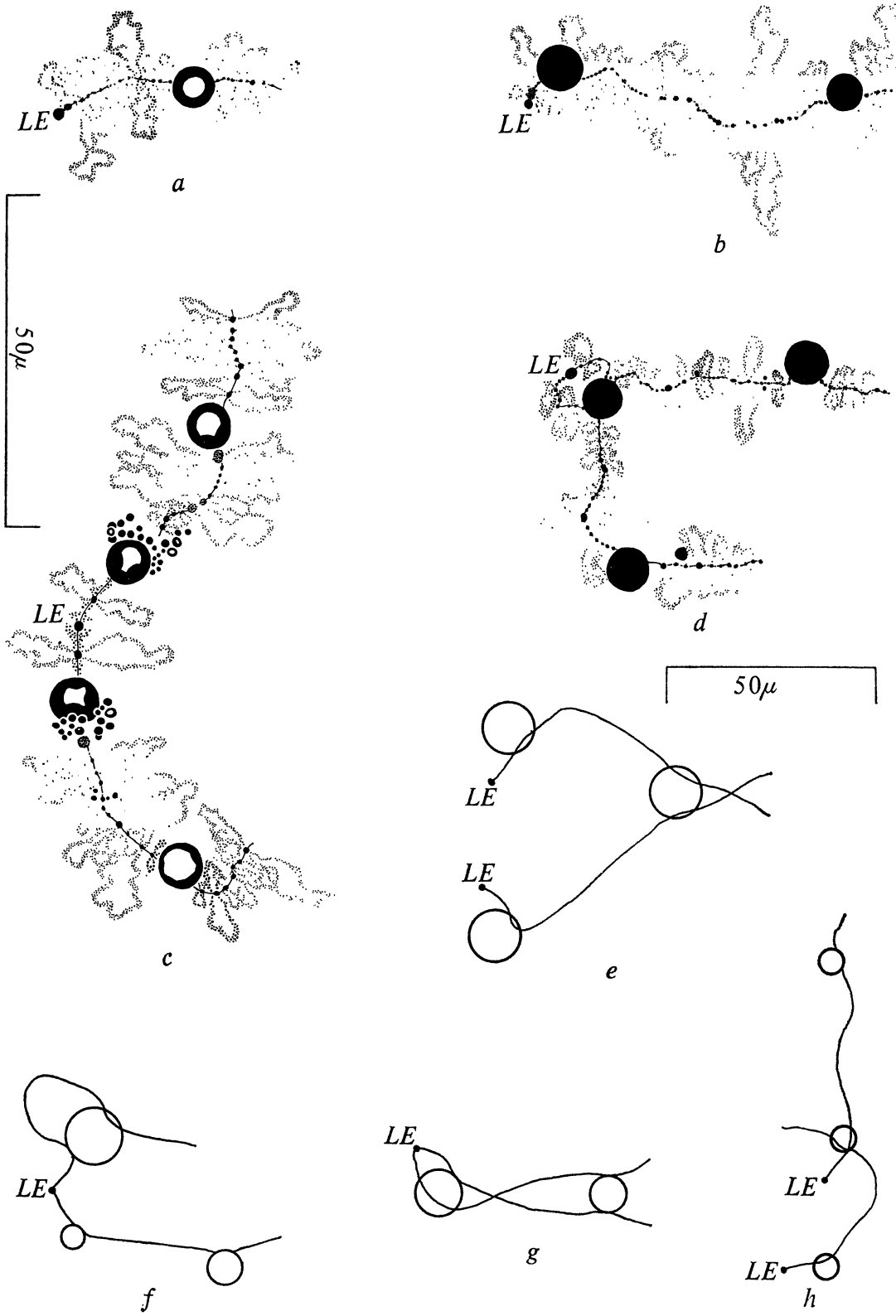

FIGURE 12. Camera lucida drawings of *T. c. carnifex* chromosome regions including the 'sphere' sites. In (*e*), (*f*), (*g*) and (*h*) the courses of chromosome axes and the spheres are outlined only. (*a*) is from ♀*D*, 0·6 mm oocyte, and shows the left end of chromosome VIII. All the remaining drawings are left ends of bivalents V; (*b*) is from ♀*D*, 0·6 mm oocyte; (*c*) is from ♀*J*, 0·8 mm oocyte, showing small refractile granules adjacent to the distal spheres and fusion of left terminal granules; (*d*) is from ♀*E*, 1·4 mm oocyte, showing fusion of homologous distal spheres and fusion of left terminal granules; (*e*) is from ♀*A*, 1·4 mm oocyte, showing fusion of homologous proximal spheres; (*f*) is from ♀*B*, 1·0 mm oocyte, showing reflected fusion of distal with proximal spheres, and fusion of left terminal granules; (*g*) is from ♀*B*, 1·0 mm oocyte, showing homologous fusions between terminal granules, distal spheres and proximal spheres; (*h*) is from ♀*O*, 1·3 mm oocyte, showing non-homologous fusion of the distal sphere of one chromosome with the proximal sphere of its partner. Terminal granules are marked *LE*. (*a*), (*b*), (*c*) and (*d*) are magnified × 1060; (*e*), (*f*), (*g*) and (*h*) are magnified × 660.

frequently at both distal and proximal sites (figures 12*g*; and 30, plate 17). The two sites on one and the same arm of chromosome V may occasionally be jointly associated with a single sphere (figure 12*f*), a combination which gives rise to a subterminal axial reflexion. Another unusual variant is two spheres attached on either side of the chromosome axis at

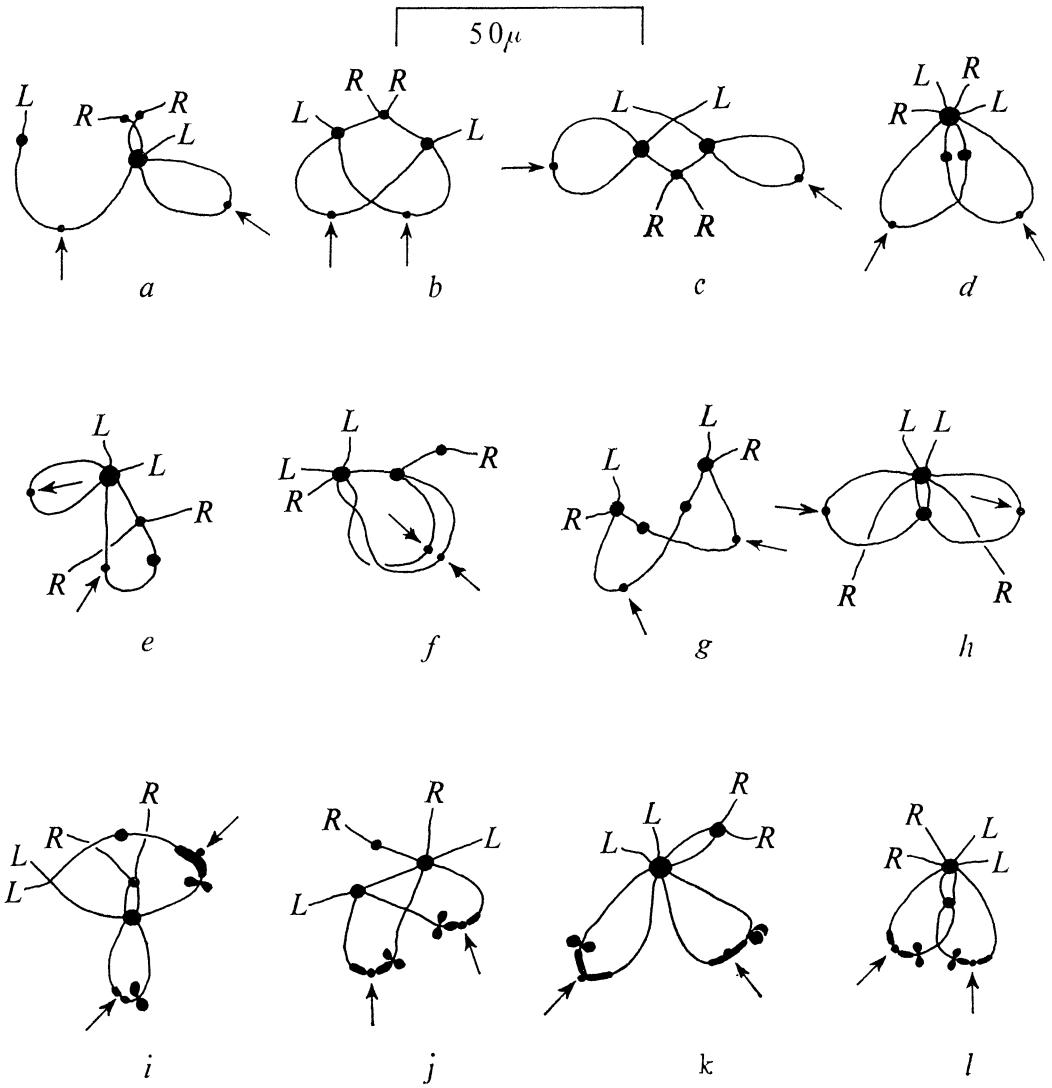

FIGURE 13. Camera lucida drawings showing the courses of chromosome axes in regions neighbouring the centromeres of lampbrush bivalents VI from oocytes in the size range 1·0 to 1·5 mm diameter: (*a*), (*b*), (*c*) and (*d*) from *T. c. carnifex*; (*e*), (*f*), (*g*) and (*h*) from *T. c. cristatus*; and (*i*), (*j*), (*k*) and (*l*) from *T. c. karelinii*. Different axial relationships established by axial granule fusions and by chiasmata [in (*b*), (*c*), (*d*), (*f*), (*g*), (*i*), (*j*) and (*l*)] are demonstrated. The centromeres are marked by arrows, and the longer (left) and shorter (right) arms of the chromosomes are marked *L* and *R*, respectively. The centric dimorphism shown in (*i*) characterizes bivalent VI of *karelinii* ♀*M*.

a single site. On rare occasions chromosome V may lack one or more of its spheres, though their sites may still be identified by the presence of clusters of tiny, highly refractile granules. When the spheres are present similar clusters of granules often lie in their immediate vicinity (figure 12*c*).

Although the spheres of chromosome V are usually most conspicuous, they tend to be smaller in smaller oocytes and in oocytes of less than 0·8 mm diameter from certain individual newts (e.g. ♀*C* and ♀*E*) they may be no more than 3 to 4 μ in diameter. When the spheres are so small they can only be recognized with difficulty, since there are other smooth axial granules of this order of size on other chromosomes. For positive identification of chromosome V one then needs to be familiar with the distribution of some rough axial granules which lie near the middle region of this chromosome (figure 59, plate 20). Three such granules are constantly present; they lie at 56, 57 and 62 units. Other axial granules more variable in their occurrence lie further to the left.

Chromosome VI: relative length 91 units.

This chromosome also possesses few striking landmarks. At 61 and 69 units are two axial granules which are very frequently fused together to produce symmetrically disposed axial reflexions on both homologues (figures 13; and 54, plate 20). There may also be cross-fusion at these sites between homologous chromosomes, with the production of a complex 'knot' (figure 13*d*). As has already been mentioned, axial reflexions also frequently occur on chromosomes II, III and IV, but those of chromosome VI are distinctive in that they are located well away from the mid-point of the chromosome, whereas the axially reflected parts of chromosomes II, III and IV are median.

Chromosome VII: relative length 87 units.

Within the mid-region of chromosome VII are some four to six places where paired lumpy objects are normally present. The sites most regularly occupied by these structures are at 41, 43·5, 50·5 and 53 units (figure 67, plate 21). In most examples of chromosome VII these objects are smaller in size and fewer in number than the similar structures on chromosome II, and it is only in small oocytes of 0·6 to 0·9 mm diameter that chromosomes VII and II are likely to be confused: the lumpy objects of chromosome VII are maximally developed in oocytes of this size. Chromosome VII lacks any structure comparable to the 'marker' loops of chromosome II, and in detail the linear distribution of lumpy objects on the two chromosomes is dissimilar.

Along the left arm of chromosome VII are two conspicuous axial granules at 15 and 26 units. These aid in the identification of chromosome VII when the median lumpy objects are of a low grade of development, and in particular prevent confusion between chromosomes VII and IX.

Chromosome VIII: relative length 75 units.

This chromosome may be identified by its left arm bearing at 3·5 units a single spherical object similar to the two spheres found on chromosome V (figures 12*a*; and 29, 30, 62, 63, plates 17 and 20). There is a correlation between the appearance of the spheres on chromosome V (size, presence or absence of vacuoles, intravacuolar granules) and the sphere on chromosome VIII, i.e. despite inter-oocyte variation, spheres on V and on VIII from a given oocyte have the same appearance. As in the case of chromosome V, each homologue of chromosome VIII may separately carry a sphere (figures 29, 62, plates 17 and 20), or alternatively the two homologues may be jointly associated with a single

sphere (figures 30, 63, plates 17 and 20): very occasionally two spheres may be attached on either side of the chromosome axis at the same site. In one preparation one sphere site of bivalent VIII was attached by a single sphere to both distal sphere sites of bivalent V.

In some newts (e.g. ♀♀*D*, *E* and *O*) a trap is provided for the unwary by the presence of another single, more or less spherical body or 'pseudo-sphere' lying at 11 units (as in figure 64, plate 20, though this is from *danubialis*). At first glance chromosomes V and VIII may be confused in such individuals; they can, however, be distinguished. The free arm projecting beyond the sphere of VIII is almost twice as long as that projecting beyond the distal sphere of V; the 'pseudo-sphere' of VIII differs in texture from the spheres of V or VIII, it has a less regular outline, does not fuse with its homologue, and is not directly attached to the chromosome axis.

There are several conspicuous rough axial granules towards the mid-region of chromosome VIII. Two of the largest of these granules lie immediately beside one another and they provide a landmark of recognition value at 48·5 units.

Chromosome IX: relative length 68 units.

This chromosome is the least distinctive member of the complement, and it can most easily be distinguished by a process of elimination once the rest of the chromosomes have been positively identified (figure 69, plate 21). There may be lumpy loops at one or two places on chromosome IX, but their presence is insufficiently regular for them to be of diagnostic value.

Chromosome X: relative length 64 units.

In oocytes ranging in diameter from 0·6 to 1·4 mm chromosome X can be recognized by five or more sites, well spaced out over its length, at which dense lumpy objects are laterally attached (figures 15*a* to *f*; and 71, 72, 74, plate 21). The sites most regularly occupied by these structures are at 24, 29·5, 36, 40·5, 47 and 53 units. The only structures with which these lumpy objects might be confused are those present on chromosomes II and VII, but in the case of both these chromosomes the lumpy objects are concentrated around the median region. Unlike the lumpy objects on chromosomes II and VII, those on chromosome X often give evidence of their derivation from loop structures; indeed in small oocytes the landmark at 53 units appears as two adjacent pairs of extremely refractile and relatively long loops having irregular swollen portions (figure 14*c*). All the conspicuous landmarks on chromosome X, with the exception of that at 18·5 units, appear in larger oocytes as groups of irregular dense lumps loosely attached around the chromosome axis at their respective places: they become progressively smaller the larger the oocyte and in oocytes of diameter 1·4 to 1·6 mm, where the lumpy objects on chromosome II are usually still conspicuous, those of chromosome X are generally small or absent.

Chromosome X is remarkable for its individual-specific characters, and once these are established they serve most readily for identification. In five females (*G*, *P*, *R*, *X* and *Y*) there are giant loops at 18·5 units on both homologues forming bivalent X (figures 15*a* and *b*; and 73, 74, 75, 78, 82, plates 21 and 22). In seven other females (*C*, *D*, *J*, *M*, *O*, *U* and *V*) there are inconspicuous structures at 18·5 units on both homologues (figures 15*c* and *d*; and 71, 72, 76, 77, plate 21). In yet other six females (*E*, *F*, *L*, *N*, *Q* and *W*) there are

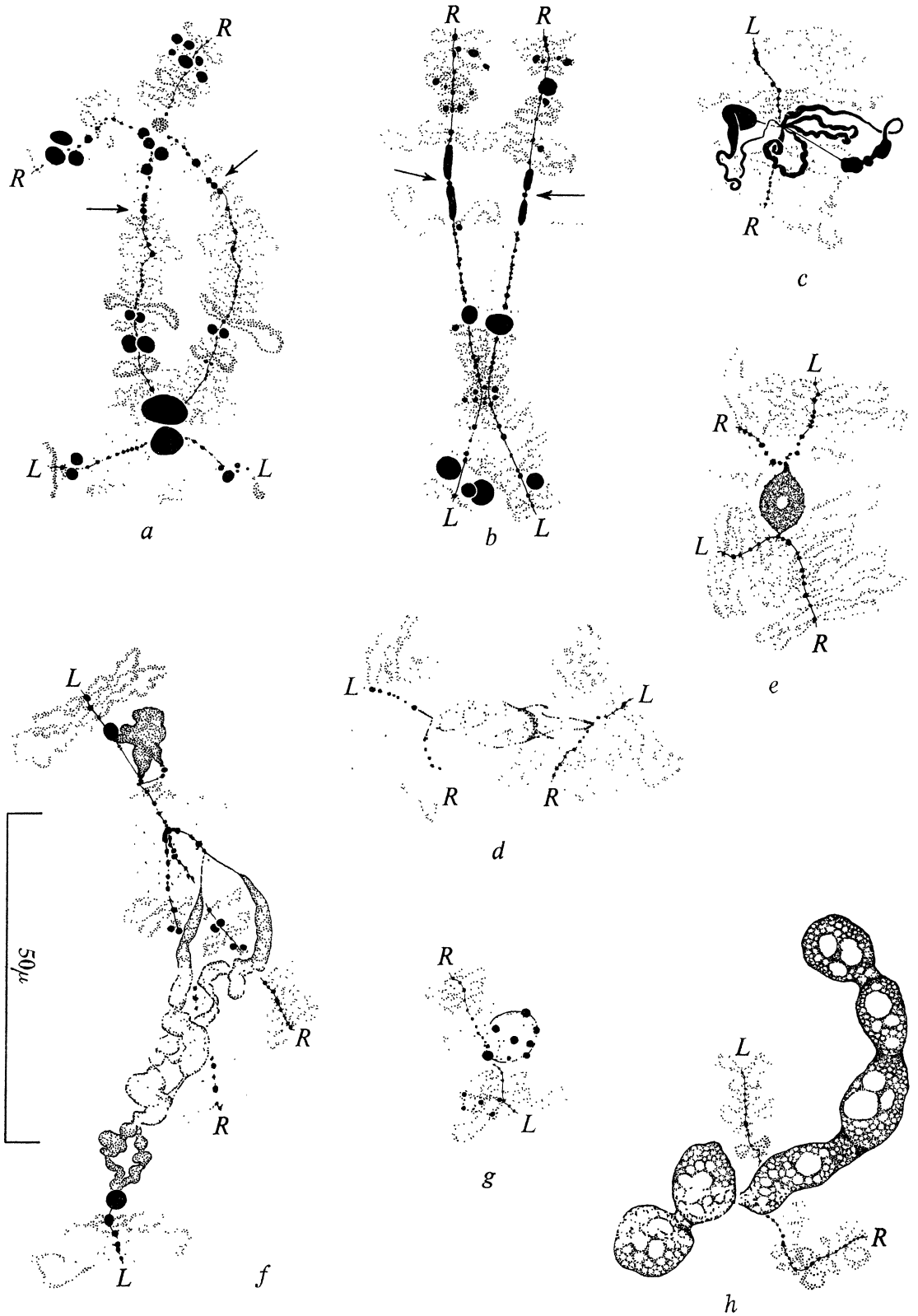

FIGURE 14. Camera lucida drawings of parts of bivalents X: (*a*) is from *T. c. carnifex* ♀*O*, 1·2 mm oocyte. It includes the centromeres, marked by arrows, and extends from about 28 to 48 units; (*b*) is from *T. c. karelinii* ♀*B*, 1·1 mm oocyte, and shows the region homologous to that depicted in (*a*); (*c*) is from *T. c. carnifex* ♀*C*, 0·7 mm oocyte, and includes the landmark located at 53 units. (*d*), (*e*), (*f*), (*g*) and (*h*) illustrate variation in morphology of the giant loops located at 18·5 units on *T. c. carnifex* bivalent X: (*d*) is from ♀*C*, 0·7 mm oocyte, (*e*) is from ♀*O*, 0·9 mm oocyte, and both show interhomologue fusion in individuals homozygous for small objects at 18·5 units; (*f*) is from a 0·7 mm oocyte of ♀*Q*, an individual heterozygous for degree of development of the giant loops, and axial breakage has occurred at the site of the larger example; (*g*) is from ♀*O* and depicts a small example of the object at 18·5 units from a large (1·3 mm) oocyte; (*h*) is from ♀*E*, an individual heterozygous for degree of development of the giant loops, and depicts a massive example of this object from a large (1·6 mm) oocyte. Orientation of the chromosome axes in relation to the working maps is indicated by *L* (left) and *R* (right).

giant loops at 18·5 units on one homologue, but inconspicuous objects at the corresponding place on its partner (figures 14*f*, 15*e* and *f*; and 79, 80, 81, 83, plate 22).

The giant loops vary greatly in appearance according to oocyte size. In oocytes 0·6 to 0·9 mm diameter sister giant loops are usually fused together forming single round objects with firm outlines and containing vacuoles and granules: in some examples of bivalent X there is interhomologue fusion at this site (figure 73, plate 21) comparable to the 'sphere'-fusions on bivalents V and VIII. In larger oocytes 1·0 to 1·4 mm diameter the objects at this site are generally not fused together between homologues (figures 74, 75, 82, plates 21 and 22) and they may give signs of being double structures (figure 79, plate 22). There remains nevertheless extensive intra-loop fusion, and the concealed loop structure only appears in preparations mounted in media A or B, when the fused material disintegrates releasing clouds of granules, exposing typical asymmetric paired loops within (figure 84, plate 22). In still larger oocytes nearing maturity, of diameter 1·6 mm and more, when the great majority of the lateral loops project only a few microns from chromosome axes, the site at 18·5 units on chromosome X may carry a great double sausage-like structure of combined length more than 100 μ and width 5 to 10 μ, with constrictions across its length and often with free structures of similar texture in its vicinity (figures 14*h*; and 78, 80, 81, 83, plate 22).

In those females which lack giant loops on one or both homologues forming bivalent X the site homologous to that bearing giant loops may not be positively identifiable, or it may carry lateral structures similar in texture and in state of fusion to the giant loops (figures 14*e*, *d* and *f*; and 76, 77, plate 21) but smaller—usually very much smaller—in size.

In a particular preparation the texture (though generally not the form) of the giant loops at 18·5 units on chromosome X resembles that of giant loops on chromosome XI. Although the appearance of the giant loops varies with oocyte size, detailed features of their form, texture and size relative to other landmarks on chromosome X can also be recognized as characteristic of individual newts. This statement holds for some of the other landmarks on chromosome X as well, and indeed these individual-specific variations may be so pronounced as to confuse the establishment of homology from one newt to another.

The telomere at the right-hand end of chromosome X is often fused to an axial granule 2·5 units away, producing a terminal axial reflexion (figure 15*f*). Care is sometimes necessary to avoid confusing this ring structure with the terminal split axis of chromosome XII.

Chromosome XI: relative length 62 units.

Chromosome XI carries a pair of giant loops on its left arm at 24 units (figures 16*b*; and 90, 91, 94, 97, 98, plate 23). In all but one (♀*R*) of the newts which we have examined, these landmarks are of similar texture and equal development on associated homologous chromosomes. As mentioned already, the giant loops on chromosomes X and XI generally resemble one another in texture; they differ, however, in that those of chromosome XI often give signs of their fundamental loop nature, even when their matrices are fused, whereas those of chromosome X are usually so extensively fused that the loop form is entirely obscured. In small oocytes 0·6 to 0·8 mm diameter the giant loops on bivalent XI are generally fused to one another and form a connecting bridge between the two

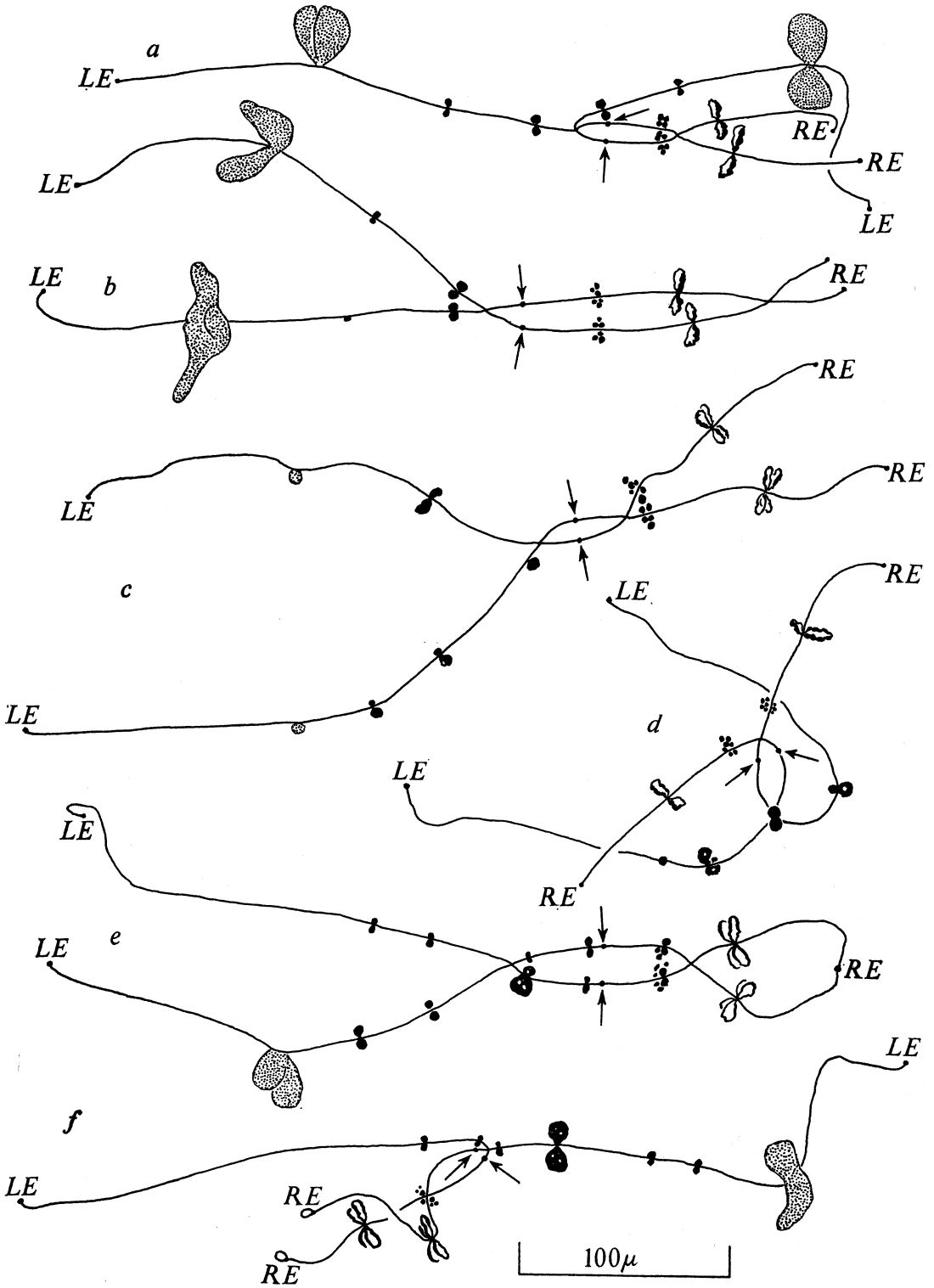

FIGURE 15. Camera lucida drawings of chromosome axes of lampbrush bivalents X from *T. c. carnifex* oocytes in the size range 1·0 to 1·3 mm diameter showing the giant loops in homozygous +/+ state [(*a*) and (*b*) from ♀*G*], in homozygous −/− state [(*c*) and (*d*) from ♀*O*] and in heterozygous +/− state [(*e*) and (*f*) from ♀*E*]. The centromeres are marked by arrows; the ends of left and right arms are indicated by *LE* and *RE*. In (*f*) the terminal granules of the right arms are fused to subterminal axial granules producing axial reflexions.

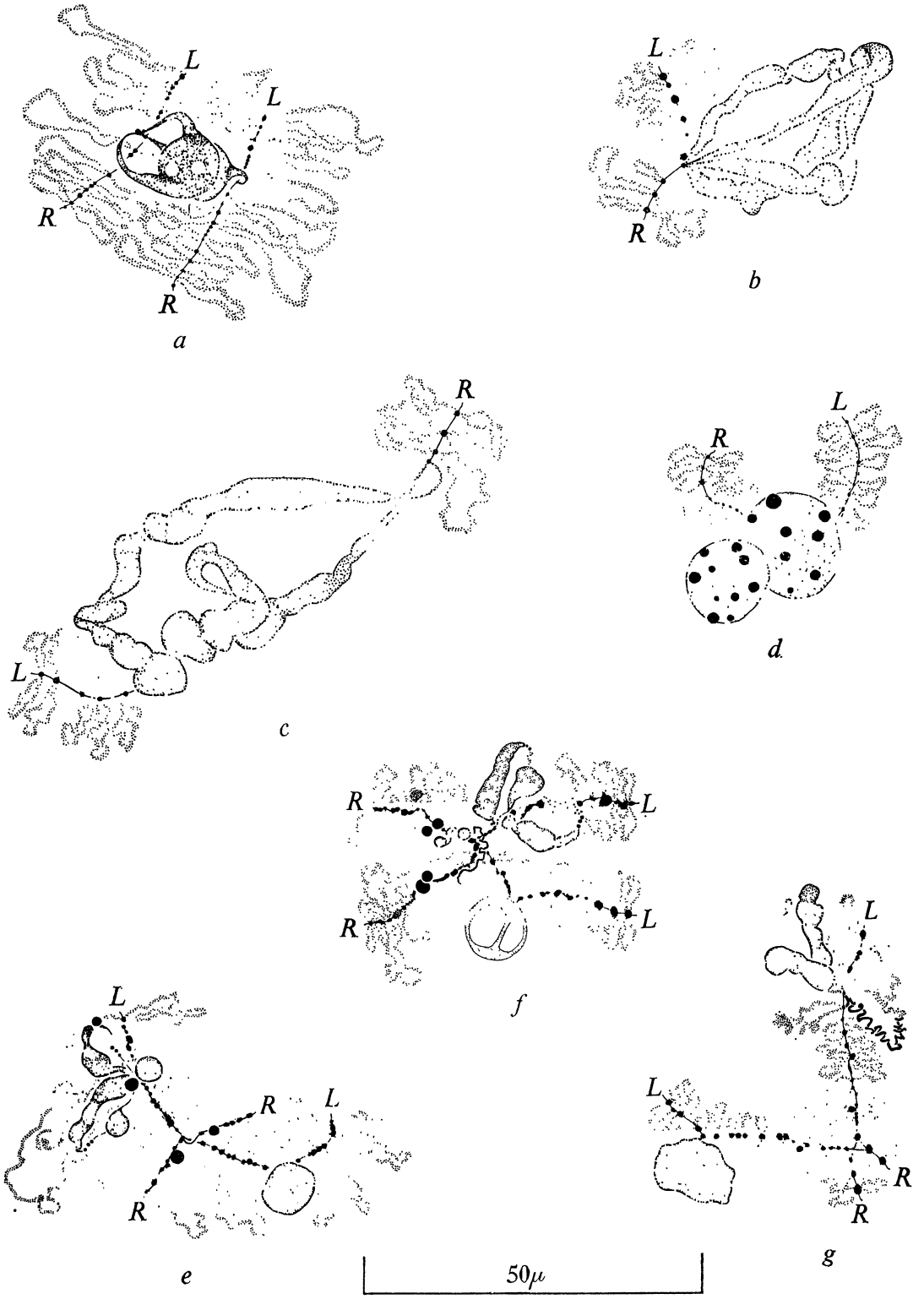

FIGURE 16. Camera lucida drawings of parts of bivalents XI of *T. c. carnifex*, illustrating variation in the morphology of the giant loops located at 24 units; (*a*) is from ♀*O*, 0·9 mm oocyte, and shows fusion between loop matrices; (*b*) is from ♀*M*, 1·1 mm oocyte, and shows the condition most frequently encountered in oocytes midway through their period of growth; (*c*) is from ♀*M*, 1·2 mm oocyte, showing axial breakage at the chromomere from which the giant loops originate; (*d*) is from ♀*O*, 1·3 mm oocyte, showing loop form entirely obliterated by matrix fusion; (*e*), (*f*) and (*g*) are from ♀*R*, 0·8, 1·2 and 1·0 mm oocytes, respectively, and demonstrate the heterozygosity typical of this individual, with sister giant loops unfused on one chromosome, fused together on its partner. Orientation of the chromosome axes in relation to the working map is indicated by *L* (left) and *R* (right).

homologues: the proximal parts of these loops are however usually separate, and thus the bridge joins the chromosome axes by four discernible 'tails' on either side (figures 16*a*; and 96, plate 23). In larger oocytes the giant loops on chromosome XI are themselves very much larger (each may reach an overall size of 100 μ) and cross-fusion between homologues is then infrequent. The site of origin of these loops suffers axial breakage particularly frequently, and when it occurs a spectacular 'double bridge' is produced (figures 16*c*; and 90, plate 23).

Carnifex ♀*R* is exceptional in that whereas sister giant loops of one chromosome XI regularly fuse to one another forming a single, roughly spherical structure, those of the partner chromosome are invariably separate from one another (figures 16*e*, *f* and *g*; and 92, 93, plate 23).

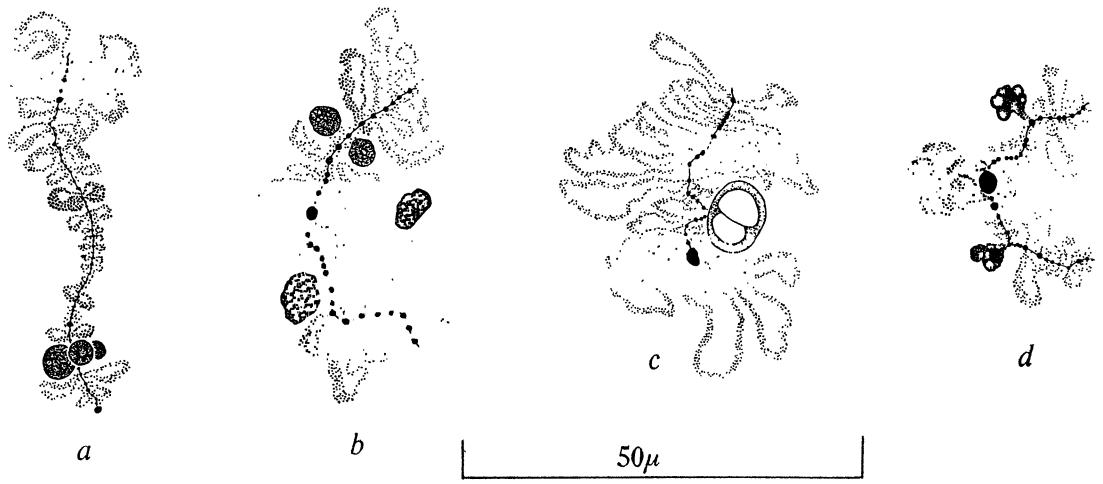

FIGURE 17. Camera lucida drawings of *T. c. carnifex* chromosome regions including 'currant buns': (*a*) is from ♀*E*, 1·1 mm oocyte, and shows the left end of chromosome III; (*b*) is from ♀*E*, 0·6 mm oocyte, and shows the left end of bivalent IV with fused terminal granules and a free 'currant bun'; (*c*) is from ♀*O*, 0·8 mm oocyte, and shows the left end of chromosome XI; (*d*) is from ♀*E*, 1·5 mm oocyte, and shows the left end of bivalent XI with fused terminal granules.

Chromosome XI lacks altogether the widely spaced out lumpy loops characteristic of chromosome X, but it has subterminal 'currant buns' at 3 units similar in appearance to those of chromosomes III and IV (figures 17*c* and *d*; and 99, 100, plate 23), and like the latter conspicuous in oocytes ranging in diameter from 0·6 to 0·8 mm and from 1·1 to 1·6 mm.

Chromosome XII: relative length 45 units.

Length alone is a sufficiently diagnostic character for chromosome XII, which is only three-quarters the length of the next shortest member of the complement. The right-hand terminal region stretching from 39 to 45 units is exceptional in possessing an evident double axis (figures 19*a* to *j*; and 123, 124, plate 25). The chromomeres of this region are, however, very small, so that casual inspection merely suggests an unusually ill-defined axis. The double axis can be made out most clearly in preparations mounted in medium A, when the granules attached to the loops of this region pass into solution. The double-axis end regions are also characterized throughout by the presence of unusually short loops,

only 3 to 4 μ in length (figures 101, 104, plates 23 and 24), which bear highly refractile granules of 0·5 to 2 μ diameter.

There may be another double-axis region in chromosome XII centred about a point at 25·5 units. The limits of this region, which is some 2 to 4 units long, are ill-defined, and it is only an evident feature when a chiasma has formed within its length (figures 18*a*, 19*d*, *g* and *i*; and 104, 107, plate 24). We may note that the smallest chromosome of *T. marmoratus* likewise has terminal and intercalary double-axis regions (Callan 1955). In oocytes approaching maximum size an axial granule lying between the two split-axis regions at 32 units is a conspicuous landmark (figures 19*a* to *j*; and 104, 105, 107, plate 24).

Like chromosome X, chromosome XII may or may not show a very striking individual-specific character. In a single *carnifex* female (*Y*) both homologues forming bivalent XII carry multiple giant loops (figures 19*a*, *b* and *c*; and 103, plate 23) at 22·5 units. In ten other females (*C*, *D*, *E*, *F*, *G*, *L*, *N*, *O*, *P* and *U*) such loops are lacking from both homologues (figures 19*d*, *e* and *f*; and 101, 102, plate 23). In yet other seven females (*J*, *M*, *Q*, *R*, *W*, *V* and *X*) there are giant loops on one chromosome XII, but inconspicuous objects at the corresponding place on its partner (figures 18*a*, *b* and *c*, 19*g*, *h*, *i* and *j*; and 94, 104, 105, 106, 107, plates 23 and 24).

The matrices of the giant loops are often fused together, but not so extensively that the multiple loop form is obliterated. Axial breakage at this locus is frequent, a fortunate occurrence since it produces a 'dissection' of what may be otherwise a highly complex structure. In one preparation the multiple giant loops of chromosome XII were found fused to the giant loops of chromosome XI (figures 18*d*; and 108, plate 24).

(*d*) *Centromere positions*

In Gall's (1954) study of the lampbrush chromosomes of *T. viridescens* centromere (= kinetochore) positions were of diagnostic value for the identification of the eleven chromosomes forming the haploid complement of this American newt. From Gall's description the centromeres of *viridescens* lampbrush chromosomes may be characterized as axial structures some 10 μ in length and 2 μ in width, which stand out in contrast to other chromosome regions owing to their lack of lateral loops. In *carnifex*, however, the centromeres of the lampbrush chromosomes are not of this appearance; they are relatively inconspicuous objects which have played little part in our identifications. Nevertheless, a knowledge of centromere positions is needed and we have been at pains to obtain this information. We made it our first task to determine the positions of the centromeres in mitotic chromosomes.

So far as we are aware there has been no study of the mitotic chromosomes of *carnifex* comparable in accuracy with Fankhauser's unpublished observations on *viridescens*; Gall used Fankhauser's data to track down the centromeres of *viridescens* lampbrush chromosomes. Through the courtesy of Professor G. Montalenti and Dr G. Vitagliano-Taddini of the Department of Genetics, University of Naples, we were provided with young larvae of *carnifex* which after natural eclosion had been kept for one day in a 1% solution of colchicine and then fixed in 3:1 absolute alcohol:glacial acetic acid. Previous experience with the larvae of *T. helveticus* and *T. vulgaris* had established the value of this pretreatment with colchicine. During the normal progress of mitosis in the squamous epithelial cells

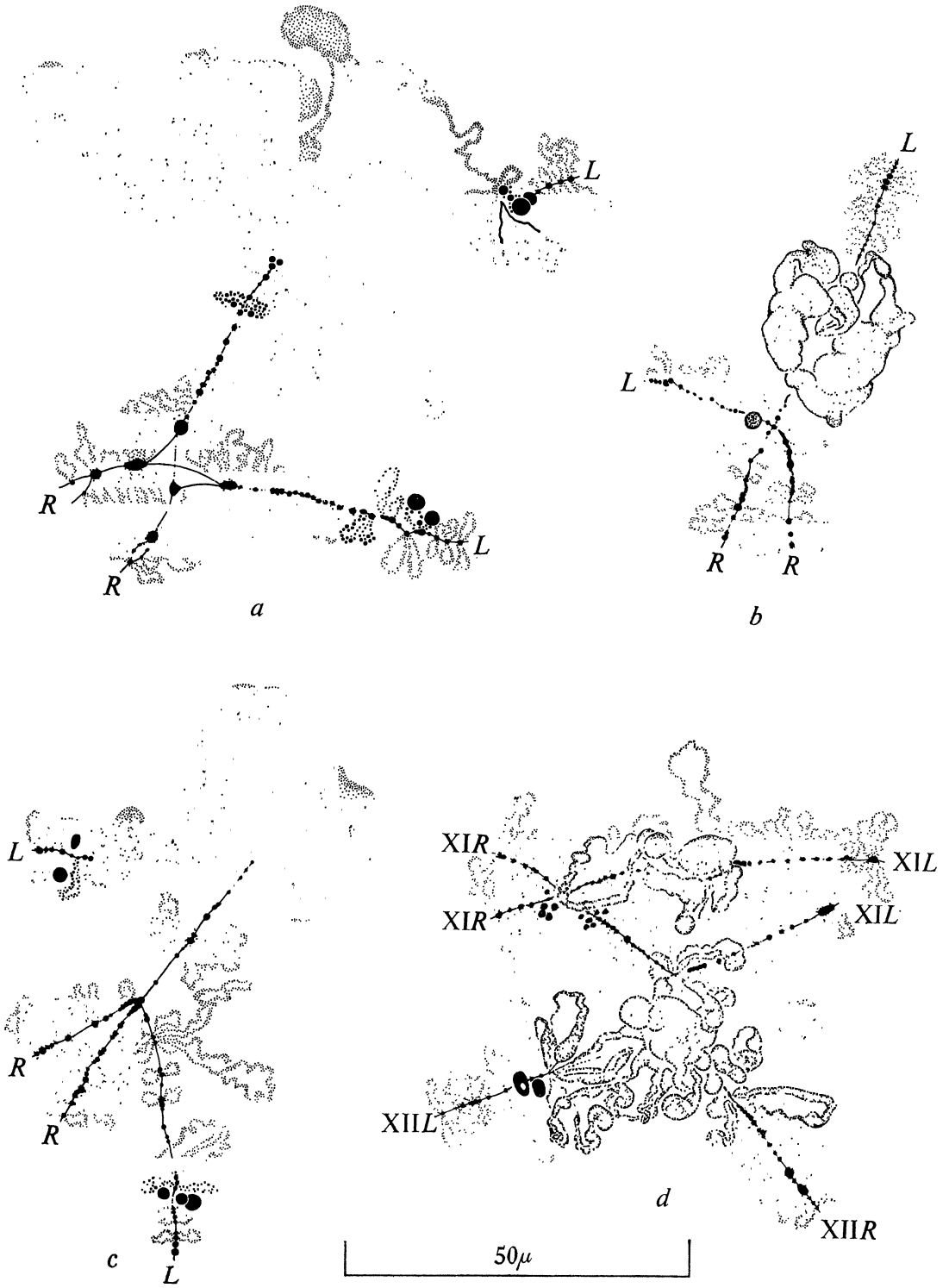

FIGURE 18. (*a*), (*b*) and (*c*) are camera lucida drawings of parts of bivalents XII, including the giant multiple loops sites, from *T. c. carnifex* females heterozygous for the giant loops: (*a*) is from ♀*M*, 1·2 mm oocyte; (*b*) is from ♀*J*, 1·1 mm oocyte; (*c*) is from ♀*M*, 0·7 mm oocyte; (*d*) is from an anonymous *T. c. carnifex* female, 1·0 mm oocyte, and shows fusion between the giant multiple loops of chromosome XII and the giant loops of chromosome XI. Orientation of the chromosome axes in relation to the working map is indicated by *L* (left) and *R* (right).

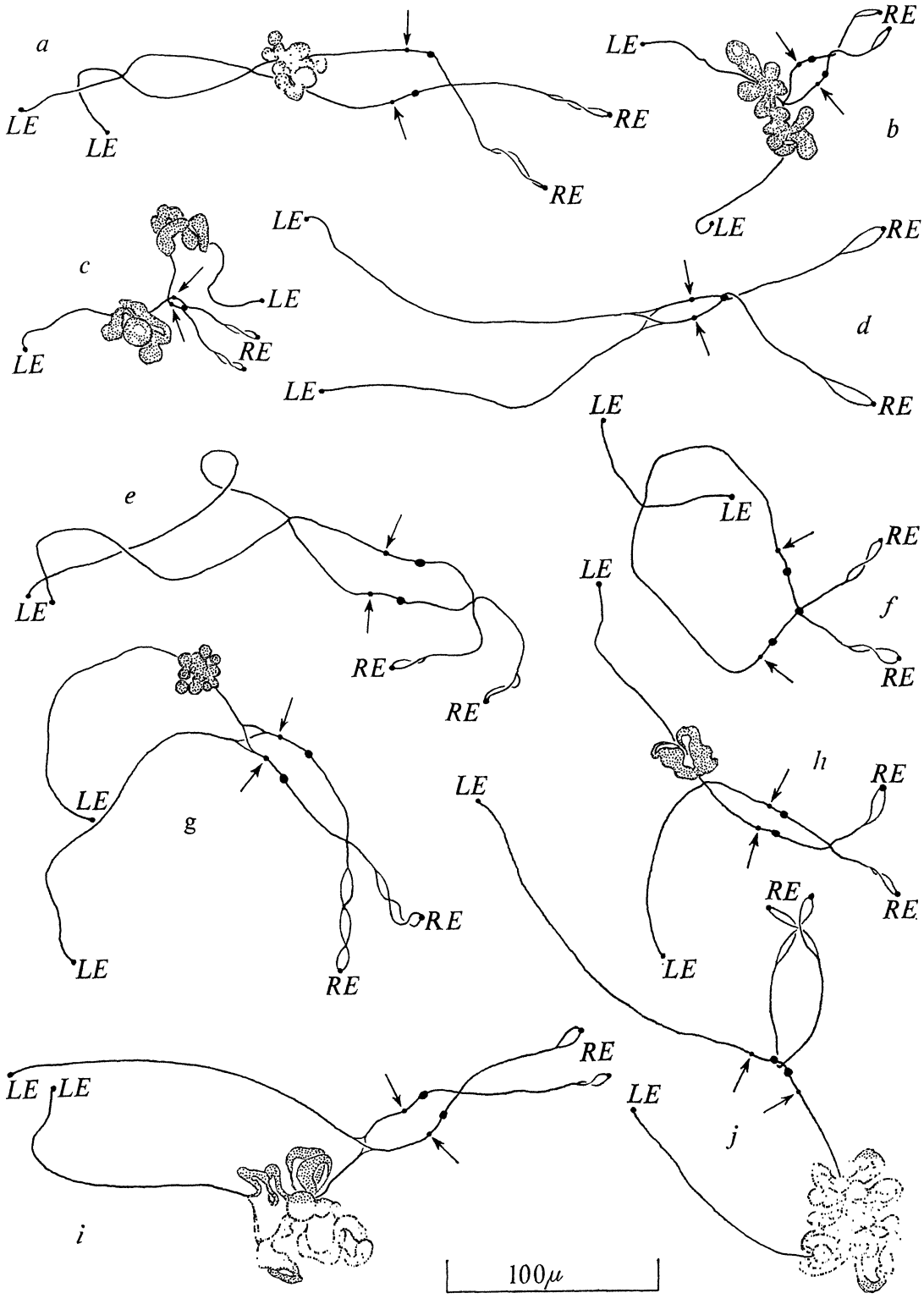

FIGURE 19. Camera lucida drawings of chromosome axes of lampbrush bivalents XII from *T. c. carnifex* oocytes in the size range 0·7 to 1·4 mm diameter showing the giant multiple loops in homozygous +/+ state [(*a*), (*b*) and (*c*) from ♀*Y*], in homozygous −/− state [(*d*) and (*e*) from ♀*E*, (*f*) from ♀*F*] and in heterozygous +/− state [(*g*) and (*h*) from ♀*J*, (*i*) and (*j*) from ♀*M*]. Chiasmata within double-axis regions are present in (*d*), (*g*), (*i*) and (*j*). The centromeres are marked by arrows; the ends of left and right arms are indicated by *LE* and *RE*.

of a newt larva's tail prometaphase is the most suitable stage for detailed study of chromosome lengths and centromere positions. At full metaphase the chromosomes are crowded on a spindle whose long axis lies in the plane of the epithelium, making accurate length measurements impossible to obtain, whilst throughout prophase the chromosome axes are contorted and centromere positions difficult to establish. The suitable stage of prometaphase is of short duration in normal mitosis, but cells at this stage can be accumulated by colchicine treatment so that each tail tip provides one or more examples.

The pretreated fixed larvae were stained entire in 1% aceto-orcein (G. T. Gurr, synthetic) for 1 h, the tails were then cut off and transferred to 45% acetic acid for softening and removal of excess stain, and were subsequently squashed out between coverslip and albuminized slide. The preparations were ringed with resin-lanolin cement and examined in this state. Twelve flat and well-spread prometaphases from ten tail tips were photographed, and positive prints made at an overall enlargement of $\times 2500$. A typical photograph is shown in figure 27, plate 17. The photographs were now compared with the original preparations and chromosome outlines inked in wherever, due to overlapping, the interpretation of the photographic detail was dubious. The arm lengths of the chromosomes in these photographs were measured to the nearest millimetre, the chromosomes were then ordered according to overall length, and paired off using overall length and centromere position (length of longer arm/total length expressed as a decimal fraction) as joint criteria of homology. Measurements made on both 'homologues' were then averaged and the seriation of these measurements for the twelve comparable mitotic sets checked against one another. When regard was paid to the estimated centromere positions of chromosomes which had been classified as comparable merely by virtue of their overall lengths relative to other members of the haploid complements, discrepancies in the seriations were apparent. In all cases of such discrepancies we decided to pay greater attention to centromere position than to relative overall lengths, since we consider centromere position to be a more stable characteristic than relative length (the mitotic chromosomes of newts continue to contract in length in cells whose spindles have been suppressed by colchicine, and the rate of this contraction may not be the same for all chromosomes).

From a practical standpoint it is fortunate that certain of the mitotic chromosomes of *carnifex* have clearly distinctive centromere positions. Thus of the two largest chromosomes in the haploid complement one has arms which are markedly unequal in length, whereas the other has arms which are virtually of equal length. Of chromosomes V, VI and VII one has arms extremely unequal in length, whereas the other two have arms of approximately equal length. The striking asymmetry of the shortest chromosome is also distinctive. On the other hand, chromosomes III, IV and V and chromosomes VII, VIII, IX and X cannot be distinguished from one another with certainty and we do not doubt that several mistaken identifications have occurred.

When the twelve series had been arranged in such a way as to give mutually compatible centromere positions for chromosomes which were adjudged homologous, relative overall lengths were calculated. In order to make these measurements comparable to those already worked out for the lampbrush chromosomes the relative lengths were based on an arbitrary length of 100 units for chromosome V. Evidence will be presented later in

TABLE 3. RELATIVE LENGTHS OF THE MITOTIC CHROMOSOMES OF *T. c. CARNIFEX* BASED ON CHROMOSOME V OF 100 UNITS, TOGETHER WITH CENTROMERE POSITIONS (LENGTH OF LONGER ARM/TOTAL LENGTH) EXPRESSED AS DECIMAL FRACTIONS

prometaphase chromosome	AA/1/2		AB/1/1		AD/1/1		AF/1/1		AL/1/1		AM/1/1		AN/1/1		AO/1/1		AO/2/1		AP/1/1		AQ/1/1		AQ/2/1		means	
I	118	0·67	118	0·67	117	0·65	115	0·66	124	0·63	118	0·64	113	0·61	116	0·65	118	0·61	116	0·65	134	0·62	118	0·67	118·8	0·64
II	111	0·50	113	0·52	114	0·52	113	0·51	115	0·51	106	0·52	111	0·52	107	0·53	117	0·50	121	0·52	124	0·51	108	0·52	113·3	0·52
III	102	0·59	114	0·56	93	0·55	108	0·55	111	0·56	100	0·65	97	0·63	100	0·56	101	0·62	109	0·58	105	0·64	102	0·60	103·5	0·59
IV	94	0·53	102	0·53	100	0·52	106	0·55	107	0·53	100	0·58	104	0·60	102	0·55	108	0·58	115	0·56	124	0·52	112	0·55	106·2	0·55
V	100	0·50	100	0·51	100	0·50	100	0·52	100	0·53	100	0·53	100	0·51	100	0·53	100	0·51	100	0·51	100	0·53	100	0·53	100	0·52
VI	85	0·74	88	0·64	88	0·74	82	0·65	81	0·66	75	0·64	72	0·73	91	0·64	84	0·67	87	0·68	88	0·70	85	0·72	83·8	0·69
VII	77	0·51	81	0·51	65	0·55	85	0·51	75	0·53	85	0·51	75	0·52	76	0·53	88	0·53	69	0·53	91	0·55	84	0·53	80·8	0·53
VIII	75	0·56	82	0·54	76	0·66	84	0·54	81	0·57	74	0·58	87	0·56	76	0·62	88	0·56	88	0·59	93	0·57	86	0·56	82·5	0·58
IX	60	0·61	66	0·55	52	0·54	59	0·56	61	0·56	58	0·51	64	0·52	61	0·57	52	0·59	62	0·64	71	0·55	58	0·57	60·3	0·57
X	44	0·62	53	0·60	49	0·62	54	0·61	56	0·60	45	0·69	55	0·61	51	0·62	52	0·62	56	0·67	59	0·63	60	0·58	52·8	0·62
XI	54	0·57	52	0·54	49	0·57	58	0·54	59	0·52	60	0·53	52	0·58	55	0·56	52	0·57	54	0·58	60	0·55	56	0·56	55·3	0·55
XII	41	0·66	49	0·64	34	0·63	41	0·63	47	0·67	35	0·65	43	0·65	44	0·70	44	0·69	41	0·65	45	0·64	42	0·67	42·2	0·66

this paper serving to show that chromosome V of the mitotic set is indeed chromosome V of the lampbrush set, and therefore that the two sets of data are comparable. Relative overall lengths of the mitotic chromosomes, together with centromere positions expressed as the decimal fraction length of long arm/total length, are set out in table 3. In order to avoid confusion the mitotic chromosome numbers I to XII given in this table are those which we now know to be equivalent to the lampbrush chromosome numbers. The seriation is not in strict accordance with a scale of diminishing mean relative overall lengths but the discordances are trivial; the transposition of chromosomes III and IV, of chromosomes VII and VIII and of chromosomes X and XI.

To juggle with figures as we have been obliged to do in this analysis is admittedly a dangerous procedure. It has, however, found practical justification in that the information so obtained aided us in our search for the centromeres of the lampbrush chromosomes. A further comment is called for. If a chromosome has arms which in reality are of equal length, experimental error will certainly on occasion lead to its being credited with a centromere position somewhat in excess of 0·50. In the matching procedure which we have adopted such errors necessarily all fall to one side of the true value and instead of cancelling out will in the long run tend to reinforce one another. That these errors have not been serious is indicated by the good agreement between the mitotic centromere positions and the centromere positions in the lampbrush chromosomes which were later established by direct observation.

Having determined the positions of the centromeres in the mitotic chromosomes we next turned our attention to the linear distribution of points of association within lampbrush bivalents. We thought that chiasma frequency would probably not be uniform along the lengths of these chromosomes and that its distribution might give a clear pointer to the whereabouts of the centromeres. Our analysis was complicated by the fact that not all points of association between lampbrush chromosomes are chiasmata; lampbrush chromosomes may also be held together by fused gene products.

The majority of chiasmate associations can be recognized without difficulty as connexions between chromosome axes. There is a tendency, just as there is at metaphase chiasmata, for homologous axes running towards a chiasma to lie in one plane, whilst beyond the chiasma they lie in a plane at right-angles to the first. Some chiasmata unquestionably lie between chromomeres. In such cases if a lampbrush bivalent happens to be under slight tension it is occasionally possible to observe, at the limit of resolution, cross-connecting fibrils at the chiasma. More frequently the physical connexion can only be inferred from the run of the chromosome strands towards one point, and from their maintenance of this relationship in spite of Brownian movement. At other clearly recognizable chiasmata homologous chromomeres are linked together, forming dumb-bell shaped structures at the point of connexion between chromosome axes.

Gene product fusions at homologous sites can be discriminated from chiasmata when they involve sufficiently bulky objects and leave the chromosome axes well separated. Fusions between telomeres, spheres on chromosomes V and VIII, lumpy loops on chromosomes II, VII and X, giant loops on chromosomes X and XI all fall within this category. Doubt arises, however, when lampbrush chromosomes are connected to one another at places where axial granules are present. A proportion of these connexions may well

involve chiasmata, whereas others are only fusions between gene products. This latter statement is justified by the occurrence of fusions between non-homologous axial granules, and between axial granules and telomeres. In our analysis of the linear distribution of associations within lampbrush bivalents all non-homologous fusions have been disregarded. Gene product fusions, chiasmata, and axial granule fusions have been separately recorded.

Camera lucida drawings of bivalents V and VIII from *carnifex* ♀♀ *A* and *B* were measured, and the positions of associations determined. A high degree of accuracy was obtained by the use of landmarks on these two chromosomes, the positions of the landmarks being first established and the positions of associations in turn related to these landmarks. For chromosome V the main landmark used was the axial granule at 57·2 units (at 0·57 fraction of length measured from the sphere-bearing end) and for chromosome VIII the double axial granule at 48·7 units (at 0·65 fraction of length measured from the sphere-bearing end). Histograms were prepared showing the distributions of associations within bivalents V and VIII, chromosome V being divided into fifty parts of equal length and chromosome VIII likewise. The histogram for forty-three examples of bivalent V, ♀*B* (figure 3*a*) shows two peaks of association frequency about the middle of the chromosome, sharply separated from one another by a trough, where no associations were recorded, lying at 0·50 to 0·52 fraction of total length as measured from the sphere-bearing end. The histogram for twenty-nine examples of bivalent V, ♀*A* (figure 4*a*) shows similar peaks with a trough at 0·50 to 0·52, though the chiasmata are less localized than are those of ♀*B*. The histogram for thirty-seven examples of bivalent VIII, ♀*B* (figure 3*b*) shows most associations restricted to the region 0·42 to 0·74 measured from the sphere-bearing end. Again the histogram for thirty-five examples of bivalent VIII, ♀*A* (figure 4*b*) shows less localized chiasmata, but when taken together with that of ♀*B* there is a well-defined trough at 0·56 to 0·58, without associations, between peaks of association frequency. These observations suggest that there is procentric chiasma localization in female meiosis in *carnifex*, at least for chromosomes V and VIII, and that the centromeres of these two chromosomes should be searched for in the regions 0·50 to 0·52 of V and 0·56 to 0·58 of VIII.

Armed with this information further preparations from oocytes of about 1 mm diameter were examined and in the appropriate regions of both chromosome V and chromosome VIII distinctive and relatively large chromomeres were observed, about 1 to 1·5 μ in diameter, *smooth in outline and without lateral loops* (figure 59, plate 20). In some examples these smooth, spherical, loop-free granules were found to be immediately flanked by axial chromomeres of about the same size or smaller, on one side or on both sides, generally but not always carrying lateral loops. The condition of the flanking chromomeres and of their loops varies considerably from preparation to preparation and even between homologues forming a single bivalent: the one unvarying feature is the smooth, loop-free granule at the appropriate place. Without prior information as to the whereabouts of the centromere, as indeed this type of chromomere has proved to be, it is improbable that we would have noticed its distinctive character. It is merely one granule amongst hundreds.

We next turned our attention to chromosomes I and II. For these two chromosomes the mitotic information suggested centromere positions at 0·64 and 0·52, respectively. Chromosome I is peculiar in that the great majority of its chiasmata (the situation is not

confused by axial granule fusions in this chromosome) lie well displaced from the middle of the bivalent (figure 7*a* to *d*). The heteromorphic arms of bivalent I are generally unassociated, though occasionally a chiasma may join them together very near the arm ends (figure 7*a*). A histogram showing the distribution of chiasmata in forty-two examples of bivalent I from ♀*P* was constructed (figure 5), this chromosome on account of its great length being divided into a hundred equal units of length. The histogram shows a well-defined trough at 0·63 to 0·65 fraction of length, measured from the ends of the heteromorphic arms, separating peaks of chiasma frequency. In constructing this histogram we made use of a landmark, a conspicuous chromomere at 86 units (0·64 fraction of length), which proved to be the centromere (figure 32, plate 18). The chromomere in question is a smooth, loop-free granule, and it stands out as a landmark, since for considerable distances on either side all other chromomeres are much smaller.

With the centromere of chromosome I accounted for at 0·64, that of chromosome II must necessarily be almost or quite median. The mid-region of chromosome II bears lateral 'lumpy' objects which may cross-fuse in various ways, and most of the chiasmata lie amongst these objects or nearby. By concentrating our attention on examples of bivalent II with simple configurations we found the characteristic loop-free granule (figures 9*a* and *b*; and 42, 43, 44, 45, plate 19) lying midway between two groups of the 'lumpy' objects at 65 units, being 0·52 fraction of length measured from the end of the arm which bears the 'marker' loops.

The striking asymmetry of the arms of chromosome XII, mitotic information placing the centromere at 0·66, suggested that the centromere of this chromosome would not be difficult to locate. An association frequency histogram for thirty-five examples of bivalent XII (figure 6*c*) shows the associations mostly concentrated between the double axis end and the middle of the chromosome, with a trough lacking associations at 0·66 to 0·68 fraction of length measured from the *single* axis end. Careful examination of many examples of bivalent XII showed that although there are several prospective candidates for the centromere amongst the axial granules in and around this region, only one fulfils the necessary requirements of invariability, size, smoothness of outline and lack of lateral loops. It lies at 30 units being 0·67 fraction of length from the single axis end. One axial granule in particular may tend to be confused with the centromere of chromosome XII. It lies slightly nearer the double axis end than does the centromere, and is much more conspicuous than the latter (figures 19*a* to *j*; and 104, 105, 107, plate 24); however, it is variable in size and is not without loops.

Another chromosome with even greater arm asymmetry than XII is chromosome VI, mitotic information placing the centromere at 0·69, and this was the next object of our attention. The diagnostic features of lampbrush chromosome VI are the two relatively large (3 to 4 μ) axial granules with rough outlines which are almost invariably fused together, producing an axial reflexion (figures 13*a* to *d*; and 54, plate 20). This region of chromosome VI is well off-centre and most of the chiasmata within this bivalent are located nearby. Conveniently, in chromosome VI there is only one granule which could possibly represent the centromere; the generality of the chromomeres of this chromosome are too small. Our chief difficulty in the case of chromosome VI was to establish with certainty the absence of loops on this one smooth granule of appropriate size, which lies

midway along the region of axial reflexion at 65 units, being 0·71 fraction of length (figure 54). At first sight this granule does not appear to be loop-free but this is because the flanking chromomeres are exceedingly small, themselves bear loops, and lie so close beside the centromere as to appear to invest this granule in loops. Clear indication of the lack of loops on the centromere granule of chromosome VI is only obtained when this region is stretched.

The centromere positions of six more lampbrush chromosomes remained to be established, III, IV, VII, IX, X and XI. In spite of the frequently complex configurations of chromosomes III and IV in their middle regions the centromeres of these two members of the complement were not difficult to find. Mitotic information placed their centromeres one at 0·59 and the other at 0·55. We feared that the centromeres of chromosome III would prove to be obscured by the stiff and very refractile loops which by their fusion complicate the middle region of this bivalent, but fortunately the smooth, loop-free granules at 66 units (0·56 fraction of length measured from the 'currant bun'-bearing ends) lie within short lengths of axis of more 'normal' appearance which are generally reflected away from the regions of fusion and thus clearly visible (figure 11*a* to *c*). The centromere granules of chromosome IV at 57 units (0·52 fraction of length measured from the 'currant-bun'-bearing ends) proved to lie symmetrically placed between the large and 'rough' axial granules which are often found fused together in this chromosome (figures 52, 53, plate 20). Axial reflexion in chromosome IV is less frequent than in chromosomes III and VI; where it occurs the centromere lies in the reflected region.

Location of the centromeres of chromosomes VII and IX was also straightforward. With the centromeres of chromosomes VI and VIII satisfactorily accounted for at positions 0·71 and 0·57, respectively, fair reliance could now clearly be placed on the unallocated mitotic determination of 0·53 for chromosome VII. The smooth, loop-free centromere granule of this chromosome lies at 47 units being 0·54 fraction of length measured from the end of the arm which bears two axial granule landmarks, with the four pairs of lumpy objects characteristic of chromosome VII symmetrically disposed on either side 3·5 and 6 units away (figure 67, plate 21). In the exceptional case of chromosome IX, whose distinctive feature is the absence of axial granules and conspicuous lateral structures, the centromere granule is itself the only regular, well-defined landmark. Mitotic information placed the centromere of this chromosome at 0·57; measurements on lampbrush chromosomes place it at 40 units, being 0·59 fraction of length.

Our greatest problem was presented by the last remaining chromosomes, X and XI. The mitotic determinations indicated centromere positions at 0·55 and 0·62, but this information was insufficient for our needs; without knowing in advance from which end to work there were four possible regions for search in each of the two chromosomes. Some of these regions could be ruled out at once owing to the absence of granules of an appropriate size, but sufficient alternative possibilities remained to leave us at first undecided. In this dilemma we had again recourse to association frequency histograms. Positions of associations were assessed with precision, use being made of all those landmarks which could be identified with certainty from preparation to preparation. The evidence from these histograms was unambiguous. In the case of chromosome X the histogram is not complicated by axial granule fusions (figure 6*a*). Most of the chiasmata fall in the region

from 0·47 to 0·98 fraction of length measured from the end of the arm which may bear giant loops, with a trough at 0·65 to 0·66. In the case of chromosome XI most of the associations fall in the region 0·35 to 0·78 fraction of length measured from the end of the arm which bears giant loops, with a trough at 0·55 to 0·57 (figure 6*b*). With the aid of this further information the centromeres were now positively identified on lampbrush chromosomes X and XI.

TABLE 4. *T. c. carnifex*. CENTROMERE POSITIONS (LENGTH OF LONGER ARM/TOTAL LENGTH EXPRESSED AS A DECIMAL FRACTION) AND RELATIVE ARM LENGTHS (BASED ON AN OVERALL LENGTH OF 100 UNITS FOR CHROMOSOME V) OF THE TWELVE LAMPBRUSH CHROMOSOMES

chromosome	centromere position	long arm	short arm
I	0·64	86	48
II	0·52	65	60
III	0·56	66	52
IV	0·52	57	53
V	0·51	51	49
VI	0·71	65	27
VII	0·54	47	40
VIII	0·57	43	32
IX	0·59	40	28
X	0·65	42	22
XI	0·56	35	27
XII	0·67	30	15

That of chromosome X is of the usual loop-free type flanked by loop-bearing chromomeres of varying size. It lies at 42 units, being 0·65 fraction of length. The variability in size of these flanking chromomeres is most deceptive, tending as it does to mask the invariability of the immediately adjacent granule. The centromere of chromosome XI, which lies at 35 units, being 0·56 fraction of length, is flanked by chromomeres which are generally a little smaller than the centromere granule itself; they bear very short loops or none at all. The flanking chromomeres are closely applied to the centromere and the little trio of granules, once located, can be easily recognized from one preparation to another.

The centromere positions in the twelve lampbrush chromosomes, established by direct measurement of ten or more examples of each member of the complement, are set out in table 4, together with arm lengths relative to the overall length of 100 units arbitrarily given to chromosome V. The positions of the centromeres in the working map are denoted by a series of arrows arranged vertically in line, with longer chromosome arms drawn to the left for uniformity of convention.

The centromeres of the lampbrush chromosomes of *T. c. carnifex* do not vary significantly in appearance in oocytes ranging in diameter from 0·5 to 1·4 mm. In oocytes of diameter 1·5 mm and upwards, regression of the generality of lateral loops, with concomitant increase in size and amalgamation of the axial chromomeres, has reached such a degree that the centromere granules can no longer be certainly identified.

In fixed preparations the centromere granules stain uniformly Feulgen-positive, and they can be discriminated from axial granules of a similar size, since the latter only show a narrow crescent staining with Feulgen's reagent, the remaining portions staining with light green if this counterstain is employed.

Final and entirely convincing proof that the granules which we have identified as centromeres are in fact the centromeres will be given in § VI of this paper, which is devoted to the lampbrush chromosomes of subspecies *karelinii*.

IV. Chromosome identification: *Triturus cristatus cristatus* (Laurenti)

We studied *T. c. cristatus* after earlier work with *carnifex*, and this experience aided us in recognizing the twelve lampbrush chromosomes of the *cristatus* complement.

An analysis of meiosis in male F_1 hybrids between *carnifex* and *cristatus* (Callan & Spurway 1951) has shown that the chromosome complements of these two subspecies are similar. These male F_1 hybrids have reduced chiasma frequencies as compared with the parent subspecies, but nearly half of the metaphases studied show all chromosomes associated as bivalents, and none of these bivalents evidently asymmetric. We have re-examined the original chiasma data and the preparations on which the 1951 paper was based, together with material from two further male F_1 hybrids which became available later, and out of a total of 120 scored metaphases, two in which all chromosomes were associated as bivalents also showed each bivalent with one or more chiasmata in both arm pairs. The two complements are nevertheless not identical. Although at the time of writing the 1951 paper we had no evidence that any translocations distinguish *carnifex* from *cristatus*, subsequent studies of meiosis in the male progeny of F_1 female hybrids back-crossed to *carnifex* males, with higher chiasma frequencies, have revealed at least one translocation.

The oocytes of *cristatus* from which we have isolated lampbrush chromosomes fall within the size range 0·8 to 1·4 mm diameter. The nuclear contents were dispersed in medium C in all preparations from which chromosome length measurements were taken and from which descriptions and illustrations of the landmarks in their normal forms were made.

Before considering the lengths and recognition characters of the *cristatus* complement in detail some general differences between *cristatus* and *carnifex* chromosomes will be mentioned. The centromeres of the *cristatus* lampbrush chromosomes are smooth, loop-free granules like those of *carnifex*, but they are smaller in size. The telomeres, on the other hand, are exceedingly conspicuous and about twice as large (diameter *ca.* 3 to 4 μ) as those of *carnifex*. Non-homologous fusion between telomeres (threefold in the case of figure 109, plate 24), between axial granules and between telomeres and axial granules together are remarkably frequent in *cristatus*, and they might well have confused our analysis had we studied this subspecies first. If reflected fusions within bivalents be disregarded, non-homologous fusions between bivalents are unusual in *carnifex*; we have noted only fifteen examples in 604 drawn bivalents. In *cristatus*, however, we have recorded twenty-three non-homologous fusions in 148 drawn bivalents.

(*a*) *Relative lengths*

The lengths of the *cristatus* chromosomes were measured in the same way as those of *carnifex*. Sufficient recognition characters are common to these two subspecies to allow the immediate identification of *cristatus* chromosomes which are homologous, at least to a first approximation, to their counterparts in *carnifex*.

In order to make a fair comparison between the *cristatus* and *carnifex* complements in terms of relative lengths, it is necessary to decide upon the arbitrary length in units of one of the *cristatus* chromosomes adjudged fully homologous to a *carnifex* chromosome. Ideally this homology could be determined directly by studying the lampbrush chromosomes of an F_1 female hybrid between the two subspecies, but unfortunately our living hybrids are as yet too young for such an analysis to be made. Chromosome V, given a length of 100 units, was the main basis for comparison of the *carnifex* chromosomes, and we would similarly have used chromosome V of *cristatus*, but for an apparent discrepancy between the positions of the centromeres on the two chromosomes. We chose instead chromosome IV of *cristatus*, whose centromere position, and landmark distribution about the centromere and towards the two chromosome ends, are in good agreement with similar characteristics of *carnifex* chromosome IV. *Carnifex* chromosome IV having a relative length of 110 units, this figure was allotted to *cristatus* chromosome IV and the lengths of the other eleven *cristatus* chromosomes calculated by proportion.

TABLE 5. *T. c. CRISTATUS*. LENGTHS OF THE LAMPBRUSH CHROMOSOMES FROM FIFTEEN OOCYTES RELATIVE TO CHROMOSOME IV OF 110 UNITS

	female *D*													female *E*			
oocyte no. ...	2	3	5	6	7	8	10	11	12	13	15	16	17	1	3	mean	s.e.±
oocyte diameter (mm)...	1·20	1·14	1·26	0·84	1·32	1·02	1·26	1·08	1·32	1·26	1·38	1·20	1·14	1·26	1·32	—	—
chromosome																	
I	—	151	—	—	129	155	154	141	140	171	154	162	123	142	147	147·4	3·9
II	—	—	144	135	124	132	117	120	120	146	145	—	130	—	126	130·8	3·2
III	—	—	—	—	125	138	—	114	129	—	119	120	118	—	—	123·3	3·1
IV	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110	—
actual length of IV (μ)	*678*	*619*	*595*	*719*	*619*	*628*	*461*	*418*	*485*	*463*	*448*	*376*	*370*	*602*	*478*	—	—
V	—	99	92	98	81	94	90	101	88	100	86	99	93	99	94	93·9	1·6
VI	98	—	76	87	86	—	—	89	84	—	92	85	81	91	—	86·9	1·9
VII	—	—	88	—	79	98	—	92	79	—	86	—	81	—	—	86·1	2·7
VIII	69	74	72	75	63	77	—	70	70	80	77	80	79	72	76	73·9	1·3
IX	66	69	—	—	63	74	—	69	67	81	70	83	53	71	—	69·6	2·5
X	60	—	—	—	—	68	56	64	54	82	68	61	58	62	—	63·3	2·5
XI	—	—	—	54	56	69	—	57	61	—	66	65	55	62	—	60·6	1·8
XII	49	46	—	51	44	—	49	52	—	53	—	50	45	—	—	48·8	1·0

The relative lengths were established from observations made on thirteen oocytes from female *D* and two oocytes from female *E*; these figures are set out in table 5. The mean relative lengths show that chromosome order based on length agrees with the order of the *carnifex* chromosomes. Differences between the respective means for the two sets of twelve chromosomes recorded in tables 1 and 5 must in part be due to sampling and experimental errors. The totals of the respective means—1080 for *carnifex*, 1095 for *cristatus*—are in good agreement, which supports our assumption that chromosomes IV of the two subspecies are genuinely homologous.

(*b*) *Recognition characters and centromere positions*

In this section general overall correspondence between the *cristatus* and *carnifex* chromosomes will be evident but will not be stressed. Emphasis will rather be laid on major points of distinction between the two subspecies. The positions of the *cristatus* centromeres were found by direct observation of the lampbrush chromosomes, without the help of evidence from mitotic chromosomes or chiasma histograms, but bearing in mind the

appearance and positions of the *carnifex* centromeres. As with *carnifex*, none of the *cristatus* centromeres are conspicuous landmarks, but all were nevertheless identified. Figure 20 is a working map of the *cristatus* chromosomes.

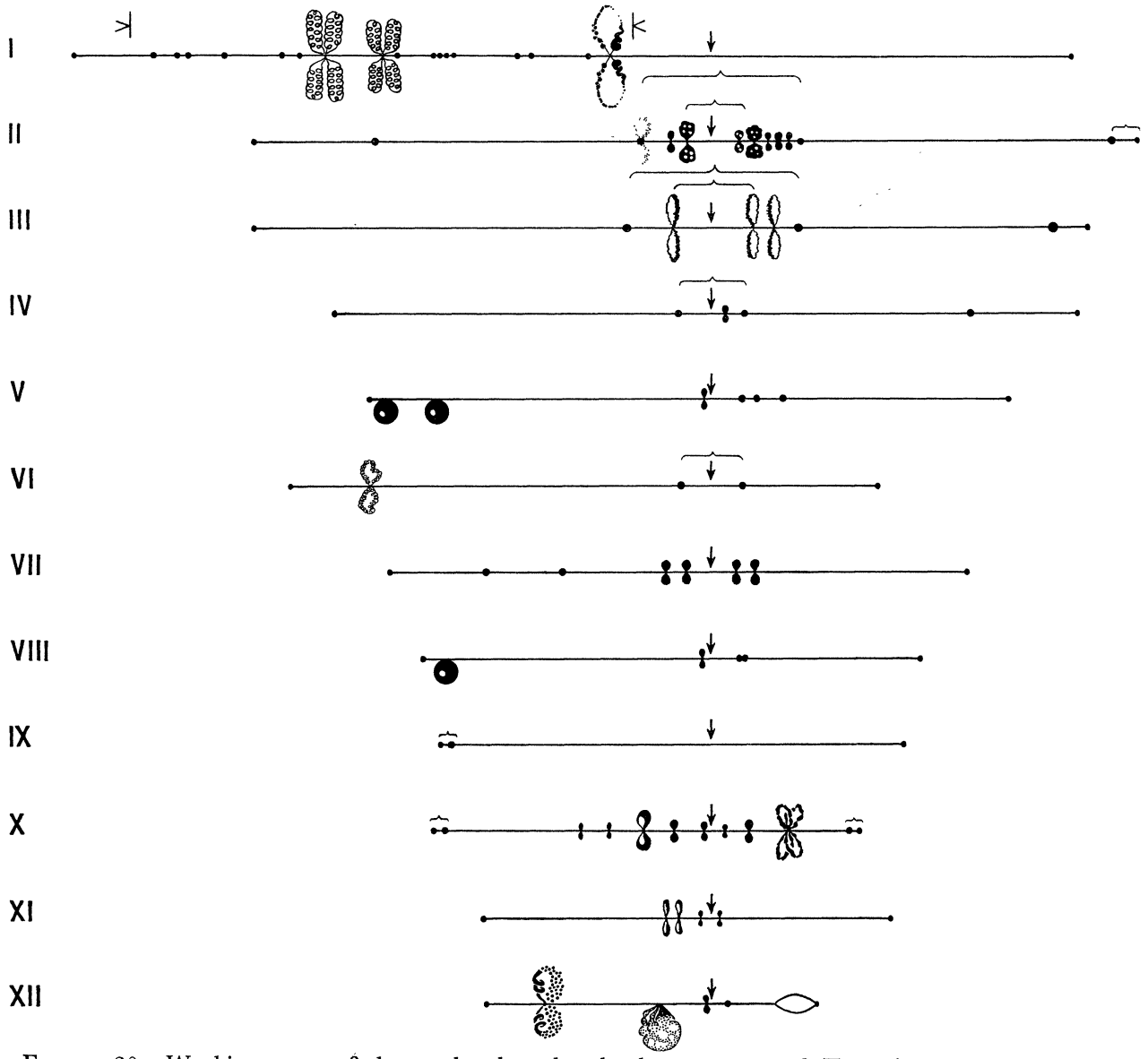

FIGURE 20. Working map of the twelve lampbrush chromosomes of *T. c. cristatus*. Centromere positions are indicated by the vertically alined arrows. Structures which often show 'reflected fusion' are linked together by brackets. The limits of the heteromorphic region of chromosome I are marked by >| and |<. Further explanation in text..

Chromosome I: relative length 147 units.

The centromere position (length of longer arm/total length) is 0·64, the arm lengths being 94 and 53 units. The partner long arms of bivalent I are of dissimilar morphologies and are never associated by chiasmata in the region between 9 and 82·5 units. Within this region are many axial granules and several distinctive lateral loops on both partner chromosomes (figure 21 *a* and *b*). One chromosome carries contorted loops, notably at 37 and 46 units, but these are less conspicuous than similar loops on *carnifex* chromosome I.

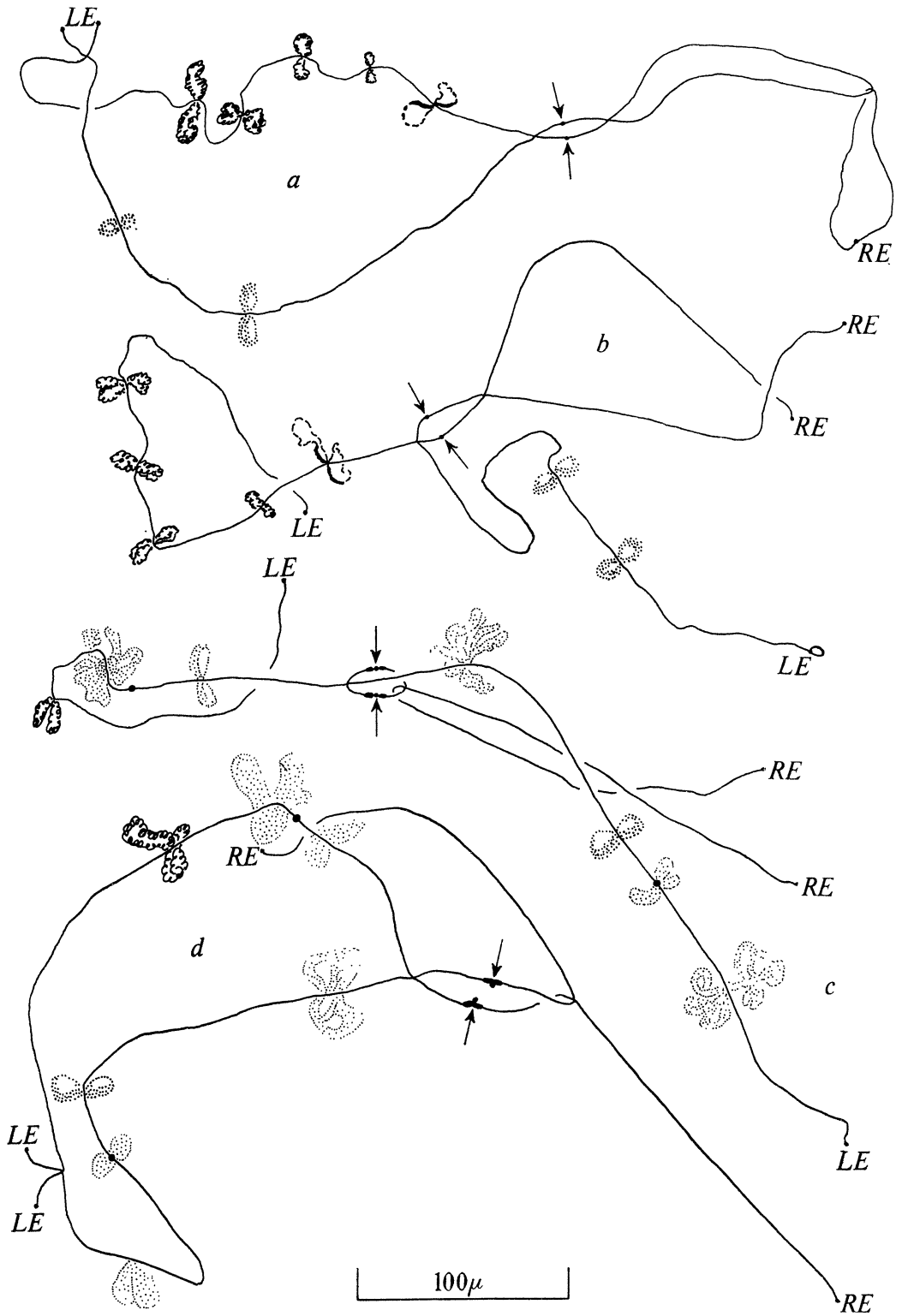

FIGURE 21. Camera lucida drawings of chromosome axes of lampbrush bivalents I from oocytes in the size range 1·0 to 1·4 mm diameter showing the more conspicuous 'heteromorphic' loops: (*a*) from *T. c. cristatus* ♀*D*; (*b*) from *T. c. cristatus* ♀*E*; (*c*) from *T. c. karelinii* ♀*H*; and (*d*) from *T. c. karelinii* ♀*F*. The centromeres are marked by arrows; the ends of left and right arms are indicated by *LE* and *RE*.

Much more striking is a large stiff loop pair at 79 units along the same arm; about the axis of this loop there is an interrupted aggregation of dense material which gives the loop a 'segmented' appearance (figures 22*e*; and 39, plate 18). The partner long arm not

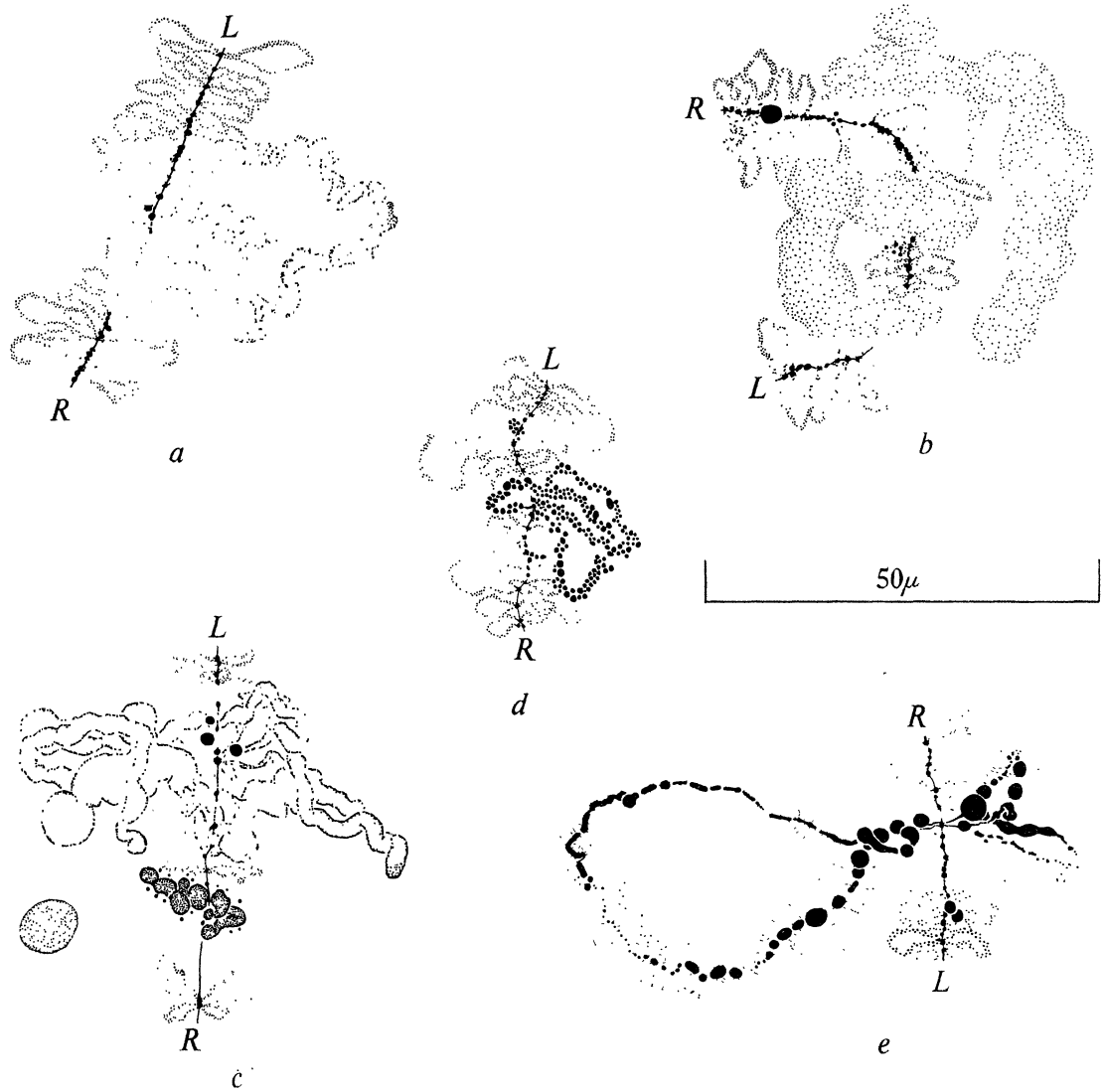

FIGURE 22. (*a*), (*b*), (*c*) and (*d*) are camera lucida drawings of distinctive lateral loops at various sites along the heteromorphic arms of bivalent I of *T. c. karelinii*: (*a*) is from ♀*C*, 0·8 mm oocyte, at 29 units; (*b*) is from ♀*C*, 1·2 mm oocyte, at 60 units on the arm *not* drawn on the working map; (*c*) is from ♀*B*, 1·2 mm oocyte, at 76 units; (*d*) is from ♀*C*, 1·1 mm oocyte, at 48 units; (*e*) shows the distinctive pair of lateral loops at 79 units along the heteromorphic arm of bivalent I of *T. c. cristatus*, from ♀*E*, 1·2 mm oocyte. Orientation of the chromosome axes in relation to the working maps is indicated by *L* (left) and *R* (right).

shown on the working map, carries some four or five large granular loops within the heteromorphic region, of which a pair situated at 44 units are most distinctive. Whereas in *carnifex* we found several striking landmarks on bivalent I characteristic of individual females, bivalents I from seven females of *cristatus* closely resemble one another.

Chromosome II: relative length 131 units.

The centromere position is 0·52, the arm lengths being 68 and 63 units. In the mid-region of this chromosome, and extending from 61 to 79 units of its length, are a series of paired lateral 'lumpy' objects which resemble those present on *carnifex* chromosome II in size, texture and distribution, and show the same tendency to fusion. There is a large 'marker' loop associated with an axial granule at 57 units, to the left of the lumpy objects, and another axial granule without conspicuous associated loops just to the right of the lumpy objects, at 81 units; similar structures occur in *carnifex* chromosome II. Whereas in *carnifex* we have rarely observed reflected fusion between these axial granules, in *cristatus* nineteen cases of reflected fusion were seen in twenty-six examples of bivalent II (figure 10*d* to *f*). In cases of reflected fusion the distal regions of the two arms of chromosome II evidently cannot be distinguished by reference to the distribution of the central landmarks. They can, however, be identified by axial granules at 18 and 126·5 units; the latter site is frequently fused to the telomere of the right arm, giving a terminal axial ring.

Chromosome III: relative length 123 units.

The centromere position is 0·55, the arm lengths being 68 and 55 units. In the mid-region of this chromosome are stiff, highly refractile loops, jagged in outline, which are generally fused together to form an irregular mass (figure 11*f*). The sites involved can only be observed accurately in examples of this bivalent not showing fusion (figure 11*d* and *e*), the major sites lying at 62, 74 and 77 units. Axial granules lie to left and right of the jagged, spiky loops, at 55 and 80 units, and reflected fusion between these granules is the normal condition. Thus as in *carnifex* chromosome III, the mid-region of *cristatus* chromosome III is generally a complex knot. There is a subterminal axial granule in the right arm at 118 units, but subterminally in the left arm there is hardly a trace of any object corresponding to the 'currant buns' of *carnifex* chromosome III.

Chromosome IV: relative length 110 units.

The centromere position is 0·51, the arm lengths being 56 and 54 units. There are axial granules to left and right of the centromere, at 51 and 60·5 units, which usually show reflected fusion, and another axial granule at 94 units. As with *cristatus* chromosome III, there is hardly a trace of a subterminal 'currant bun' on the left arm of chromosome IV.

Chromosome V: relative length 94 units.

The centromere position is 0·53, the arm lengths being 50 and 44 units. We are not sure whether the short arm of this chromosome is genuinely shorter than the corresponding arm of *carnifex* chromosome V; our data merely suggest that this is so. In other respects *cristatus* chromosome V closely resembles its *carnifex* counterpart, with spheres at 2 and 11 units, and axial granules at 55, 57 and 61 units. The spheres may be fused in all the combinations mentioned when describing *carnifex* chromosome V, and in two bivalents out of fifteen we have observed fusion between one of the spheres on chromosome V and a sphere on chromosome VIII. The centromere of *cristatus* chromosome V can be readily located as it lies immediately to the right of a pair of small but very dense loops situated at 49·5 units.

Chromosome VI: relative length 87 units.

The centromere position is 0·72, the arm lengths being 63 and 24 units. This chromosome has axial granules to left and right of the centromere, at 58 and 67 units, between which reflected fusion is the rule (figure 13*e* to *h*). It possesses a further striking landmark, not evident in *carnifex* chromosome VI, in the form of long coiled loops at 12 units.

Chromosome VII: relative length 86 units.

The centromere position is 0·56, the arm lengths being 48 and 38 units. There are pairs of lumpy objects symmetrically disposed about the centromere at 41, 44, 51 and 54 units; though similar to the lumpy objects on chromosome II, reflected fusion between those of chromosome VII has not been observed. There are conspicuous axial granules at 14·5 and 25·5 units.

Chromosome VIII: relative length 74 units.

The centromere position is 0·58, the arm lengths being 43 and 31 units. This chromosome carries a sphere at 3·5 units, a double axial granule at 47·5 units, and a pair of dense loops just to the left of the centromere at 41·5 units. As in *carnifex*, although the appearance of the spheres may vary considerably from oocyte to oocyte and from newt to newt, within a single nucleus the spheres on chromosomes V and VIII are very much alike in size and texture.

Chromosome IX: relative length 70 units.

The centromere position is 0·59, the arm lengths being 41 and 29 units. In *cristatus* as in *carnifex* this is the least distinctive member of the complement. Some examples carry small lumpy loops at a few places, but being inconstant these have no diagnostic value.

Chromosome X: relative length 63 units.

The centromere position is 0·65, the arm lengths being 41 and 22 units. Well spaced out over two-thirds of the length of this chromosome are lumpy loops at 22, 26, 31, 36, 40, 43, 47 and 53 units. The biggest of these objects lie at 31 and 53 units and are regular in their occurrence; smaller objects lie at the other sites, and in some examples of chromosome X these may be inconspicuous. *Cristatus* lacks altogether the giant loops at 18·5 units which characterize some individuals of *carnifex*. There are subterminal axial granules in both arms at 2 and 61·5 units, to which the telomeres are often reflected and fused.

Chromosome XI: relative length 61 units.

The centromere position is 0·56, the arm lengths being 34 and 27 units. This chromosome may carry subterminal 'currant buns' in its left arm, but it differs from *carnifex* chromosome XI in lacking giant loops (figure 95, plate 23). There are four places close to the centromere, at 27, 29, 32 and 35 units, where small dense loops are usually present, and the clustering of these structures at the middle of the chromosome serves best to distinguish *cristatus* XI from X. Chromosomes XI and IX are less readily distinguishable, and altogether these three chromosomes are more easily confused in *cristatus* than they are in *carnifex*.

Chromosome XII: relative length 49 units.

The centromere position is 0·68, the arm lengths being 33 and 16 units. The right arm terminates in a double axis region, with short loops each carrying one or two 0·5 to 2 μ highly refractile granules. To the right of the centromere, at 36 units, is a conspicuous

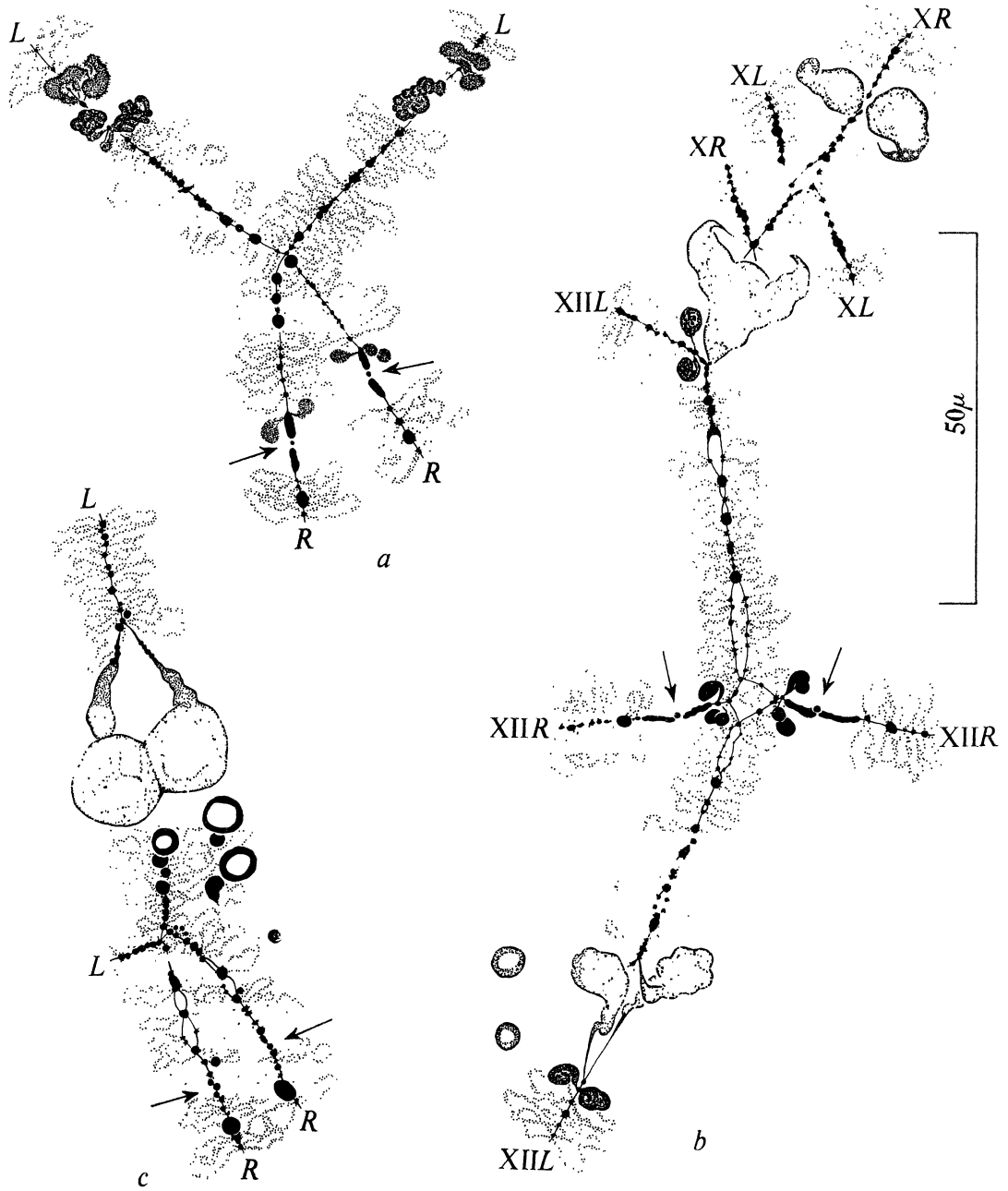

FIGURE 23. (*a*) Camera lucida drawing of part of bivalent XII, including the centromeres and giant loops, from *T. c. karelinii* ♀*A*, 1·1 mm oocyte. The giant loops, which lie towards the top of the drawing, are in a low state of development; (*b*) is from *T. c. karelinii* ♀*D*, 1·1 mm oocyte, and shows fusion between giant loops of chromosome XII and giant loops of chromosome X; (*c*) is from an anonymous *T. c. cristatus* female, 1·2 mm oocyte, and shows part of bivalent XII including the centromeres and one of the pairs of giant loops. Orientation of the chromosome axes in relation to the working maps is indicated by *L* (left) and *R* (right). The centromeres are marked by arrows.

axial granule (figures 23*c*; and 110, plate 24). Immediately to the left of the centromere, at 32·5 units, there is frequently a pair of dense lumpy objects which may obscure the centromere by their phase halo.

There is a giant loop pair at 25·5 units (figures 23*c*; and 110, 114, plate 24), homologous to the multiple giant loops of chromosome XII in some *carnifex* females. However, unlike

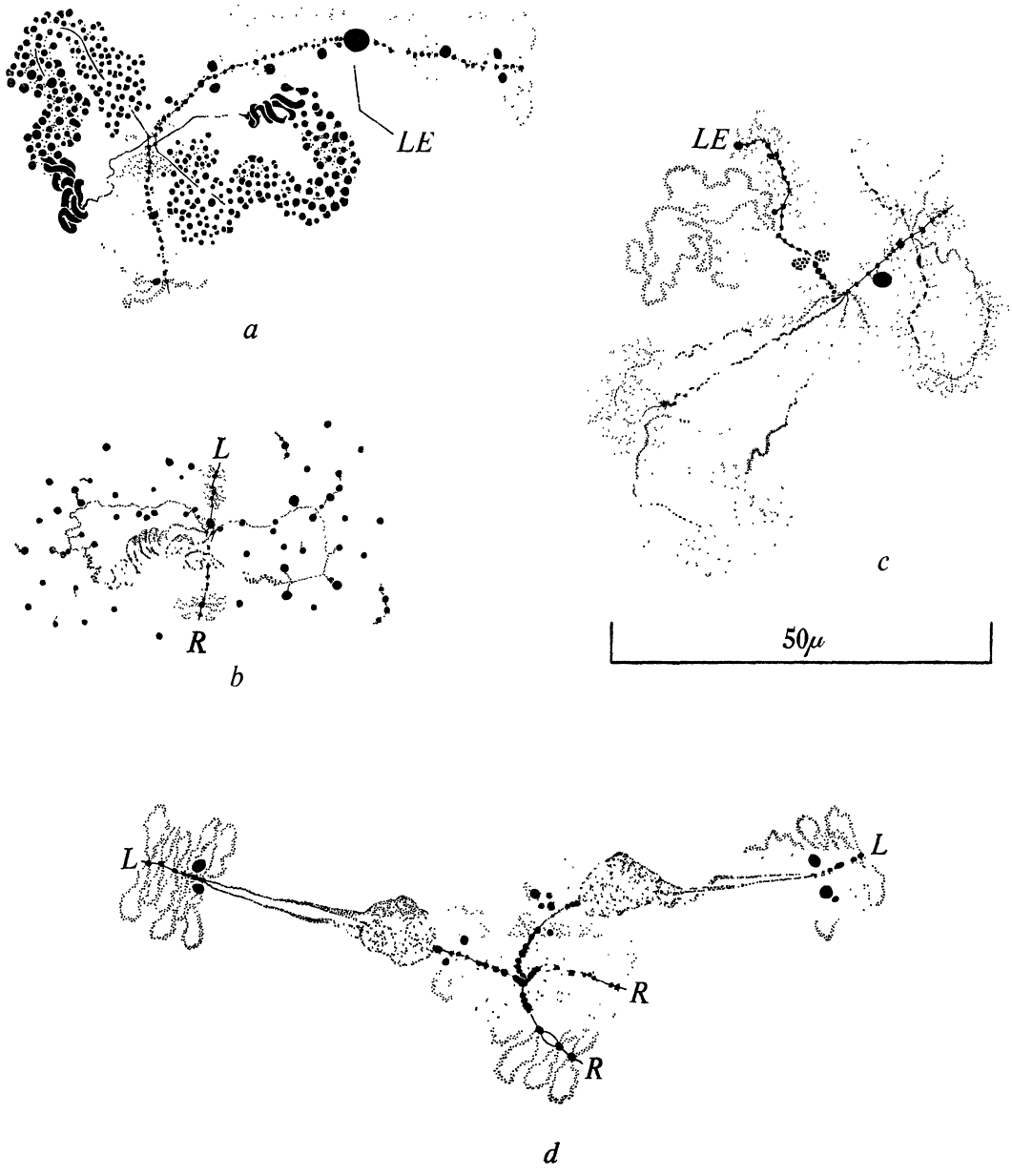

FIGURE 24. (*a*) Camera lucida drawing of part of bivalent XII, including the left arm terminal granules (fused) and the giant granular loops at 9 units from *T. c. cristatus* ♀*G*, 1·1 mm oocyte; (*b*) shows the characteristic pattern of disintegration of giant granular loops like those drawn in (*a*) 28 h after isolation from a 0·8 mm oocyte of *T. c. cristatus* ♀*D*; (*c*) shows part of chromosome XII, including the left-arm terminal granule and the two pairs of large 'fine fibrous' loops at 4·5 and 7·5 units, from *T. c. karelinii* ♀*H*, 0·9 mm oocyte; (*d*) shows part of bivalent XII including the giant loops at 24·5 units from *T. c. danubialis* ♀*F*, 1·1 mm oocyte. Terminal granules are marked *LE*.

the situation in *carnifex*, where we have observed the multiple giant loops present on both homologues, on one homologue only, or on neither, in *cristatus* the giant loops are always present on both homologues. Also unlike *carnifex*, these giant loops are simple pairs, not multiples. The sister loops are invariably fused together, and the homologous loops are often cross-fused between partner chromosomes as well. In the great majority of preparations there are axial breaks at the sites of the giant fused loops, the narrow tails of the loops being always attached to the distal portion of the chromosome arm, i.e. away from the centromere (figures 23*c*; and 114, plate 24). This condition is so frequently encountered that it can hardly be accidental; indeed we think that axial breakage at the giant loops site occurs naturally in life. Frequent breakage at this site is readily explicable; the giant fused loops are a source of origin of large free bodies in *cristatus* oocytes. Numbers of these objects are generally aggregated about the middle of bivalent XII (figure 110, plate 24). These bodies are stiff structures, and as they grow *in situ* they must tend to force the bases of the loops apart.

Finally, we come to a most striking landmark, a giant granular loop pair at 9 units (figures 24*a*; and 110, 111, 112, 113, plate 24). These loops are characteristic of *cristatus*. Other chromosomes, both of *carnifex* and *cristatus*, may carry granular loops at many places, but the granular loops of *cristatus* chromosome XII are of enormous size, each 60 μ or more in length and often 20 μ in breadth at the widest part. The narrow part of the loop is dense and contorted, whilst the wider part consists of a hundred or more granules each 1 to 2 μ in diameter.

When first isolated in medium C the giant granular loops are compact structures, but after a few hours they become somewhat disaggregated and it may then be possible to make out their axes, which run from the dense contorted region straight through the middle of the granular region and back to the chromomere of origin (figure 24*a*). The transition zone between the dense contorted region and the disperse granular region is often occupied by some twenty neatly arranged refractile rods whose bases are attached to the loop axis and whose free ends terminate in round knobs similar in density and in size to the granules occurring in the neighbouring region of the loop. These rods are visible in figure 113. The pattern of disintegration of the giant granular loops observable a day or more after isolation also suggests that the granules are mounted on stalks which project from the loop axis, as can be seen in figure 24*b*. We have not observed fusion between sister granular loops, nor axial breakage at this site. The chromomere from which the giant granular loops originate is, however, often split into two lengthwise.

V. Chromosome identification:

Triturus cristatus danubialis (Wolterstorff)

Male F_1 hybrids between *carnifex* and *danubialis* form fewer chiasmata than do the parent subspecies, but there is no evidence from analyses of meiosis in F_1 and back-cross males that these subspecies differ from one another by translocations (Callan & Spurway, unpublished).

We have examined the lampbrush chromosomes of *danubialis* oocytes ranging from 0·7 to 1·3 mm diameter. Most nuclei were dissected in medium C, a few in medium D. We have rarely found the centromeres of *danubialis* lampbrush chromosomes. Chromo-

some II of *danubialis* carries lumpy and 'marker' loops in its mid-region distributed like the comparable landmarks of *carnifex* chromosome II. This fixes the probable centromere site with precision, but the granule at this site is less than 0·5 μ in diameter and being comparable in size to neighbouring loop-bearing chromomeres it cannot be identified with assurance. The same is true of other *danubialis* chromosomes in which, by homology with *carnifex*, the anticipated centromere loci can be readily fixed (see figure 121, plate 25).

Another general characteristic, which differentiates *danubialis* lampbrush chromosomes from those of the other three subspecies, is the presence of spherical homogeneous structures of low refractility associated with some of the axial granules and telomeres. These objects, usually 2 to 4 μ in diameter ranging up to 8 μ, are found in every preparation though not consistently at all axial granules and telomeres.

TABLE 6. *T. C. DANUBIALIS.* LENGTHS OF THE LAMPBRUSH CHROMOSOMES FROM TWELVE OOCYTES RELATIVE TO CHROMOSOME IV OF 110 UNITS

	female *A*				female *B*	female *D*	female *E*		female *J*					
oocyte no. ...	2	3	4	6	1	2	2	3	1	3	4	6	mean	S.E. ±
oocyte diam. (mm) ...	0·90	1·08	1·08	1·08	0·72	0·84	1·08	1·02	1·14	0·72	0·84	1·02	—	—
chromosome														
I	—	131	156	135	138	155	143	140	148	—	—	133	142·1	3·0
II	—	117	—	130	125	130	132	132	125	139	—	—	127·5	2·4
III	135	117	134	133	—	127	119	110	—	141	—	124	126·7	3·3
IV	110	110	110	110	110	110	110	110	110	110	110	110	110	—
actual length of IV (μ)	*415*	*517*	*447*	*549*	*580*	*496*	*524*	*552*	*440*	*521*	*489*	*388*	—	—
V	110	95	105	102	93	96	87	87	96	93	97	97	96·5	1·9
VI	92	92	—	101	—	107	87	—	93	88	89	86	92·8	2·3
VII	—	79	—	87	90	99	80	77	89	—	91	92	87·1	2·4
VIII	—	67	77	71	71	80	65	—	72	68	—	77	72·0	1·4
IX	—	67	—	66	—	65	65	63	72	68	74	73	68·1	1·3
X	—	59	66	61	57	57	63	—	68	65	65	59	62·0	1·3
XI	64	65	—	66	—	68	66	54	68	61	63	63	63·9	1·3
XII	—	53	55	54	—	57	52	44	52	45	51	50	50·6	1·2

Danubialis telomeres are about 2 μ in diameter. Non-homologous telomere fusions are uncommon: we have noted eight examples in 200 drawn bivalents, two of these being threefold fusions. Apart from reflected fusions within bivalents, we have observed no other non-homologous fusions in this subspecies.

(*a*) *Relative lengths*

The lengths of the *danubialis* chromosomes were measured in the same way as those of *carnifex* and *cristatus*. Homologies with the *carnifex* chromosomes are evident, and the *danubialis* chromosomes were numbered accordingly. Chromosome IV was chosen as the basis for relative lengths and was allotted 110 units to make the *danubialis* and *carnifex* data comparable. The choice was made after preliminary study of one of Dr H. Spurway's F_1 hybrids 1951*C*♀13 (*danubialis* ♀ × *karelinii* ♂). This hybrid demonstrated apparent full homology between *danubialis* IV and *karelinii* IV, and as will be seen in § VI another F_1 hybrid demonstrated homology between *karelinii* IV and *carnifex* IV.

The relative lengths were established from observations made on twelve oocytes from five females. These figures are set out in table 6. The order of mean relative lengths agrees

with the *carnifex* order, apart from the transposition of chromosome X and XI. The total of means—1099—is in reasonable agreement with the *carnifex* total of 1080 and the *cristatus* total of 1095.

(*b*) *Recognition characters*

As in § IV*b* when dealing with the recognition characters of *cristatus* chromosomes, in this section we will mention correspondence between *danubialis*, *carnifex* and *cristatus* chromosomes but will stress features which distinguish *danubialis*. Not having identified the centromeres of *danubialis* we have not constructed a working map for this subspecies. However, by taking account of homologies between *danubialis* and *carnifex* we have kept to the established left-right convention in the following descriptions.

Chromosome I: relative length 142 units.

Partner left arms are not associated by chiasmata in the region between 9 and 72 units. There are many axial granules along these arms, and zones of axis bearing exceptionally long but otherwise normal lateral loops on both partners. These zones do not correspond in position on partner chromosomes, but their limits are ill-defined. Contorted loops like those of *carnifex* and *cristatus* are not present on *danubialis* I, nor fused loops of low refractility such as are found in *karelinii*. There is a useful landmark at 64 units on one homologue only—a pair of short loops each carrying some twenty granules of up to 3 μ diameter.

Chromosome II: relative length 128 units.

The mid-region carries 'lumpy' objects distributed as in chromosomes II of the other three subspecies. The largest of these objects lie at 60 and 70 units and may show inter-homologue and/or reflected fusion. There are exceedingly conspicuous 'marker' loops at 56 units—more striking than those of the other three subspecies—which in *danubialis* are particularly useful for distinguishing between chromosome II and some examples of chromosome III. Like *cristatus* and *karelinii* but unlike *carnifex*, reflected fusion may occur between the marker loop's axial granule and another axial granule lying just to the right of the series of lumpy objects. Subterminally in the right arm at 126 units is a pair of loops with stiff, fused and vacuolated matrix. These may be confused with the 'currant buns' of chromosomes III, IV or XI, but are generally more elongate objects.

Chromosome III: relative length 127 units.

There are several sites bearing stiff, refractile loops in the mid-region of chromosome III, and axial granules lie to left and right of this region. Reflected fusion between these axial granules occurs more often than not, but there tends to be less extensive fusion between the stiff loops than exists in the other three subspecies. The stiff loops are, moreover, less jagged, more compact, consequently in *danubialis* chromosomes II and III may at first glance be confused. *Danubialis* has smaller 'currant buns' than *carnifex*, and those lying subterminally in the left arm of chromosome III are systematically smaller than those of chromosomes IV and XI. The axial region lying to the right of the 'currant bun' site is bare of lateral loops: this is a useful recognition character. In one preparation where chromosome III was without reflected fusion we noticed a short region, close to the anticipated centromere where the axis was clearly double and with chromomeres bearing single lateral loops.

Chromosome IV: relative length 110 units.

As in the other three subspecies there are axial granules in the mid-region of chromosime IV lying on either side of its probable centromere. These granules cross-fuse between homologues, but reflected fusion is infrequent and this is distinctive of *danubialis*: we have noted only one case in sixteen drawn examples of bivalent IV. Small 'currant buns' are regularly present at a subterminal site in the left arm, and there is a distinctive axial granule at 96 units in the right arm.

Chromosome V: relative length 97 units.

Chromosome V carries 'spheres' at 2 and at 8 units. These can be as large as in *carnifex*, but are generally smaller. The only type of cross-fusion we have recorded is between homologous distal spheres. Spheres may be absent from one or the other of the sites on chromosome V where they are formed: this is a rare occurrence in *carnifex* and *cristatus*, but distinctively frequent in *danubialis* where we have recorded 23 sites lacking spheres in 22 drawn bivalents. Possibly once a sphere is shed it is reconstituted more slowly in *danubialis* than in *carnifex* or *cristatus*. In females *E*, *F* and *J* each attached and each free sphere has one or two 'caps' applied to its surface. The caps are homogeneous in texture and smaller than spheres. They presumably consist of material similar to that of the spheres and reflect some peculiarity in the way this material accumulates. There is another spherical structure of homogeneous texture regularly lying at 66·5 units on chromosome V. This object may reach 8 μ diameter and is distinctive of *danubialis*.

Chromosome VI: relative length 93 units.

As in the other three subspecies there are two axial granules well displaced from the mid-region of chromosome VI, at 61 and 72 units, between which reflected and/or interhomologue fusion is the usual condition.

Chromosome VII: relative length 87 units.

The lumpy objects in the mid-region of chromosomes VII of the other three subspecies are absent or very poorly developed in *danubialis* VII. Lack of these lumpy objects, and the variable occurrence of up to twelve axial granules between the left arm end and the middle of the chromosome, make *danubialis* VII difficult to identify.

Chromosome VIII: relative length 72 units.

There is a 'sphere' site at 4 units, and sphere fusions occur as in *carnifex* and *cristatus*. Spheres with 'caps' are regular features of females *E*, *F* and *J*. In *danubialis* chromosome VIII is easily confused with chromosome V, since it regularly carries a large 'pseudo-sphere' at 12 units (figure 64, plate 20). Two pseudo-sphere fusions have been recorded in nineteen drawings of bivalent VIII, but when by fusion and in texture the pseudo-sphere resembles a true sphere, it can be distinguished by its connexions to the chromosome axis, which are loop bases. Spheres are attached directly to the chromosome axis.

Chromosome IX: relative length 68 units.

There are no landmarks of recognition value on this chromosome.

Chromosome X: relative length 62 units.

There are several lumpy objects distributed over the length of chromosome X, the largest of these lying at 41 units. As in the other three subspecies there is a conspicuous landmark in the right arm at 56 units—extremely refractile long stiff loops of irregular outline. *Danubialis* resembles *cristatus* and differs from *carnifex* and *karelinii* in lacking giant loops in the left arm of chromosome X.

Chromosome XI: relative length 64 units.

Of the poorly developed 'currant buns' found in *danubialis*, those carried subterminally in the left arm of chromosome XI are systematically larger than those of chromosomes III and IV and are always useful for identification. Distinction between chromosomes XI and IX would otherwise be difficult, since *danubialis* like *cristatus* and *karelinii* lacks the giant loops present in *carnifex* XI.

Chromosome XII: relative length 51 units.

As in the other three subspecies, the right arm of *danubialis* XII terminates in a double-axis region with short lateral loops, there is another double-axis region about the middle of the chromosome and a conspicuous axial granule at 36 units (figure 121, plate 25). *Danubialis* XII may carry giant loops at 24·5 units (figure 24*d*) and as in *carnifex* but unlike *cristatus* or *karelinii* these may be present on both homologues (♀*F*), on one homologue only (♀♀ *A*, *B*, *D*, *H* and *K*) or on neither (♀♀ *E*, *G* and *J*). The texture of the giant loops on *danubialis* XII is characteristic: each consists of a long strand of uniform thickness closely packed on itself and conglomerated. As might be expected of such a structure, comparable objects are not found free in the nuclear sap. Axial breakage at the giant loops site is frequent, and when it occurs the thin ends of the loops always point towards the left-arm end.

VI. Chromosome identification:

Triturus cristatus karelinii (Strauch)

Analyses of meiosis in male F_1 and back-cross hybrids involving *karelinii* show that this subspecies differs from *carnifex* by at least one translocation, from *cristatus* by at least two, and from *danubialis* by at least one (Callan & Spurway 1951, and later unpublished observations).

We have examined the lampbrush chromosomes of *karelinii* oocytes ranging from 0·7 to 1·6 mm diameter. With the exception of the first few oocytes from ♀*A*, whose nuclear contents were dispersed in medium B, medium C was used throughout for this subspecies.

The most remarkable of the general distinctive features of *karelinii* lampbrush chromosomes concerns their centromeres. The centromeres of *karelinii* are easily located, since on either side of each smooth, loop-free centromere granule there are highly refractile 'axial bars' which also lack lateral loops. These axial bars are approximately 1 μ thick, and they range from 1·5 to 8 μ in length, being longer in larger oocytes. The centromere granules tend to be smaller (less than 1 μ diameter) in smaller oocytes, and in such oocytes they lie in the chromosome axis in line with the axial bars on either side (figures 14*b* and 23*a*; and 87, 118, plates 22 and 25). However, in larger oocytes of 1 to 1·5 mm diameter the

axial bars are often continuous through the centromere, with the result that the centromere granule, which may range up to 1·5 μ in diameter, is displaced laterally from the chromosome axis (figures 23*b*; and 34, 65, 66, 70, 88, plates 17, 21 and 22). Growth of the axial bars during oocyte development results from the progressive incorporation of neighbouring chromomeres into their substance, the lateral loops of these chromomeres regressing as incorporation proceeds, and the chromosome axis shortening. Occasionally homologous axial bars grow out of step, in which case the length of chromosome axis between the centromere and a neighbouring landmark may be appreciably different in two homologues forming a bivalent (figure 118, plate 25). The centric regions of *karelinii* lampbrush chromosomes thus resemble those of *T. viridescens* and of *Amblystoma tigrinum* described by Gall (1954), and differ markedly from the centric regions of *carnifex*, *cristatus* and *danubialis*.

The reader will recall that several of the *carnifex* centromeres were originally located by studying the linear distributions of chiasmata and other interhomologue associations; associations were found not to occur at the granules assumed to be the centromeres, whereas neighbouring regions showed peaks of chiasma frequency. The same is true of *cristatus*, but this statement does not hold for *karelinii*, where fusion between homologous centromere granules occurs at high frequency (figures 10*g* and *i*; and 47, 60, 89, 116, 119, plates 19, 20, 22 and 25). Out of 148 drawn *karelinii* bivalents, 30 were associated precisely at their centromeres, i.e. at the centromere granules, not in the adjacent axial-bar regions—in fact we have not observed chiasmata within the axial bars. Gall (1954) has similarly recorded fusions between homologous 'kinetochores' in *T. viridescens*.

In view of this surprising observation we must reconsider whether the *carnifex* and *cristatus* centromeres have been correctly identified, since one of the main criteria by which their sites were established is not valid for *karelinii*. Chromosomes II, III, IV, VI, VII and XII of *karelinii* bear landmarks close to their centromeres which resemble landmarks on corresponding chromosomes of the other three subspecies. Relative to these landmarks the assumed centromeres of *carnifex* and *cristatus* occupy positions which tally with the positions of the *karelinii* centromeres (compare figures 66 and 67, plate 21). This evidence supports our claim to have correctly identified the centromeres of *carnifex* and *cristatus*; stronger evidence comes from a preliminary study of one of Dr Spurway's F_1 hybrids, 1948*B*♀2 (*carnifex* ♀ × *karelinii* ♂). In the lampbrush chromosomes of this hybrid, centromeres derived from the *karelinii* parent can be recognized, and for several of the bivalents the positions of the known and assumed centromeres tally exactly. Moreover, in one example of bivalent VIII we have observed fusion between *carnifex* and *karelinii* centromere granules.

We have been unable to decide whether the fusions between *karelinii* centromere granules have genetic significance. In fixed preparations the centromere granules and axial bars stain uniformly Feulgen-positive; centromere granule fusions thus appear to differ from axial granule and sphere fusions, where Feulgen-negative 'gene-product' materials are involved. It is conceivable that these centric fusions are chiasmata, but if this be so they should lead to configurations at first meiotic metaphase which have hitherto not been observed in any organism. Moreover, we have recorded two examples of fusion between non-homologous centromere granules, one threefold involving both centromeres

of chromosome VII with one centromere of chromosome II (figure 9*c*), the other involving one centromere of chromosome III with one centromere of chromosome IX. This again suggests that the centric associations are not chiasmata.

The telomeres of *karelinii* lampbrush chromosomes, usually less than 2 μ in diameter, are smaller than those of *carnifex* or *danubialis*, considerably smaller than those of *cristatus*. Homologous telomere fusions are relatively infrequent; we have only noted 27 cases in 148 drawn bivalents, as compared with 67 cases in 148 drawn bivalents of *cristatus*. Fusions of all kinds associating non-homologous chromosomes are also infrequent; 6 examples in 148 bivalents, which is approximately the proportion found in *carnifex* but less than a third that found in *cristatus*.

TABLE 7. *T. c. KARELINII.* LENGTHS OF THE LAMPBRUSH CHROMOSOMES FROM FOURTEEN OOCYTES RELATIVE TO CHROMOSOME IV OF 110 UNITS

	female *A*				female *B*				female *G*			female *H*				
oocyte no....	1	2	3	8	1	4	5	6	1	2	4	1	2	7	mean	S.E. ±
oocyte diam.(mm)...	1·38	1·38	1·14	1·14	1·50	1·50	1·08	1·56	1·32	1·08	1·38	0·96	0·96	1·02	—	—
chromosome																
I	150	151	150	144	—	—	—	144	154	143	144	124	134	130	142·5	2·9
II	124	—	119	—	—	128	—	—	140	—	130	119	122	124	125·8	2·5
III	128	—	—	—	—	—	—	—	118	123	—	103	—	116	117·6	4·2
IV	110	110	110	110	110	110	110	110	110	110	110	110	110	110	110	—
actual length of IV (μ)	*685*	*781*	*719*	*774*	*602*	*390*	*730*	*420*	*389*	*498*	*437*	*530*	*452*	*498*	—	—
V	95	—	89	95	95	—	—	102	103	105	91	82	95	95	95·2	2·0
VI	86	—	82	—	96	—	95	—	—	90	87	80	85	93	88·2	1·9
VII	86	—	88	—	—	87	—	—	95	95	83	79	85	73	85·7	2·3
VIII	75	—	78	—	—	69	68	91	80	—	85	70	75	70	76·1	2·4
IX	72	66	—	—	—	—	—	90	86	69	65	71	73	69	73·4	2·9
X	65	54	58	57	64	71	64	67	69	61	65	55	61	59	62·0	1·7
XI	71	55	60	53	—	66	63	68	63	69	69	60	63	66	63·5	1·5
XII	—	—	48	40	50	—	—	52	56	56	55	53	53	49	51·2	1·8

Another distinctive general feature of *karelinii* lampbrush chromosomes concerns their chromomeres, which are noticeably larger and more refractile than those of the other three subspecies when chromosomes having the same degree of lateral loop development are compared. Fixed and stained preparations confirm this impression; whereas only occasional and large chromomeres of *carnifex* or *cristatus* lampbrush chromosomes are clearly Feulgen-positive, in Feulgen preparations of *karelinii* lampbrush chromosomes long sequences of stained chromomeres can be readily defined.

(*a*) *Relative lengths*

Recognition characters, which are common to *carnifex* and *karelinii*, aided in the recognition of the *karelinii* lampbrush chromosomes. As a first approximation the *karelinii* chromosomes were assumed homologous to those of *carnifex*, and were numbered accordingly. Preliminary study of F_1 hybrid 1948*B*♀2 (*carnifex* ♀ × *karelinii* ♂) gave direct evidence of homology between chromosomes IV. This chromosome was therefore chosen as the basis for *karelinii* relative length measurements, as in *cristatus* and *danubialis*, and was allotted a length of 110 units to make the *karelinii* data comparable with that of the other three subspecies.

The relative lengths were established from observations made on fourteen oocytes from four females. The figures are set out in table 7. The order of mean relative lengths agrees with the *carnifex* order apart from the transposition of chromosomes X and XI. The total of mean lengths is 1092 units, in reasonable agreement with the *carnifex* total of 1080, the *cristatus* total of 1095 and *danubialis* total of 1099. The positions of the centromeres on the *karelinii* chromosomes, together with relative arm lengths, are set out in table 8 for comparison with corresponding data for the *carnifex* chromosomes displayed in table 4.

TABLE 8. *T. c. KARELINII.* CENTROMERE POSITIONS (LENGTH OF LONGER ARM/TOTAL LENGTH EXPRESSED AS A DECIMAL FRACTION) AND RELATIVE ARM LENGTHS (BASED ON AN OVERALL LENGTH OF 110 UNITS FOR CHROMOSOME IV) OF THE TWELVE LAMPBRUSH CHROMOSOMES

chromosome	centromere position	long arm	short arm
I	0·66	94	49
II	0·53	67	59
III	0·56	66	52
IV	0·52	57	53
V	0·50	48	47
VI	0·72	63	25
VII	0·55	47	39
VIII	0·57	43	33
IX	0·58	42	31
X	0·66	41	21
XI	0·57	36	28
XII	0·67	34	17

(*b*) *Recognition characters and centromere positions*

In this section resemblances between the *karelinii* and *carnifex*, *cristatus* and *danubialis* lampbrush chromosomes will be evident, but stress will once again be laid on distinguishing features. Figure 25 is a working map of the *karelinii* chromosomes.

Chromosome I: relative length 143 units.

The centromere position is 0·66 (figures 33, 34, plate 18), the arm lengths being 94 and 49 units. The partner long arms of bivalent I are of dissimilar morphologies and they are not associated by chiasmata in the region between 9 and 82·5 units. In this heteromorphic region the axial granules are fewer and less conspicuous than in the other three subspecies, but there are several highly distinctive loops on both partner chromosomes (figures 21*c* and *d*; and 33, plate 18). There is a characteristic granular loop (figure 22*d*) at 48 units on the chromosome shown in the working map, but of greater distinction are the structures at 16, 29, 39 and 76 units (figures 22*a* and *c*; and 40, 41, plate 18). These are large loops of very low refractility, and they shed rounded lumps of similar low-refractile material into the nuclear sap. Straight, uncontorted axes are sometimes visible in these loops; it will be recalled that the conspicuous loops present in the heteromorphic parts of chromosomes I of *carnifex* and *cristatus* are contorted structures, but those of *karelinii* have different morphologies. Their matrices are very faintly granular, have relatively even and firm outlines, and may be interrupted at intervals. Loop axis spanning a gap in the matrix

is thicker and more refractile than a part of the axis imbedded in matrix, as though coated with some material which gives rise to matrix by swelling. Loop form is obliterated by fusion at the sites at 16 and 39 units; the object at 76 units (figures 22*c*; and 40, 41, plate 18) consists of multiple loops arising from several discrete chromomeres.

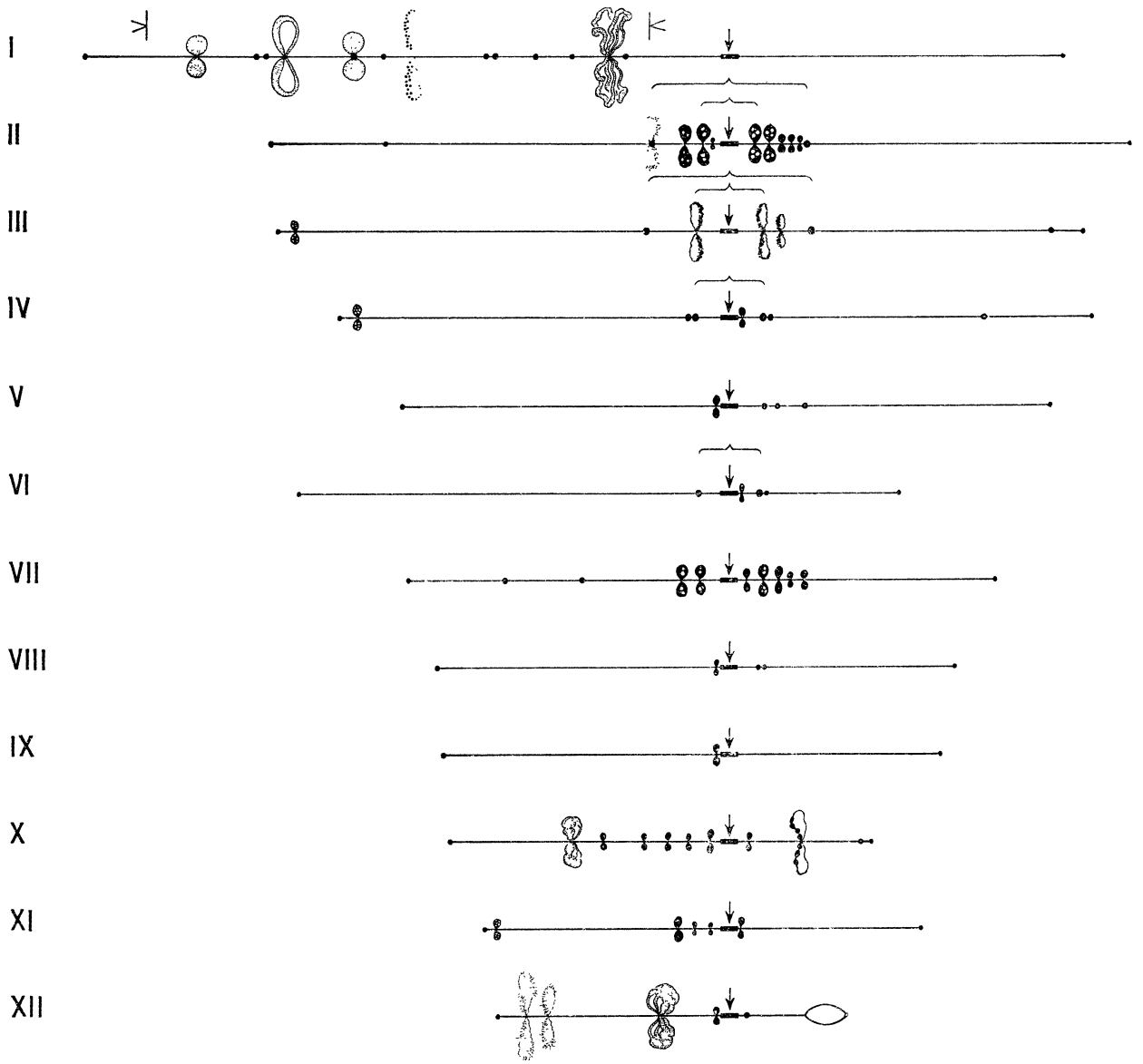

FIGURE 25. Working map of the twelve lampbrush chromosomes of *T. c. karelinii*. Centromere positions are indicated by the vertically alined arrows. Structures which often show 'reflected fusion' are linked together by brackets. The limits of the heteromorphic region of chromosome I are marked by >| and |<. Further explanation in text.

The partner chromosome I, not shown on the working map, bears similar loops of low refractility, a larger at 60 (figure 22*b*) and a smaller at 67 units. At both sites the loop form is largely obliterated by fusion of matrix. At 49 units on this chromosome there is a single pair of large loops having irregular contorted axes and coarse fibrous matrix. We have not noticed individual-specific variation in *karelinii* bivalent I.

Chromosome II: relative length 126 units.

The centromere position is 0·53 (figure 47, plate 19), the arm lengths being 67 and 59 units. The lumpy objects in the middle of this chromosome resemble structures present on corresponding chromosomes of the other three subspecies and those at 63 and 71 units regularly undergo reflected fusion. As in *cristatus* and *danubialis* but unlike *carnifex*, reflected fusion between the 'marker' loops' axial granule at 56 units and the axial granule at 78 units is frequent (figures 10*g*; and 47, plate 19). Moreover, interhomologue fusion also occurs at these places (figures 10*h* and *i*; and 48, plate 19), and in eight out of thirteen examples of bivalent II all four granules are fused together.

Chromosome III: relative length 118 units.

The centromere position is 0·56, the arm lengths being 66 and 52. Reflected fusion between the axial granules at 54 and 78 units is a regular feature (figures 11*g*, *h* and *i*), and there is always extensive fusion between the stiff spiky loops lying within this reflected region as well. The complexity of chromosome axis relationships here is generally too great for full analysis, though the short reflected lengths of axis including the centromeres are usually not masked. The 'currant buns' at 2·5 units are smaller than is typical of *carnifex* (figure 51, plate 19); subterminally in the short arm at 113 units is a conspicuous axial granule.

Chromosome IV: relative length 110 units.

The centromere position is 0·52, the arm lengths being 57 and 53 units. Reflected fusion and interhomologue fusion between four major axial granules symmetrically disposed on either side of the centromere is frequent, though it is of less regular occurrence than the fusions on chromosome II and III. Some examples of bivalent IV simulate bivalent III because of fusions between short stiff loops which lie just beyond the major axial granules. These loops are, however, very variable in their development (for this reason they are not indicated on the working map) and it is generally possible to follow the complete runs of chromosome axis through the middle of bivalent IV. A small dense loop pair lying just to the right of the centromere allows the middle region to be orientated in cases of reflected fusion. Left and right arms of bivalent IV can be identified by small 'currant buns' at 2·5 units and an axial granule at 94 units.

Chromosome V: relative length 95 units.

The centromere position is 0·50, the arm lengths being 48 and 47 units. It will be recalled that two 'spheres' situated subterminally in the left arm of chromosome V are amongst the most striking features of the chromosome complements of *carnifex*, *cristatus* and *danubialis*. *Karelinii* ♀*F* has similar spheres at 2 and 11 units (figures 56, 58, plate 20) (and also spheres on chromosome VIII), but nine other *karelinii* females either lack all trace of spheres, or have the spheres so ill-developed that they lack diagnostic value. These latter females also lack spheres on chromosome VIII, so the rule established in *carnifex* that variations in morphology of the spheres on bivalent V are matched by the spheres on bivalent VIII is maintained to the extreme in *karelinii*. As in *cristatus* there is a small dense loop pair just to the left of the centromere (figure 60, plate 20). As well as

the three characteristic axial granules to the right of the centromere which are drawn on the working map, there are other axial granules, more variable in development, which lie to the left of the centromere. Occasional examples of bivalent V show reflected fusion between one or other of these granules about the centromere.

Chromosome VI: relative length 88 units.

The centromere position is 0·72, the arm lengths being 63 and 25 units. Reflected fusion and/or interhomologue fusion between the axial granules at 58 and 67 units has been observed in most of the possible combinations (figures 13*i*, *j*, *k* and *l*; and 55, plate 20), half the examples of bivalent VI which have been studied showing fusion of all four granules together. There is a small dense loop pair just to the right of the centromere which aids in tracing chromosome axis sequence through reflected fusions. In *karelinii* ♀ *M* the centromere granule and neighbouring axial bars of one chromosome VI are consistently much smaller than the homologous structures of its partner (figure 13*i*).

Chromosome VII: relative length 86 units.

The centromere position is 0·55, the arm lengths being 47 and 39 units. In *karelinii* the lumpy loops in the mid-region of chromosome VII are almost as large as those of chromosome II (figure 66, plate 21); moreover, in comparison with the other three subspecies more places are occupied by such structures, and their asymmetrical distribution about the centromere mimics the condition in chromosome II. Interhomologue fusion of these lumpy loops occurs, and most exceptionally reflected fusion across the centromere. Nevertheless, the middle regions of chromosomes VII and II and may be readily distinguished, since VII lacks axial granules just beyond the lumpy loop region which in II so frequently fuse to produce axial reflexions enclosing all of the lumpy loops. In one oocyte fusion between the lumpy loops at 52 units on bivalent VII with those at 63 and 71 units on bivalent II was noticed, these two bivalents being also united by a threefold centromere fusion (figure 9*c*). There are conspicuous axial granules at 14·5 and 25·5 units.

Chromosome VIII: relative length 76 units.

The centromere position is 0·57, the arm lengths being 43 and 33 units. The presence or absence of the sphere on this chromosome has already been mentioned when discussing chromosome V. When present as in ♀ *F* (figure 61, plate 20), the sphere lies at 3·5 units, and when a 'pseudo-sphere' at 11 units is also developed the left arm of chromosome VIII mimics the left arm of chromosome V as it does in the other three subspecies. The two adjacent axial granules to the right of the centromere are regular features, as is the small dense loop pair just to the left of the centromere. Further to the left are several more axial granules which are, however, variable in their development.

Chromosome IX: relative length 73 units.

The centromere position is 0·58, the arm lengths being 42 and 31 units. As in the other three subspecies this is the least distinctive member of the complement (figure 68, plate 21). A small dense loop pair just to the left of the centromere is its only conspicuous character (figure 70, plate 21).

Chromosome X: relative length 62 units.

The centromere position is 0·66, the arm lengths being 41 and 21 units. As in the other subspecies this chromosome (figures 14*b* and 23*b*; and 85, 86, 87, 88, 89, plate 22) can be recognized by the dense lateral objects spaced out over much of its length. Whereas the landmark at 51 units consists of recognizable loops, at the other landmarks matrix fusion is extensive and the loop form usually obliterated.

The landmark at 18 units is homologous to the giant loop of *carnifex* chromosome X, and as in *carnifex* this structure shows individual-specific variation in size. Large examples, 20 to 30 μ across, are single or paired objects of irregular shape and uneven refractility, which gives a marbled appearance in phase contrast, and they may contain a few vacuoles (figure 23*b*). In eight *karelinii* females (*C*, *D*, *F*, *G*, *H*, *K*, *L* and *M*) both homologues forming bivalents X carry such structures, whereas in two other females (*A* and *B*) whilst one chromosome X regularly carries a large object at this site its homologue carries a much smaller structure (figures 85, 86, plate 22). In *karelinii* the giant loops on chromosome X never reach the great dimensions often encountered in *carnifex*, nor are their forms so bizarre.

We have also noticed regular size dimorphism of the refractile objects located at 44 units in bivalent X from ♀*G* (figure 87, plate 22).

Chromosome XI: relative length 64 units.

The centromere position is 0·57, the arm lengths being 36 and 28 units. Since like *cristatus* and *danubialis* XI this chromosome lacks the giant loops which are so characteristic of *carnifex* XI, it can easily be confused with chromosome IX. It can be identified most readily by four sites bearing small dense objects in the mid-region at 28, 30·5, 33 and 37·5 units, and by tiny subterminal 'currant buns' at 2 units.

Chromosome XII: relative length 51 units.

The centromere position is 0·67, the arm lengths being 34 and 17 units. The right arm terminates in a very evident double axis region, with short loops carrying few 0·5 to 2 μ granules (figures 115, 116, 122, plate 25). To the right of the centromere at 36·5 units is a large axial granule, and to the left of the centromere, where the axial bar terminates, is a pair of dense loops (figures 23*a* and *b*; and 118, 119, 122, plate 25). Between these loops, which lie at 32 units, and giant loops situated at 24 units, the chromosome axis is in some examples double (figures 23*b*; and 116, 118, 119, 120, plate 25), in others apparently single (figures 23*a*; and 115, plate 25). There may be a string of large axial granules, as many as eight in immediate succession, plastering the chromomeres in this region; in ♀*G* these axial granules are a particularly striking feature (figure 120, plate 25).

As in the other three subspecies there are giant loops on *karelinii* chromosome XII. They lie at 24 units, and in texture they resemble the giant loops on chromosome X. We have observed two cases of fusion between the giant loops of X and XII, one of which is drawn in figure 23*b*. The size of bivalent XII's giant loops varies considerably from one female to another, but in any one newt they appear to be equally developed on the two homologous

chromosomes. Large examples from ♀*D* are shown in figure 23*b* and from ♀*B* in figures 116, 117 and 119, plate 25, small examples from ♀*A* are shown in figure 23*a* and from ♀*G* in figures 115 and 120, plate 25. We have not encountered the 'presence-absence' type of heterozygosity as it exists in *carnifex* and *danubialis*, but in *karelinii* ♀*H* there is fusion heterozygosity comparable to that present at the giant loops loci of bivalent XI in *carnifex* ♀*R*.

It will be recalled that there are several pairs of loops at the giant loops site of *carnifex* chromosome XII, but a single pair in *cristatus* and *danubialis*. Two pairs of loops constitute the giant loops site of *karelinii* chromosome XII, and together with chromosome X's giant loops they appear to be sources of origin of large free bodies. In some bivalents XII there are size and textural differences between the two neighbouring pairs of giant loops, which may be due to their cycles of production of free bodies being out of step.

Subterminally in the left arm at 4·5 units is a pair of large loops with very fine fibrous matrix of low refractility and with clearly defined axes (figures 24*c*; and 115, plate 25). These loops are reminiscent of the big heteromorphic loops on chromosome I in that intercalary portions of their axes are bare of matrix. At 7·5 units is another pair of large loops with similar faintly fibrous, though uninterrupted matrix. These latter may be homologous to the giant granular loops situated subterminally on the left arm of *cristatus* chromosome XII, some examples having highly refractile granules interspersed in the matrix. These granules are, however, less than 0·5 μ in diameter, much smaller than those of the giant granular loops of *cristatus*.

We have noticed presence-absence heterozygosity for loops located at 40 units in the right arm of bivalents XII from *karelinii* ♀♀*F*, *G*, *K* and *L*, these loops of irregular shape projecting some 10 μ from the chromosome axis and having a stiff refractile matrix enclosing fine granules (figures 120, 122, plate 25). The homozygous +/+ condition at this site was noted in ♀♀*D* and *H*, and the −/− condition in ♀♀*A*, *B* and *C*.

VII. Interpretation and speculation

Our intention in writing this paper has been to describe the lampbrush chromosomes of four related subspecies of newts so as to establish a basis for future genetical and physiological studies. However, it would be unreasonable to leave entirely unconsidered the implications of our recorded observations, and in this chapter we will survey some of the questions which these observations pose.

The lampbrush chromosomes of *Triturus cristatus* are morphologically complex—and variable. Some of their structural variability has been shown to be correlated with different stages of oocyte development, and we may surmise that further variability is a reflexion of varying physiological states of oocytes, i.e. varying according to the rate of oocyte development at the time of dissection. It should be equally evident, however, that there is a genetically determined foundation which supports the morphological diversity of lampbrush chromosomes, giving that degree of consistency to our observations without which the mere recognition of individual chromosomes and parts of chromosomes would not be possible. We have been able to recognize individual-specific features of lampbrush chromosome complements because of their constancy. Thus, for example, all oocytes of *carnifex* ♀*E* were heterozygous for giant loops on bivalent *X* and homozygous for lack of

giant loops on bivalent XII when an ovary sample from this female was first studied in March 1956; the same picture obtained at a later sampling in January 1957 and again in October 1959. Similarly all oocytes of *carnifex* ♀ *J* were homozygous for lack of giant loops on bivalent X and heterozygous for giant loops on bivalent XII in October 1956, again in March 1957 and in December 1959.

If we assume that the 'presence' or 'absence' of giant loops on *carnifex* chromosome X is directly determined by genes ' + ' or ' − ' at this site, and if we make a similar assumption regarding the 'presence' or 'absence' of multiple giant loops on *carnifex* chromosome XII, we may inquire whether the frequencies of the combinations within and between bivalents which we have observed accord with Hardy–Weinberg expectations. The data for twenty-two *carnifex* females is set out in table 9. The reasonable agreement between observed and expected figures, though statistically without significance, at least does not discredit the assumption that these alternative giant loop characters assort and combine like two pairs of Mendelian alleles in a random mating population.

TABLE 9. TWENTY-TWO FEMALES OF *T. C. CARNIFEX* CLASSIFIED ACCORDING TO GIANT LOOP CONSTITUTIONS OF BIVALENTS X AND XII. HARDY–WEINBERG EXPECTANCY FIGURES ARE GIVEN IN BRACKETS

	bivalent X			
bivalent XII	+/+	+/−	−/−	totals
+/+	1 (0·07)	0 (0·53)	0 (0·46)	1 (1·14)
+/−	2 (1·01)	2 (3·59)	4 (3·12)	8 (7·72)
−/−	2 (1·74)	4 (6·07)	7 (5·32)	13 (13·13)
totals	5 (2·90)	6 (10·19)	11 (8·91)	22

Moreover, although we have so far made only a preliminary study of the lampbrush chromosomes of interracial hybrids, we have already found that the large loops of low refractility characteristic of *karelinii* chromosomes I, and the axial bars neighbouring the centromeres of all chromosomes of this sub-species, are recognizable in heterozygous state in an F_1 ♀ hybrid *carnifex* ♀ × *karelinii* ♂ and in another F_1 ♀ hybrid *danubialis* ♀ × *karelinii* ♂. How other lampbrush chromosome characters are inherited, to what degree they are stable in hybrids and the extent to which their morphologies reflect qualities of the total gene complex, are questions which await future study both in race hybrids and within races. Whatever the answers to these questions, the very existence of site-characteristic morphologies implies that these morphologies are 'phenotypic' expressions of local genetic specificity—and as such of particular interest because of the intimate relationship here existing between phenotype and genotype.

Throughout this paper we have tacitly assumed that from certain chromosome sites objects large enough to be seen with the light microscope are released into the nuclear sap. The evidence might be read the other way round, i.e. to suggest that certain objects appear first in the nuclear sap and then later become attached to the chromosomes, but if this be so we are entitled to wonder how it is that objects identifiable by details of form and texture regularly proceed to certain places on the chromosomes but not to others. Rejecting this unlikely alternative, we will list the crucial evidence for release from specific sites. The

following are sites where structures, closely resembling certain restricted types of objects found free in the nuclear sap, are laterally attached to the lampbrush chromosomes:

(*a*) The 'currant bun' sites of chromosomes III, IV and XI of *carnifex* (e.g. figures 17*b*; and 49, plate 19).

(*b*) The sites of the large loops of low refractility in the heteromorphic region of chromosome I of *karelinii* (e.g. figure 22*c*).

(*c*) The sphere sites on chromosomes V and VIII of *carnifex*, *cristatus* and *danubialis*, and of such *karelinii* females as possess large spheres (e.g. figures 29, 30, plate 17). The identity of free and attached spheres is especially clear since variations in attached sphere structure from one animal to another, such as vacuolation, presence of dense bodies inside the vacuoles, presence of 'caps' on the spheres, is precisely matched by the free spheres.

(*d*) Some of the lumpy loops sites in the middle region of chromosome II of all four races.

(*e*) In *carnifex*, *cristatus* and *karelinii* the giant loops sites on some or all of chromosomes X, XI and XII—according to individual and race (e.g. figures 81, 83, 110, 117, plates 22, 24 and 25).

There are many other free bodies not covered by the list above, notably the tiny granules or globules 2 μ and less in size, visible in figure 28, plate 17, which vary greatly in number from one oocyte to another. These may form of their own accord in the nuclear sap, but it seems more logical to assume that they are liberated from the chromosomes. Indeed we have direct visual evidence of the release of granules or globules from telomeres, from the short loops on the right arm of chromosome XII (in the terminal double-axis region), from the giant granular loops on chromosome XII of *cristatus*, and from many of the axial granules used as landmarks, in preparations mounted in medium C and observed over a few hours.

The history of the free bodies after their release from the chromosomes is obscure. They remain for a time scattered through the nucleus, where we suppose that they give rise to nuclear sap and disappear by the end of this process. Gall (1955) has emphasized how, in Amphibia, the germinal vesicle nucleus continues to increase in volume throughout the period of growth of an oocyte, and the synthesis of specific nuclear proteins may well be mediated, at least in part, by the *RNA*-rich free bodies which are used up as synthesis proceeds. Certainly the various free bodies do not merely accumulate inside the oocyte nuclei. If they did, and if they are being continually produced by the chromosomes, the greatest number of free bodies would be found in the largest oocytes. In fact the nuclei of mature oocytes are strikingly deficient in free bodies, there being merely a few objects scattered amongst and immediately surrounding the tiny central cluster of chromosomes, the main bulk of nuclear sap being glassy clear. The variation in granularity of immature oocyte nuclei suggests rather that objects visible with the light microscope detach from lampbrush chromosomes *only if they are being synthesized more rapidly than they are transforming into nuclear sap*. We must take a dynamic view of the structure of lampbrush chromosomes, acknowledging that the appearance of the various structures in a particular preparation depends on the balance between rate of synthesis and rate of transformation at the moment of dissection. Thus a *carnifex* oocyte of diameter less than 0·8 mm has very small spheres on bivalents V and VIII, and no free spheres. Only in larger oocytes, when the attached spheres are larger, are free

spheres also present—presumably because sphere material is now being produced faster than it is undergoing transformation. And in those *karelinii* females which at no time form large spheres we never observe free spheres, large or small—presumably in such females the production of sphere material never exceeds the rate of its transformation into nuclear sap. It is significant that in elasmobranch oocytes, where the later stages of cytoplasmic growth (largely due to the accumulation of yolk) are not accompanied by germinal vesicle growth, the chromosomes revert from an extended lampbrush to a condensed form at the end of the period of nuclear growth (Maréchal 1907). This suggests that the lampbrush form stands in particular functional relationship to the synthesis of nuclear material rather than to yolk production.

We will now consider lateral loop structure. The great majority of axial chromomeres bear pairs, or multiples of pairs, of lateral loops. In life, and when isolated in medium C, the fundamental loop form of many lateral structures is not immediately apparent but it becomes apparent when oocyte nuclei are dissected in salines of lower concentration. The only objects attached to axial chromomeres which genuinely lack a loop basis are telomeres, axial granules and spheres. There is a thin fibre forming an axis in each lateral loop and a variety of tests have shown that the continuity of this axis can be enzymically destroyed by *DNA*ase only. This loop axis therefore consists, wholly or in part, of a continuous *DNA* fibril. Surrounding the loop axis there is the material which we have termed 'matrix', which dissolves in salines of low concentration, which is attacked by proteolytic enzymes and by *RNA*ase, and whose texture may differ strikingly from one lateral loop to another. We have assumed that this matrix represents the product of gene-directed synthesis, and we suppose that the loop axis is the genetic material.

Matrix appears to be symmetrically disposed around loop axis, but it is not uniformly distributed with respect to a length of axis. Irrespective of the particular textures of the matrices of loops at particular sites, all lateral loops have one thin end leading from a chromomere, the other end thicker—often very much thicker. In 'normal' loops the gradation from thin to thick end is more or less uniform. There are loops of highly irregular outline and in which the thickest parts are intercalary, nevertheless such loops too have thinner and thicker insertions in chromomeres. At sites where matrix fusion is extensive, partial solution of matrix by enzymes or dilute saline reveals not only the existence of loops, but asymmetrical loops. Loop asymmetry is thus a general and characteristic feature. What does this imply?

The polarity of a loop's asymmetry with respect to the long axis of a chromosome cannot be assessed unless its chromomere of origin breaks, leading to separation of the loop insertions and therefore to a 'double-loop bridge'. All double-loop bridges which we have observed show sister loops with corresponding polarity, i.e. the thinner ends of both attached to one part of a fragmented chromomere, and the thicker ends of both attached to the other part. At least with regard to the giant loops, natural or accidental double-loop bridges have been observed sufficiently often to justify our assertion that loop polarity is constant. Our recorded evidence is as follows:

(*a*) Giant loops on *carnifex* chromosome X at 18·5 units: seven cases from three females, all showing the thin end of the loops directed towards the centromere (e.g. figure 14*f*).

(*b*) Giant loops on *carnifex* chromosome XI at 24 units: eleven cases from six females, all showing the thin end of the loops directed towards the centromere (e.g. figures 16*c*; and 90, plate 23).

(*c*) Multiple giant loops on *carnifex* chromosome XII at 22·5 units: eight cases from five females, all showing the thin ends of one *or more* pairs of loops directed towards the left arm end (e.g. figure 18*a* and *c*).

(*d*) Giant loops on *cristatus* chromosome XII at 25·5 units: seventeen cases from three females, all showing the thin ends of the loops directed towards the left arm end (e.g. figures 23*c*; and 114, plate 24).

(*e*) Giant loops on *danubialis* chromosome XII at 24·5 units: eight cases from two females, all showing the thin ends of the loops directed towards the left arm end (e.g. figure 24*d*).

The asymmetry of lateral loops might be determined by diversity of genetic information along a length of loop axis, so disposed that for full synthesis of gene product at the site in question the material being elaborated must travel past an ordered sequence of regions of loop axis. The morphology of the giant loops at 25·5 units on *cristatus* chromosome XII (figures 23*c*; and 114, plate 24) gives some support to this idea, since the finished products—large free bodies—appear always to slough off from the thick ends of the loops which are nearer the centromere. However, amongst the sites from which free products large enough to be observable with the light microscope detach, these giant loops of *cristatus* chromosome XII are exceptional. At other such sites, although the free products do not accumulate at and detach from regions which are very close to the thinner ends of the loops, detachment is not confined to the thicker ends: it also occurs at intercalary positions. This is particularly clear in the case of the large loops of low refractility on *karelinii* chromosome I (figure 22*c*), and it is probably responsible for the peculiar shapes of those lateral loops which have highly irregular outlines: just such a loop, drawn from *T. marmoratus*, was figured by Callan (1955). If we accept that a structure which detaches from a lateral loop is a 'fully-fashioned' gene product, then accumulation at and detachment from intercalary regions leads us to suppose that intimate contact with a full length of lateral loop axis is not a requisite for complete synthesis. Furthermore, at one and the same site the lateral loops may be long or short in different oocytes, depending on developmental stage and possibly on physiological state. If there is diversity of genetic information throughout the length of a lateral loop, full synthesis only becomes possible at maximum loop extension. Yet at a particular site the full range of textural differentiation can be evident both in short and in long loops, suggesting that complete synthesis can be occurring even though loops are not maximally extended.

Loop asymmetry may alternatively depend on a polarized system of loop development. Chromomeres are Feulgen-positive; they are at their smallest when the loops attached to them are maximally extended, and increase in size as the loops regress in length. Loop axes contain *DNA*, and thus it seems very probable that loop axis extends at the expense of chromomere substance, though how this extension occurs is obscure. If loop axis extends from a chromomere *at one end only*, then there will be 'new' and 'old' regions of axis, with greater accumulation of gene product in older than in younger regions. Such a scheme would account very well for the regional differences in texture at sites such as the giant granular loops at 9 units on *cristatus* chromosome XII (figures 24*a* and *b*; and 111,

112, 113, plate 24). The thin, supposedly new, end of this loop leads on to a zone where dense material invests a contorted stretch of axis; farther on this dense material splits up into several bars loosely wrapped about the axis; farther on still these bars project radially from the loop axis and are constricted so that the dense material is disposed as granules on strings (see figure 113); finally we reach an extensive 'mature' zone, fully half the length of the loop, where the connexions between granules of dense material and the loop axis are very tenuous—it is from this zone that the granules break free. Certainly the morphology of this loop—exceptional only in size—suggests a developmental sequence in granule

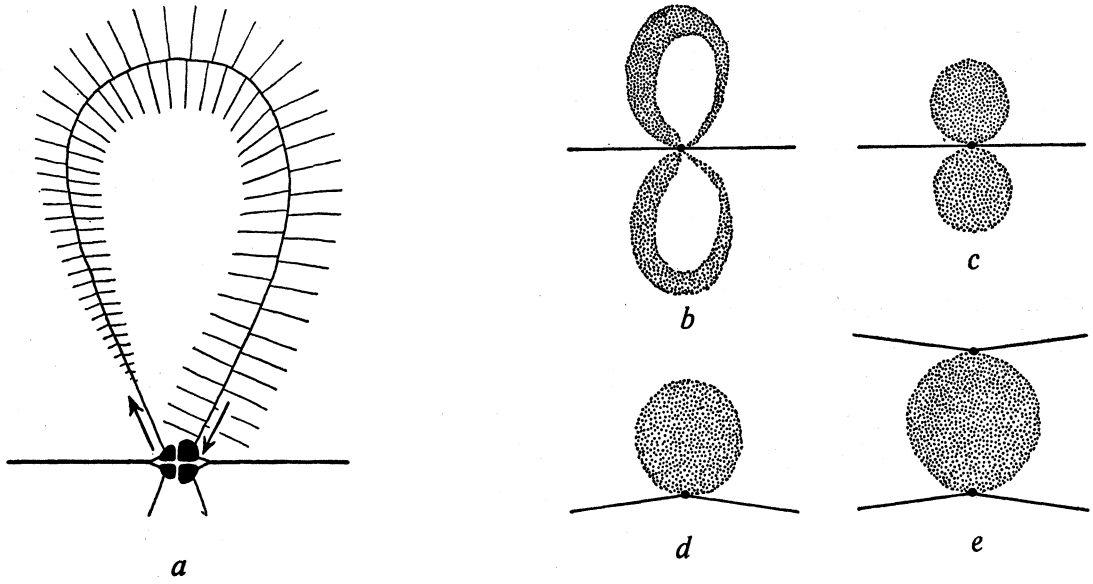

FIGURE 26. (*a*) Diagram illustrating a theory of polarized lateral loop extension and retraction put forward in § VII to account for the asymmetry typical of lampbrush loops. The chromomere is drawn quadripartite, indicating the planes in which it is known to be capable of cleavage. (*b*) to (*e*) are diagrams illustrating degrees of matrix fusion, loop form being conserved in (*b*), obliterated in (*c*), sister loops fused in (*d*), and sister plus homologous loops fused in (*e*).

production. Now it is possible that the dense bars move along the loop axis as they differentiate into granules, but we consider this explanation for the zonation less plausible than to suppose that the axis *itself moves*, there being always a recently drawn or extended portion of axis in association with which new synthesis of granule material starts up. However, if this latter explanation is to hold, then as a corollary we must suppose that the oldest region of loop axis is continually being reincorporated into the parent chromomere. Were this not so the loop would continue to increase in overall length throughout oocyte development, whereas in fact it is relatively constant in size in oocytes ranging from 0·8 to 1·4 mm diameter. Where loops do vary greatly in size from oocyte to oocyte—compare, for example, figure 101 with figure 102, plate 23—the variations could be explained as due to changing balance between new loop-axis production and old loop-axis reincorporation, the balance being in some way determined by the rate at which raw materials and energy supplies are provided to the nucleus.

The evidence at present available does not warrant much further speculation, but it should be realized that acceptance of this second explanation for loop asymmetry, shown

diagrammatically in figure 26*a*, includes the assumption that there is no diversity of genetic information *within* individual lampbrush loops; the information carried by a particular region of a chromosome may rather be serially repeated along a loop axis.

It is currently assumed that genetic information consists of a coded sequence of nucleotides (Watson & Crick 1953), a code which at most replications is faithfully copied and which may give rise to a mutation if miscopying occurs. The smallest genetic recombination fractions so far measured suggest that mutational sites may be only a few nucleotide pairs long (Benzer 1955, 1957; Pontecorvo & Roper 1956). However, mutation occurring in a permanent, serially repeated nucleotide sequence would presumably only be detectable when most, if not indeed all repeats of the code were similarly altered from the original.

This logical impasse can be avoided if we make the further assumption that there is at each chromomere a 'master copy' of the genetic code which owes its specificity not only to a particular nucleotide sequence, but also to a particular structural form (see discussion in Pontecorvo 1959) in which this sequence is arranged. Such a master copy might act as a template, repeatedly handing on the appropriate form to loop axis extending from a *DNA* 'store' in the chromomere. The *DNA* in a loop axis, now fully specified, would act as the template for synthesis of gene product, during which process its form specificity might be degraded and finally lost as the thread is packed up for transit through the later stages of meiosis. Given such a mechanism, single-mutational events could be detectable if they occurred in master copy *DNA* and affected its form, since we assume specificity of form to be conserved during cell division by the master copy only.

Several authors (e.g. White 1954) have remarked on the immense differences between animal species in quantity of *DNA* per nucleus (Mirsky & Ris 1951). These differences are certainlv not correlated with grade of organization, and are therefore unlikely to be correlated with degree of genetical complexity—in crude terms total number of genes—per haploid chromosome set. Mirsky & Ris (1951) and Ris (1957) have accounted for such differences by postulating different degrees of 'strandedness' of the chromosomes characteristic of particular organisms; they might instead be attributable to differing proportions between 'master copy' *DNA* and *DNA*, specified as to form by the master, which acts as the immediate template for synthesis of gene product.

We will now consider the various kinds of fusions between lampbrush chromosomes, first of gene products and secondly chiasmata. Fusions do not occur between what we have termed 'normal' lateral loops. As mentioned in §III*c* these, the great majority of lampbrush loops, consist of loop axis bearing a polarized distribution of radially arranged fibres. We suppose that the product synthesized by such loops transforms into nuclear sap as fast as it forms, without accumulating as aggregates visible in the light microscope. The fine fibres projecting from the axes of such loops are extremely sensitive to proteolytic enzymes and *RNA*ase, and also readily dissolve in dilute salines. Occasional oocyte nuclei have granular loops at so many places that we must assume that 'normal' loops in exceptional physiological states bear granules. Fusions do not occur between granular loops.

The matrices of loops which do undergo fusions have as common properties:

(*a*) A texture so dense that loop axis and associated fine fibres are not evident.

(*b*) A sharply defined boundary between matrix and nuclear sap. The latter property is a feature also of those other components of lampbrush chromosomes which take

part in fusions, namely telomeres, axial granules, centromere granules (in *karelinii*) and 'spheres'.

Evidently if matrix fusions are to occur, matrix must accumulate where it is being synthesized, the objects fusing must lie close together, and there must be certain textural qualities of the matrix permitting it to amalgamate.

There are several degrees of matrix fusion, shown schematically in figure 26*b* to *e*:

(*a*) Within single loops, when the loop form is partly obscured or totally obliterated.

(*b*) Between sister loops, producing single instead of the more usual paired objects at particular sites.

(*c*) Between homologous loops or other structures on partner chromosomes.

(*d*) Between genetically non-homologous structures which, however, possess some textural similarity. Fusions of this fourth degree have particular interest. Telomeres can fuse with homologous telomeres, with non-homologous telomeres of the same bivalent, with telomeres of other bivalents—such fusions occasionally involving more than two telomeres (figure 109, plate 24)—and with at least certain axial granules (figure 73, plate 25). Spheres can fuse with homologous spheres, with non-homologous spheres of the same bivalent, and fusions between the spheres of chromosomes V and VIII have been observed once in *carnifex* and twice in *cristatus*. The lumpy loops about the centromere of chromosome II can fuse with homologous loops, with non-homologous loops of the same bivalent, and a fusion between these and similar loops about the centromere of chromosome VII has been observed once in *karelinii* (figure 9*c*). In *karelinii* we have twice observed fusions between giant loops of chromosomes X and XII (figure 23*b*), whilst in *carnifex* we have once observed fusion between giant loops of XI and XII (figures 18*d*; and 108, plate 24).

Evidently non-homologous fusions do not occur at random. Thus spheres only fuse with other spheres, not with telomeres despite their being near neighbours. Indeed if we take the evidence from fusions as suggestive that certain genetically non-homologous sites synthesize identical or at least similar products, then although these newts considered overall have diploid constitutions they must in part be polyploid. It is conceivable that chromosomes II and VII, both with lumpy loops about the centromeres, have a common evolutionary ancestry; the same may be true of chromosomes V and VIII, both carrying subterminal spheres.

Spatial proximity as well as textural affinity is required if gene products are to fuse, and it is not surprising that interhomologue fusions are much more frequent than non-homologous fusions, since homologous sites lie side by side during the zygotene preceding the lampbrush stage. Pericentric reflected fusions such as occur so frequently in bivalents II, III, IV and VI are more puzzling. However, the more or less symmetrical distribution of landmarks on either side of the centromeres of these chromosomes may possibly indicate an evolutionary history of centric misdivision followed by isochromosome formation (Rhoades 1938; Darlington 1939); mitotic anaphase orientation persisting through to early meiotic prophase would then cause structures on either side of the centromere having genetic affinities to lie close together.

Having discussed gene product fusions we now turn to chiasmata. The problem of how to discriminate between chiasmata and gene-product fusions was raised in § III*d*, prior to

examining the linear distributions of chiasmata along lampbrush chromosomes. Since some gene products are dissolved by pepsin plus hydrochloric acid when these agents are used under certain conditions which do not cause lampbrush chromosomes to coagulate (Macgregor, personal communication), and since pepsin does not break interchromomeric fibrils nor lateral loop axes, this enzyme can be used experimentally to identify gene product fusions: unions between homologous chromosomes which break down during pepsin digestion must be of gene products. However, we cannot be sure of the converse, i.e. that unions withstanding pepsin digestion are necessarily chiasmata. Macgregor (personal communication) has found that centromere fusions in *karelinii* survive pepsin digestion, yet as discussed in § VI we think it unlikely that these fusions are chiasmata.

The shattering of lampbrush chromosomes by *DNA*ase, but not by other enzymes (Callan & Macgregor 1958), is evidence that a *DNA* fibre runs continuously from one end of the chromosome, out into each lateral loop, and between each chromomere, until it reaches the other end. We may inquire whether genetic recombination at chiasmata is restricted to regions of the *DNA* fibre between lateral loops (and their parent chromomeres), or whether it can also occur within the lengths of lateral loops (and their parent chromomeres). As mentioned in § III *d*, chiasmata always involve connexions between chromosome 'main' axes, either at interchromomeric regions or at chromomeres. We have never observed chiasmata within the lengths of lateral loops, though we should certainly expect to recognize such relationships between homologues if they existed. This observation may be taken as implying that all genetic exchanges fall between successive lateral loops (or their parent chromomeres), but if so the chiasmate associations at chromomeres, which unquestionably exist, require explanation. Rather than speculate that such chromomeres are compound structures, with the genetic exchanges nevertheless still occurring between parent chromomeres of successive lateral loop pairs, we prefer to leave the question open. To answer this question with assurance we require further knowledge of chromomere fine structure and of the mechanism by which loop axis is generated.

Finally, let us very briefly consider the problem presented by peripheral 'nucleoli'. Having satisfied ourselves that several classes of free bodies in these newt oocyte nuclei arise by detachment from particular sites on the lampbrush chromosomes, we held for a long time to the preconceived notion that the peripheral 'nucleoli', most conspicuous of all the free bodies, must also originate in this way. In half-grown and older oocytes of subspecies *cristatus* the bodies which detach from the giant loops' site at 25·5 units on chromosome XII resemble 'nucleoli' in size, refractility and texture, and so, in some though not all oocytes, do the bodies which detach from the giant loops' sites on chromosomes X, XI and XII of subspecies *carnifex*. In *karelinii* there is less resemblance between 'nucleoli' and bodies which detach from the giant loops' sites on chromosomes X and XII, while in *danubialis* there are no sites anywhere along the lampbrush chromosomes which produce objects remotely resembling 'nucleoli'. Furthermore, in tiny oocytes of all four subspecies, when the peripheral 'nucleoli' are smaller, morphologically and texturally different, and tightly attached to the nuclear membrane, there are no lateral structures on the lampbrush chromosomes in any way similar to such 'nucleoli'.

By applying enzymes to the contents of unfixed oocyte nuclei Macgregor (personal communication) has shown that the reactions of peripheral 'nucleoli' and of the material

accumulated at the giant loops' sites of chromosomes X, XI and XII of *carnifex* are different, and has supplied further evidence forcing us to conclude that the 'nucleoli' are not generated at the chromosomes. If so, then the peripheral 'nucleoli' of newt oocytes are not comparable to nucleoli of somatic interphase nuclei, and this is the reason why, whenever we have used the term 'nucleolus' in relation to oocytes, we have placed this word in inverted commas. Gall gave a similar opinion in his preliminary (1952) paper on the lampbrush chromosomes of *Triturus viridescens*, though he tentatively reversed this judgement as a result of later observations (1954) made on *Amblystoma tigrinum*. We are indeed now convinced that the peripheral 'nucleoli' of oocytes are unrelated to somatic nucleoli; but unfortunately we have not yet been able to determine which structures on the lampbrush chromosomes do correspond to the latter objects.

An attack on the ultra-structure of lampbrush chromosomes is necessary to establish the relationships between loop axis and matrix; between chromonema, chromomere and loop axis; and the degree of 'strandedness' of lampbrush chromosomes' *DNA*. Such a study might well throw light on the general problem of chromosome replication, since one can conceive of the possibility that in lampbrush chromosomes replication is actually occurring as *pairs* of loop axes extend from *single* parent chromomeres. Of the other challenging problems raised by the structure of lampbrush chromosomes, we may in conclusion mention that no correlation has yet been established between site-specific morphologies of lampbrush chromosomes and characters of adult newt phenotypes, nor has the synthesis of any substance having a high degree of chemical specificity been demonstrated in amphibian oocyte nuclei. Since site-specific morphologies imply chemically specific syntheses, it will be of interest to establish to what extent amphibian egg cytoplasm receives raw materials pre-specified for embryo-building from the germinal vesicle nucleus. The relative or complete inability of sperm chromosomes to survive and replicate in foreign cytoplasm suggests pre-specification of a high degree.

We are indebted to the many people with whom we have discussed the structure and functional significance of lampbrush chromosomes; and in particular we wish to thank Mr D. R. R. Burt, Dr J. M. Dodd, Dr G. A. Horridge, Mr H. C. Macgregor, Dr C. Muir and Professor G. Pontecorvo, F.R.S., for detailed criticism of the manuscript of this paper.

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Description of plates 17 to 25

Plate 17

Figure 27. The twenty-four mitotic chromosomes in tail epithelium of a *Triturus cristatus carnifex* larva, from a 'squash' preparation fixed in acetic-alcohol and stained in aceto-orcein. Each chromosome is marked with its most probable identity.

Figure 28. Intact nucleus from a *T. c. carnifex* oocyte of 1 mm diameter isolated in 0·2 M-potassium chloride, with a trace of magnesium chloride added to improve the visibility of the lampbrush chromosomes. Notice also the peripheral 'nucleoli', and the many tiny granules surrounding the central chromosome group.

Figures 29 and 30. Low-power phase-contrast photographs of entire isolated *T. c. carnifex* lampbrush chromosome complements, with each bivalent identified. Figure 29 is from ♀*D*, 1·5 mm oocyte, in medium A (see text). Notice the two free spheres marked *f.s.* and the attached *not fused* spheres on bivalents V and VIII. Most of the other free objects in the photograph are partially disintegrated peripheral 'nucleoli'. Bivalent III is shown at a higher magnification in figure 50, plate 19. Figure 30 is from ♀*R*, 0·9 mm oocyte, in medium C (see text). Again notice the free spheres, and the attached *fused* spheres on bivalents V and VIII. The other relatively large free objects are peripheral 'nucleoli'. Bivalent X is shown at a higher magnification in figure 73, plate 21.

Negatives of the photographs shown in plates 18 to 25 were taken with phase contrast at one or other of two objective magnifications: × 40, subsequently referred to as low power (LP); and × 95, subsequently referred to as high power (HP). All these negatives were subsequently printed at the same photographic enlargement. Scales are marked on figures 31 (LP) and 32 (HP), the respective overall magnifications being × 257 and × 610.

Where present and identifiable, centromeres are marked by arrows. Where included in the photographs, the ends of left and right arms of the various chromosomes are marked '*LE*' and '*RE*', respectively; the orientation of portions of chromosome axes not including ends is given by the marks *L* (left) and *R* (right); the left/right convention is defined in the text.

In the descriptions of figures 31 to 124, newts are referred to by their subspecific names only, followed by the identity of the female and by the diameter of the oocyte, in brackets, from which the chromosomes were derived. Unless otherwise stated, the photographed chromosomes were isolated in medium C (see text).

Plate 18

Figure 31. LP *carnifex* ♀*J* (1·1 mm). Bivalent I entire. Notice the very conspicuous contorted loop pair, marked *HL*, lying at 24 units in the heteromorphic region of one of the left arms. An outline drawing of this bivalent is shown in figure 7*b*, p. 155.

Figure 32. HP *carnifex* ♀*O* (1·2 mm). Region around the centromeres of bivalent I, with two chiasmata. The large white objects are 'nucleoli'.

Figure 33. LP *karelinii* ♀*F* (1·0 mm). Bivalent I entire, with chiasmata on either side of the centromeres and another chiasma close to the ends of the left arms. An outline drawing of this bivalent is shown in figure 21*d*, p. 184, where the more conspicuous loops in the heteromorphic regions are indicated.

Figure 34. HP *karelinii* ♀*F* (1·0 mm). Region around the centromeres of the bivalent I shown in figure 33, with two chiasmata. Notice the refractile axial bars about the centromere granules, which are distinctive of *karelinii*.

Figure 35. HP *carnifex* ♀*D* (0·6 mm). Left end of chromosome I, including a large loop pair with striking asymmetry lying at 6 units. A detailed drawing of this loop pair is shown in figure 8*a*, p. 156.

FIGURE 36. HP *carnifex* ♀*C* (0·7 mm). This photograph includes one of a large pair of loops lying at 12 units in chromosome I. A detailed drawing of this region is shown in figure 8*b*, p. 156.

FIGURE 37. HP *carnifex* ♀*O* (1·0 mm). Focused on the chromosome axis, this photograph includes a typical example of a pair of the contorted loops present in the heteromorphic region of bivalent I. These contorted loops probably correspond to the large loop shown in figure 36; they lie at 12 units.

FIGURE 38. HP *carnifex* ♀*J* (1·1 mm). This photograph includes the highly refractile contorted loop pair of the bivalent I shown in figure 31. A detailed drawing of this object is shown in figure 8*e*, p. 156.

FIGURE 39. HP *cristatus* ♀*E* (1·2 mm). This photograph includes one of the pair of loops located at 79 units in the heteromorphic region of chromosome I which are distinctive of *cristatus*. A detailed drawing is shown in figure 22*e*, p. 185.

FIGURES 40 AND 41. HP *karelinii* ♀*B* (1·2 mm). These photographs include the multiple large loops of relatively low refractility located at 76 units in the heteromorphic region of chromosome I which are distinctive of *karelinii*, figure 40 being focused on the chromosome axis and figure 41 on outlying parts of the loops. A detailed drawing is shown in figure 22*c*, p. 185.

PLATE 19

FIGURE 42. LP *carnifex* ♀*G* (1·2 mm). Middle region of bivalent II including the centromeres and 'lumpy' loops.

FIGURE 43. LP *carnifex* ♀*G* (1·2 mm). Middle region of bivalent II including the centromeres and 'lumpy' loops.

FIGURE 44. HP *carnifex* ♀*N* (1·1 mm). Middle region of bivalent II including the centromeres and 'lumpy' loops.

FIGURE 45. HP *carnifex* ♀*CA* (0·8 mm). Middle region of bivalent II including the centromeres and 'lumpy' loops.

FIGURE 46. LP *karelinii* ♀*F* (1·1 mm). To the left, middle region of bivalent II and to the right, middle region of bivalent VII, both including centromeres and 'lumpy' loops. See also legend to figure 47.

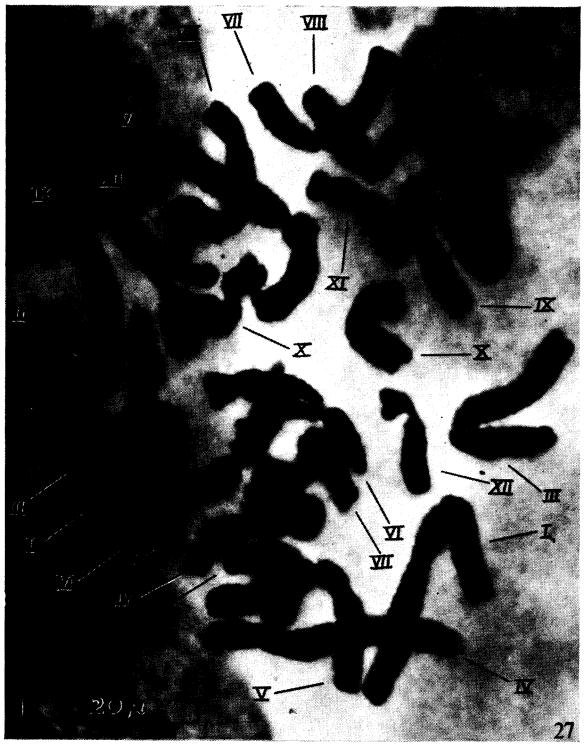
FIGURE 47. HP *karelinii* ♀*F* (1·1 mm). This photograph includes the middle region of the bivalent II in figure 46, and shows reflected fusion between pericentric axial granules, marked *RF*, and centromere fusion. An outline drawing of this configuration is shown in figure 10*g*, p. 159.

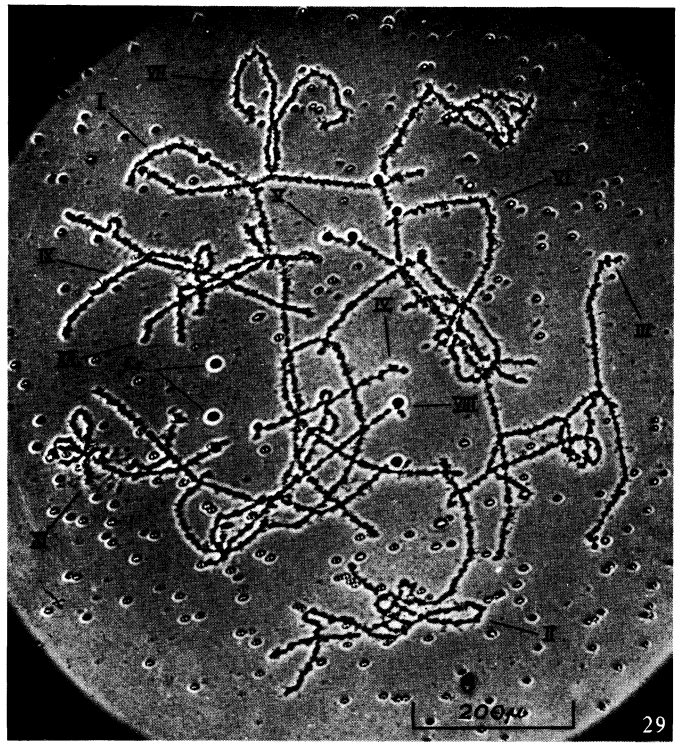
FIGURE 48. HP *karelinii* ♀*F* (1·2 mm). This photograph includes the middle region of bivalent II, and shows reflected plus interhomologue fusion (*RF*) of pericentric axial granules. An outline drawing of this configuration is shown in figure 10*i*, p. 159.

FIGURE 49. HP *carnifex* ♀*D* (1·6 mm). This photograph includes one of the left arm ends of the bivalent III in figure 50, with a pair of subterminal 'currant buns' and two identified free products.

FIGURE 50. LP *carnifex* ♀*D* (1·6 mm). Bivalent III entire, showing subterminal 'currant buns' on the left arms, reflected fusion between pericentric axial granules, marked *RF*, and reflected fusion of terminal to subterminal axial granules in the right arms, marked *REF*.

FIGURE 51. HP *karelinii* ♀*F* (1·0 mm). This photograph shows fusion between homologous terminal granules on the left arms of bivalent III, with neighbouring 'currant buns'.


VII
VIII
XI
IX
X
X
XII
III
VI
I
VIII
V
IX
27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VIII
200μ
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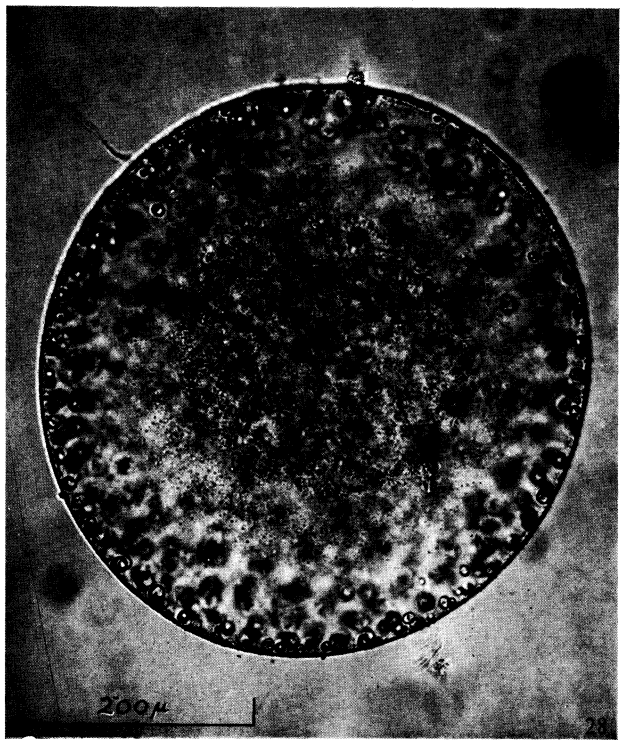
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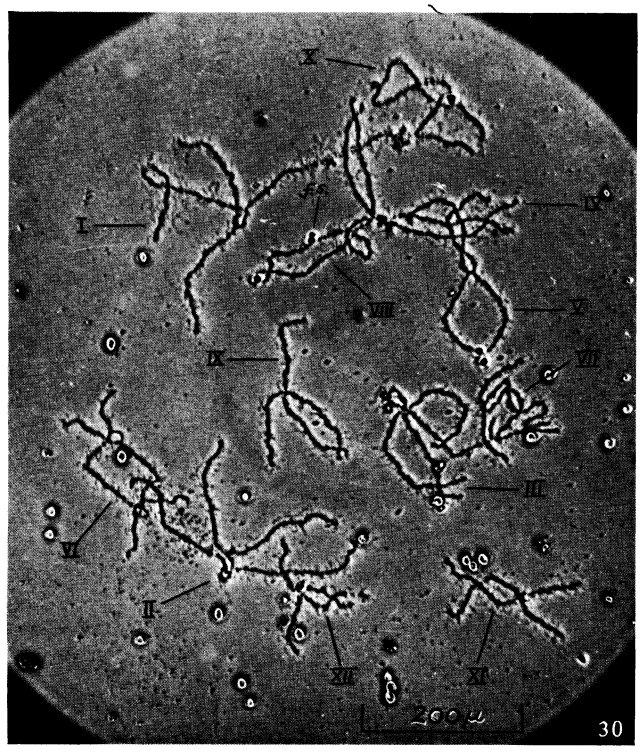
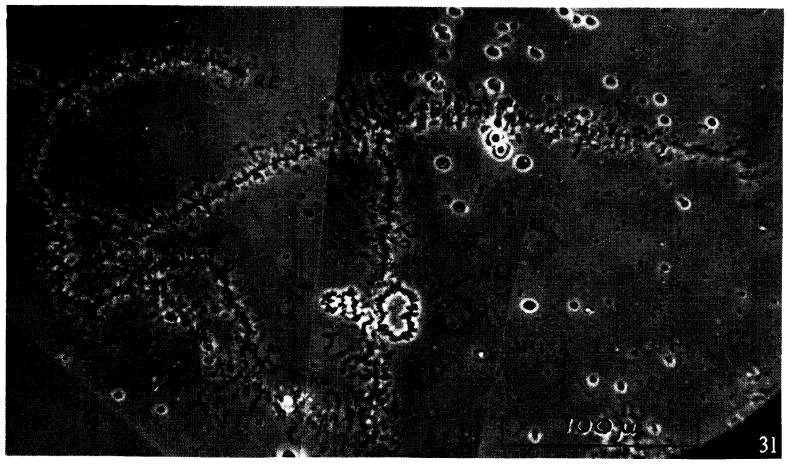

X
I
IX
VIII
V
IX
VII
III
VI
II
XII
XI
200μ
30

(*Facing p.* 214)


100μ
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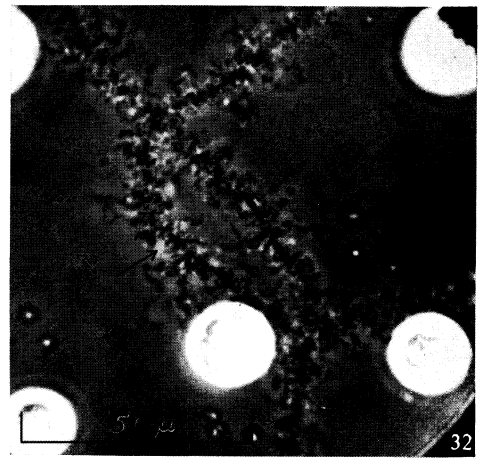
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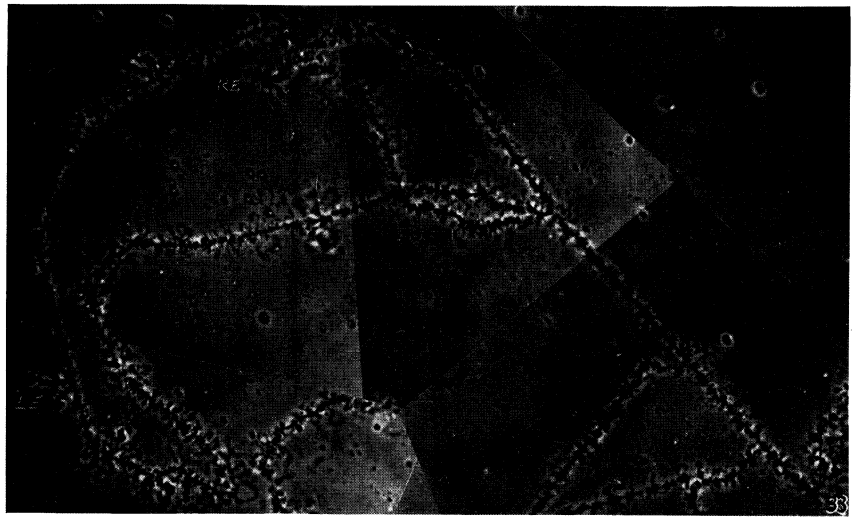
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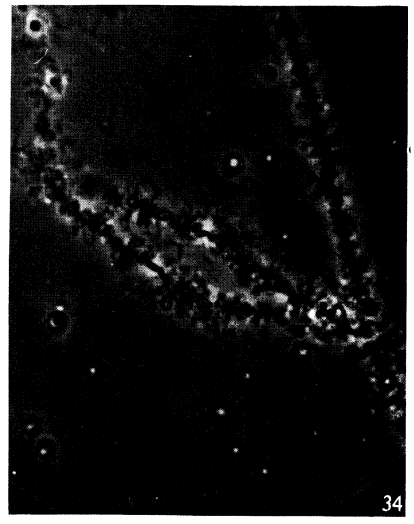
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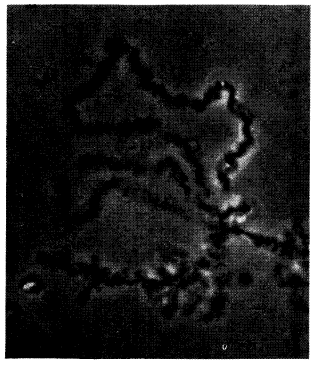

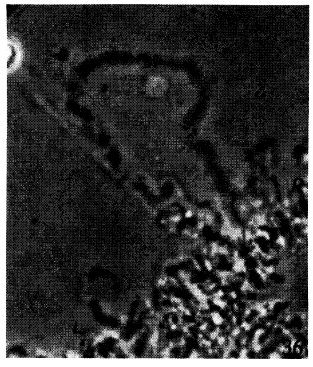

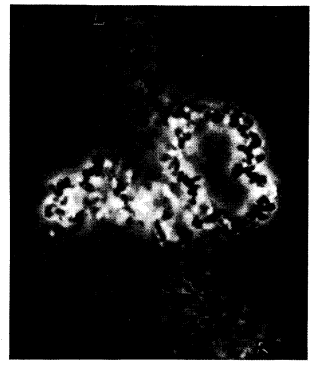

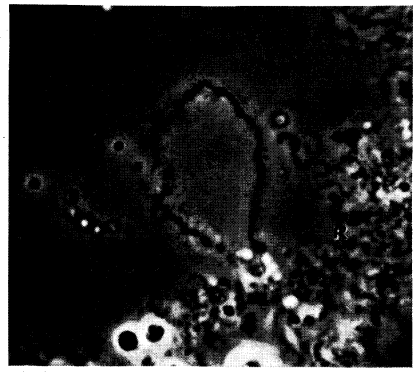

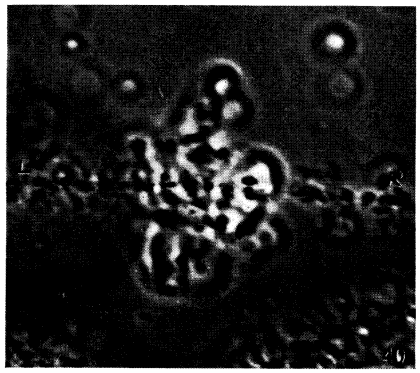

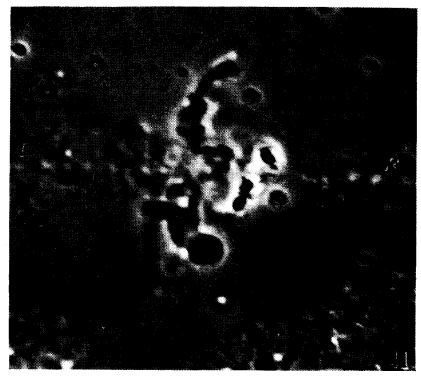

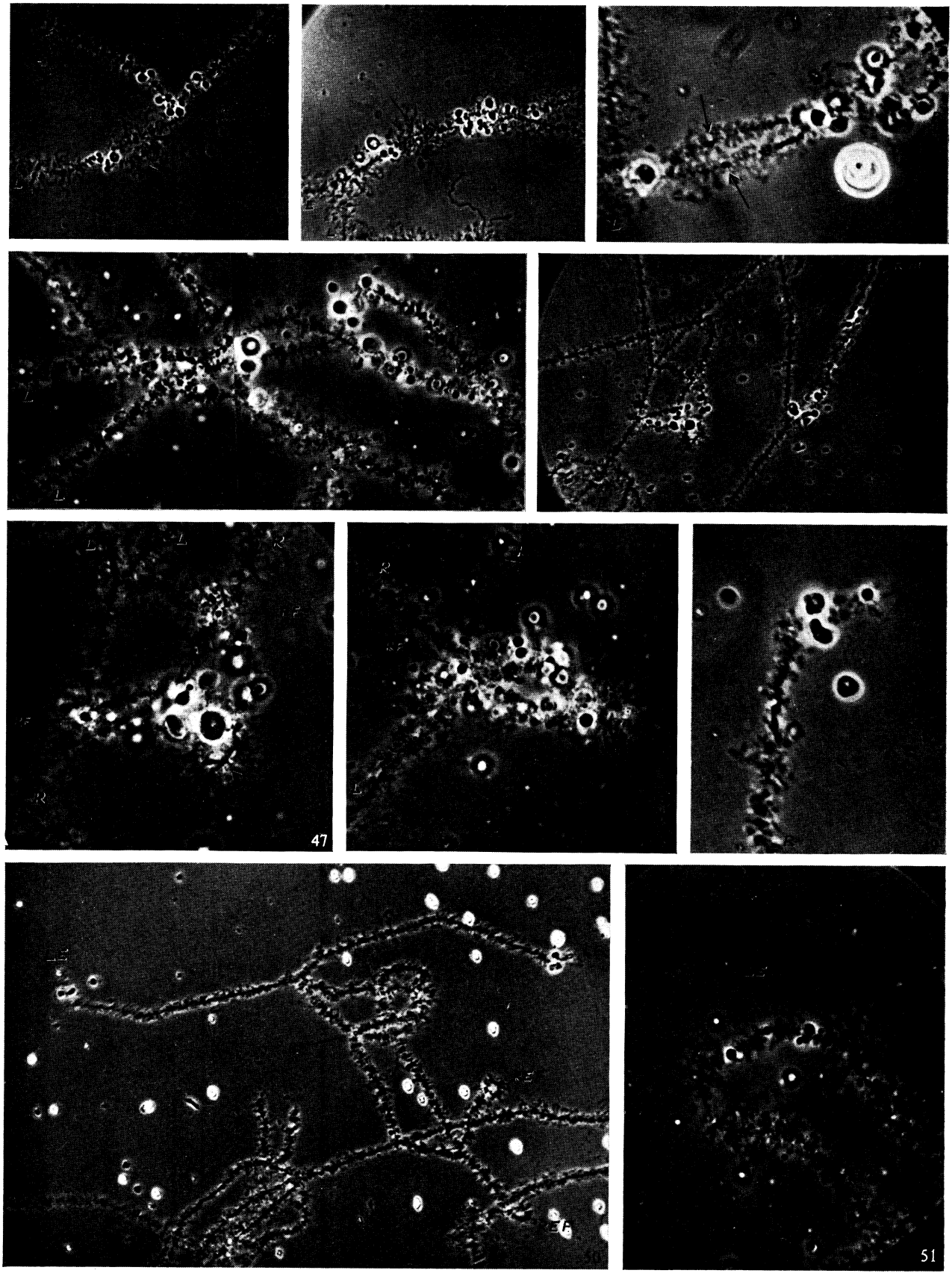
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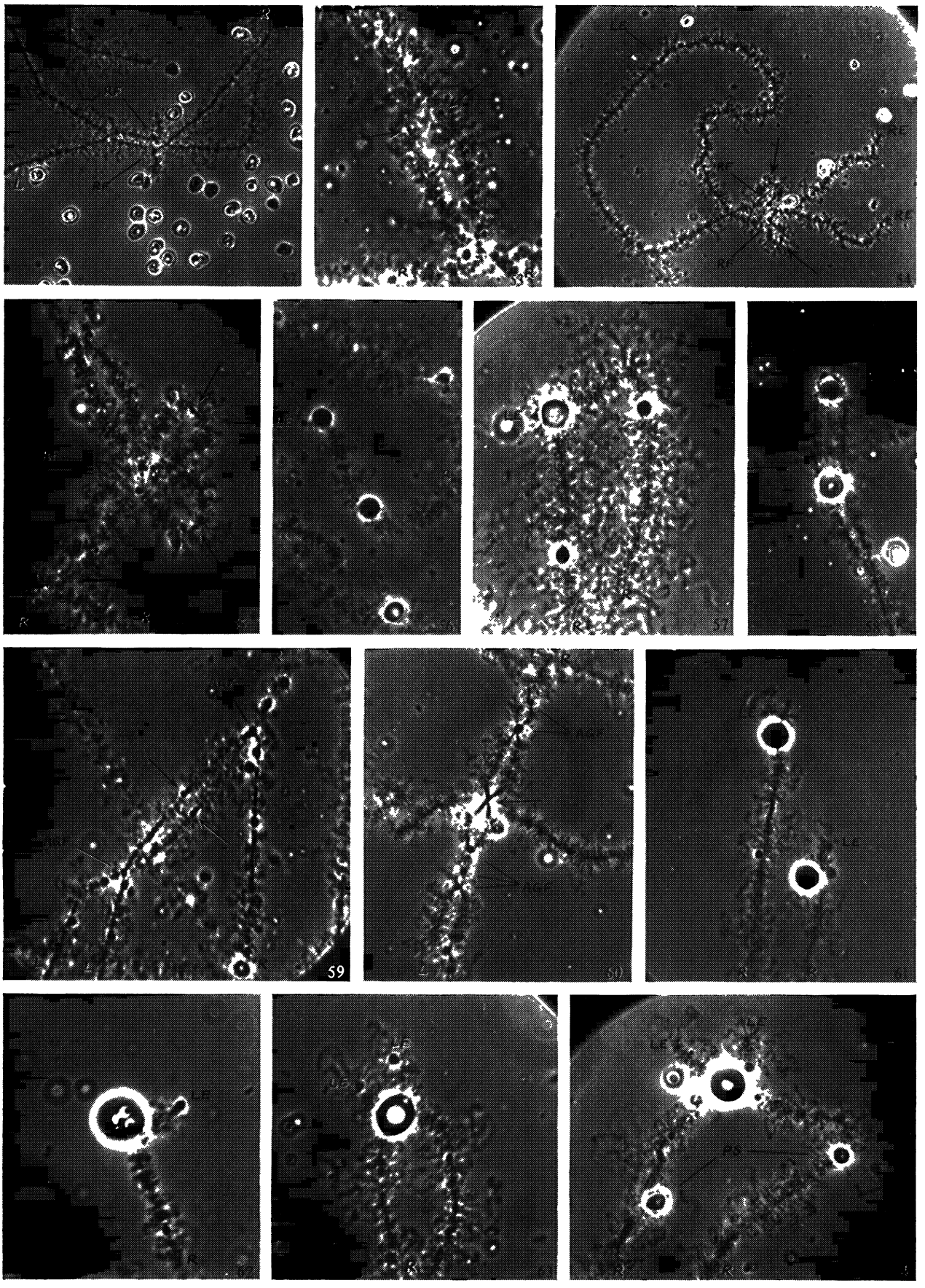
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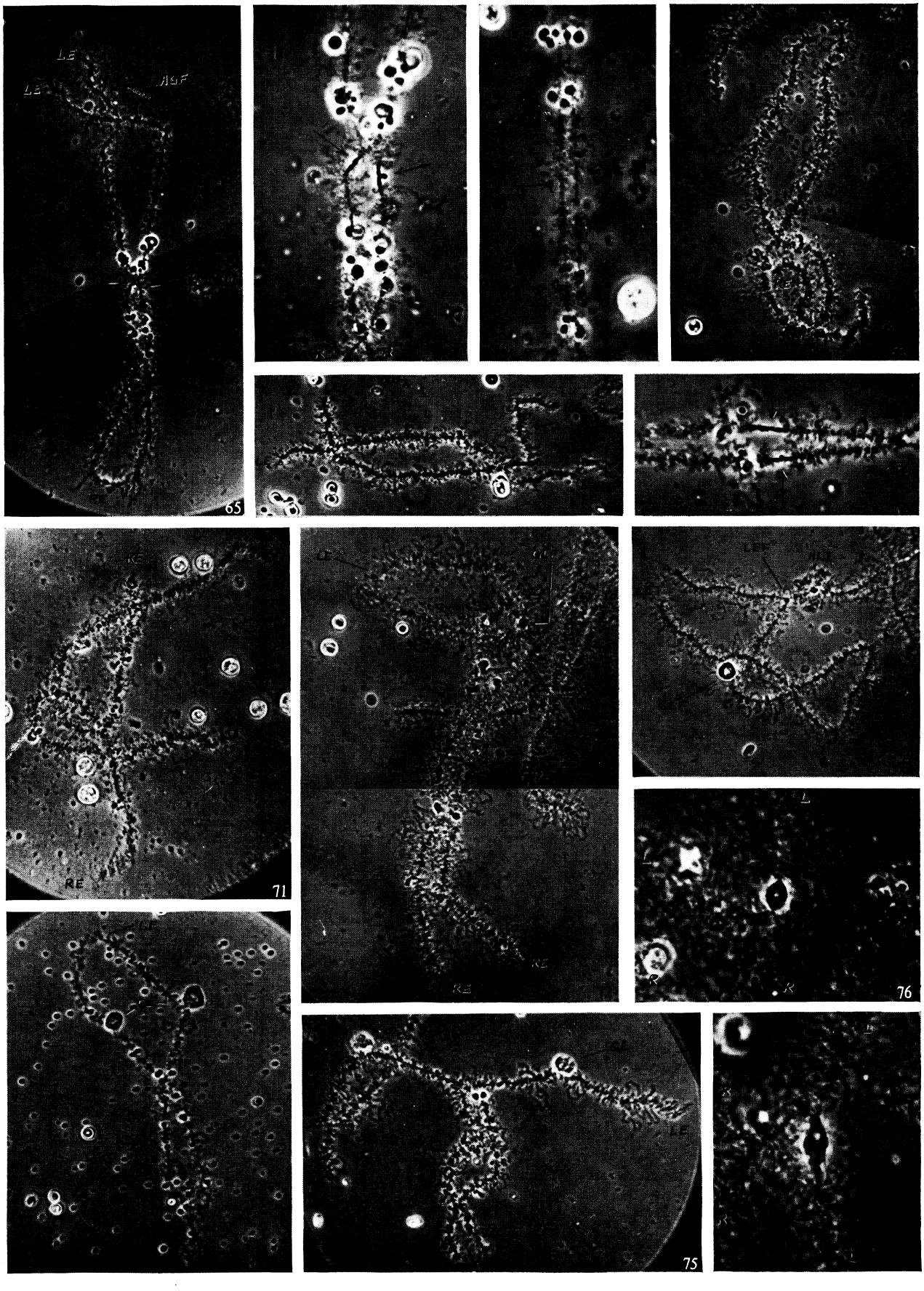
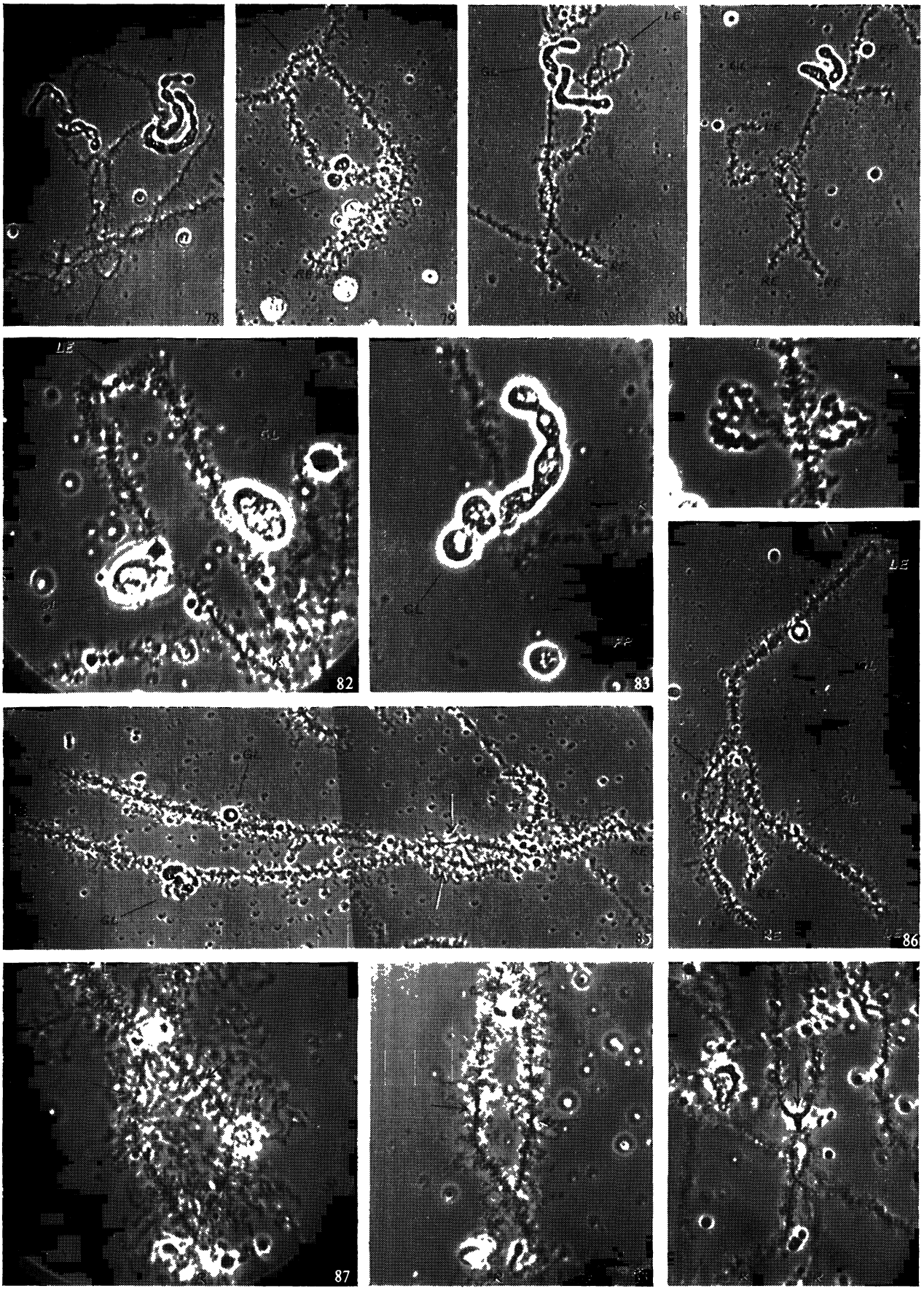

LE
LE
ASF
65
71
RE
RE
RE
75
76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LE
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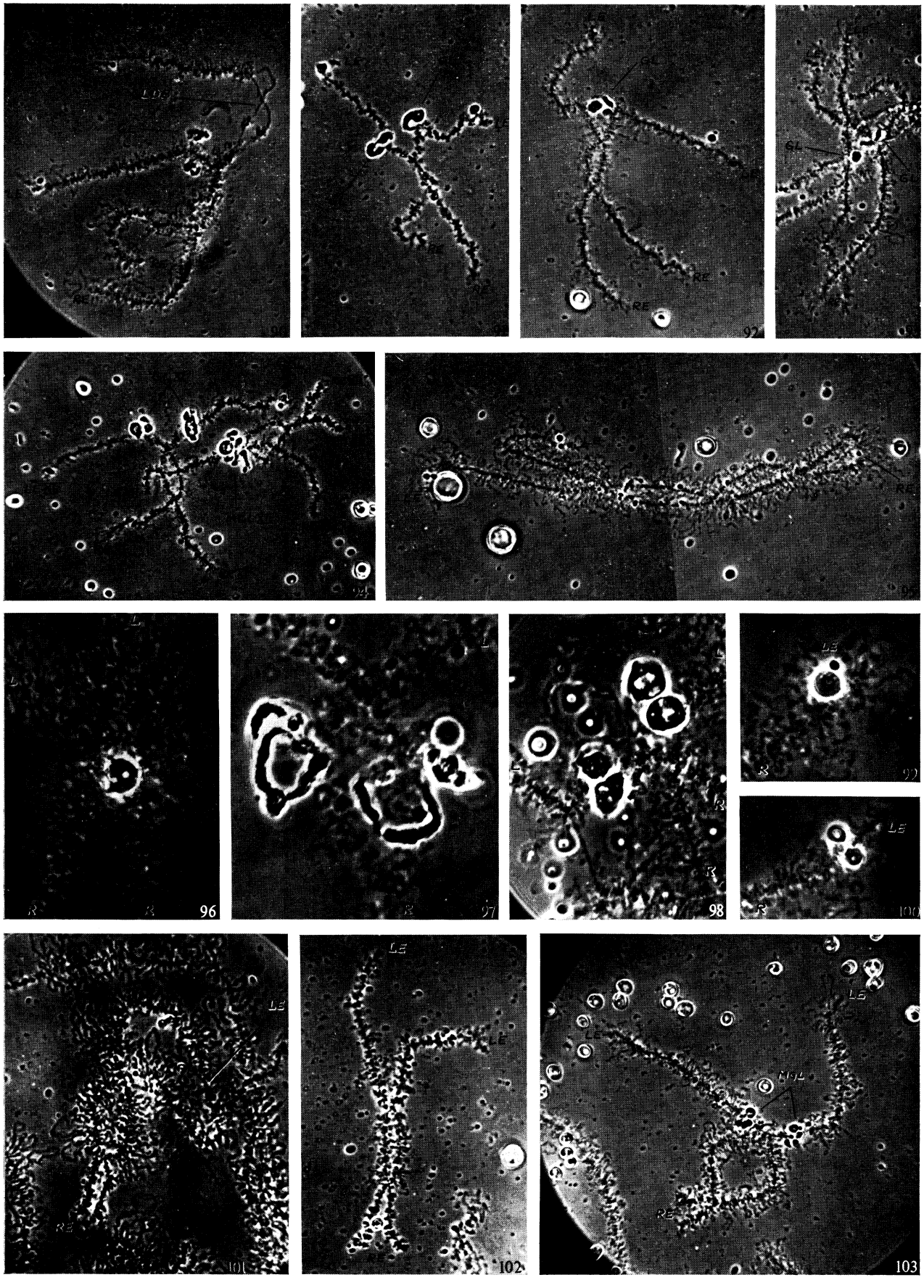
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GL
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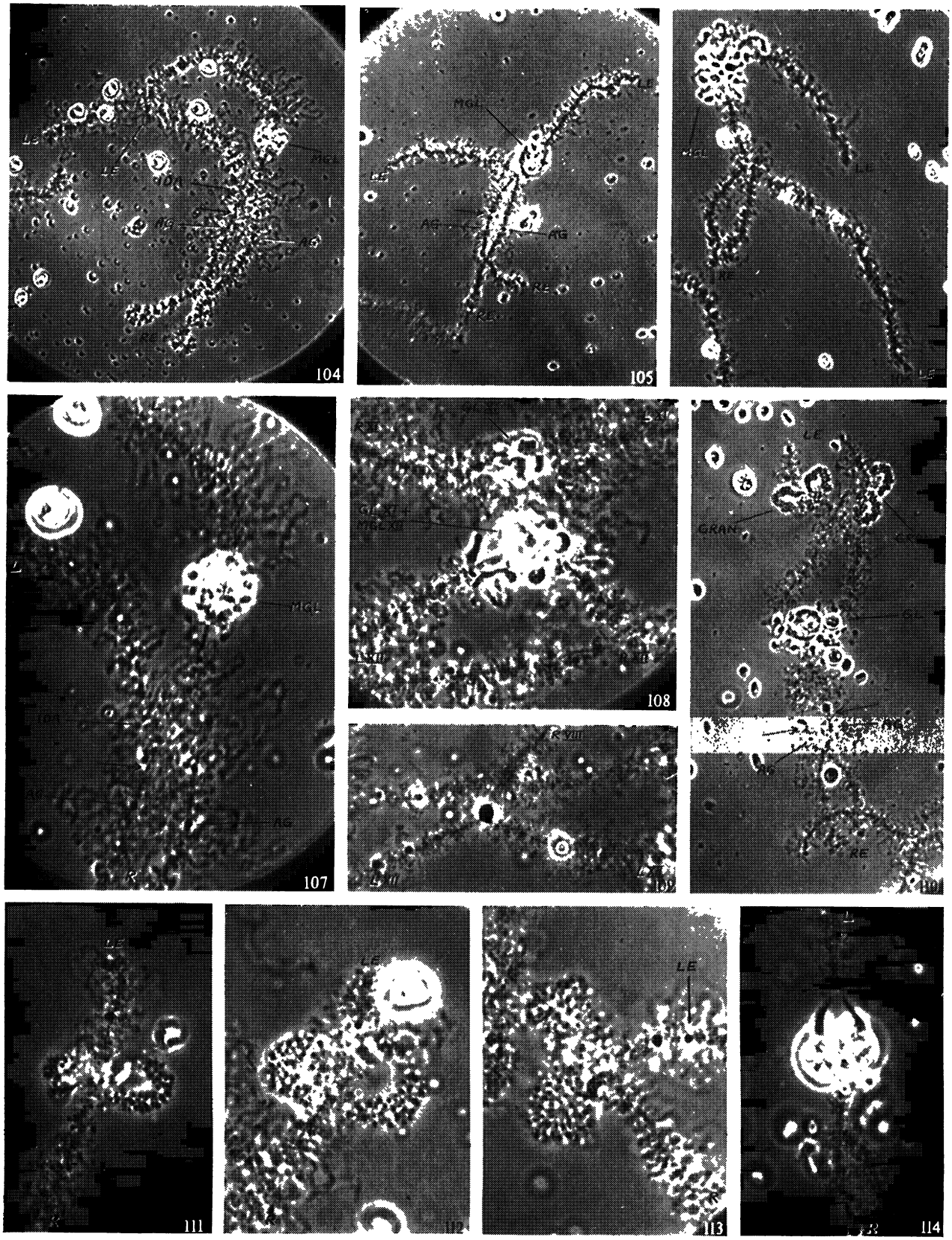
98
92
100
101
102
103
MGL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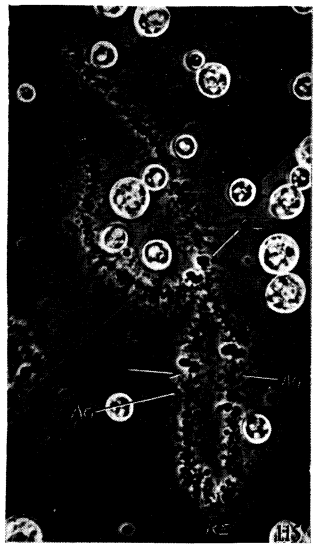

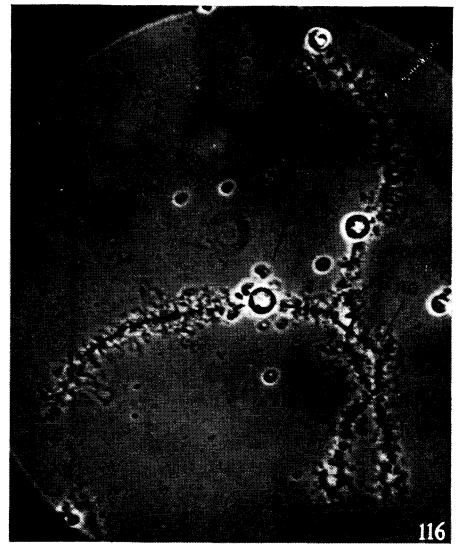
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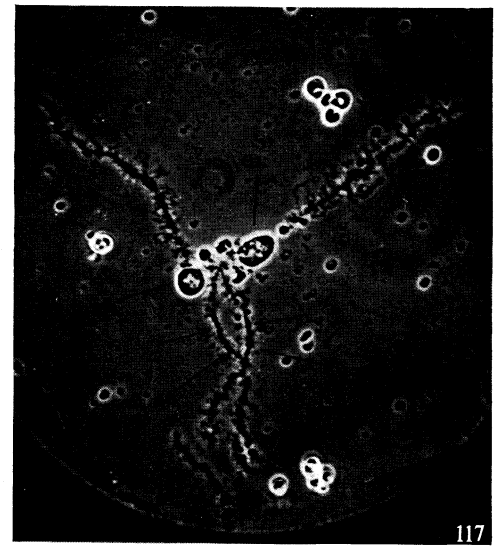
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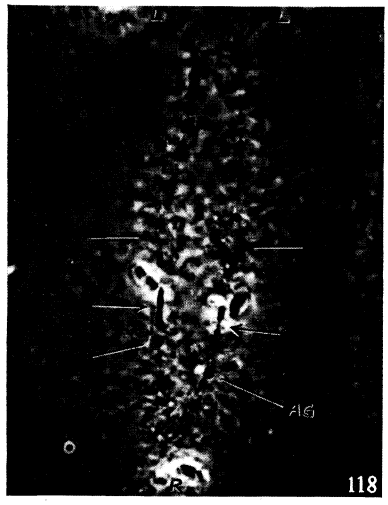
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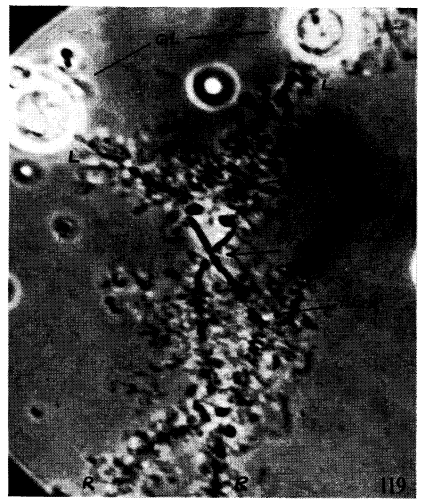
GL
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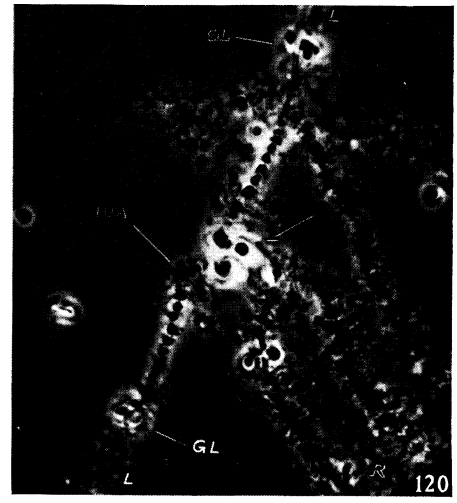
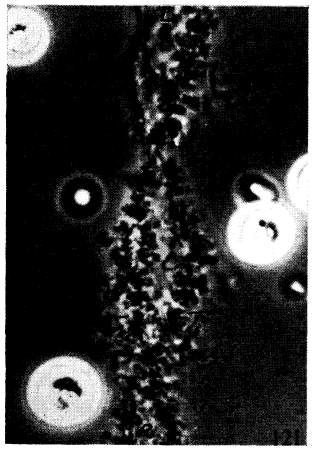

GL
GL
120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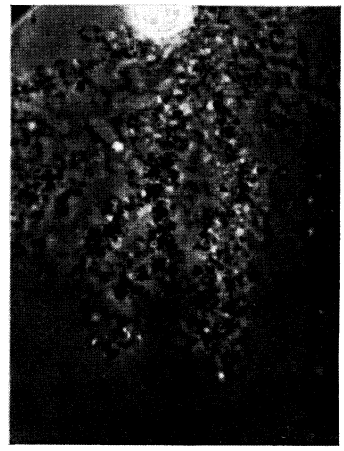


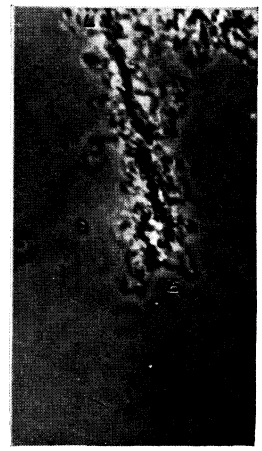


PLATE 20

FIGURE 52. LP *carnifex* ♀ anonymous (0·9 mm). This photograph includes the middle region of bivalent IV and shows reflected fusion (*RF*) of pericentric axial granules, with chiasmata to left and right.

FIGURE 53. HP *carnifex* ♀*O* (1·1 mm). Region around the centromeres of a bivalent IV not showing pericentric reflected fusion.

FIGURE 54. LP *carnifex* ♀*F* (1·2 mm). Bivalent VI entire showing interhomologue fusion of left-arm terminal granules, and reflected fusion (*RF*) of pericentric axial granules, with chiasmata to left and right. An outline drawing of a similar configuration is shown in figure 13*c*, p. 163.

FIGURE 55. HP *karelinii* ♀*F* (1·0 mm). Region around the centromeres of a bivalent VI showing reflected plus interhomologue fusion (*RF*) of pericentric axial granules. An outline drawing of a similar configuration is shown in figure 13*l*, p. 163.

FIGURE 56. HP *karelinii* ♀*F* (1·2 mm). End of left arm of chromosome V showing terminal granule and the two 'sphere' sites.

FIGURE 57. HP *carnifex* ♀*D* (0·6 mm). Ends of left arms of bivalent V showing fused terminal granules, fused distal 'spheres' and smaller, not fused proximal 'spheres'.

FIGURE 58. HP *karelinii* ♀*F* (1·2 mm). Ends of left arms of bivalent V showing fused terminal granules, fused distal 'spheres' and fused proximal 'spheres'.

FIGURE 59. HP *carnifex* ♀*L* (1·3 mm). Region around the centromeres of bivalent V showing interhomologue fusion of axial granules (*AGF*) to left and right. Chiasmata may accompany such fusions.

FIGURE 60. HP *karelinii* ♀*F* (1·1 mm). Region around the centromeres of bivalent V showing several interhomologue axial granule fusions (*AGF*) and centromere fusion. Notice also the highly refractile lumpy objects which lie just to the left of (below) the centromere bars.

FIGURE 61. HP *karelinii* ♀*F* (0·8 mm). Ends of the left arms of bivalent VIII showing separate subterminal 'spheres'.

FIGURE 62. HP *carnifex* ♀*D* (1·6 mm). End of the left arm of chromosome VIII showing terminal granule and subterminal 'sphere'.

FIGURE 63. HP *carnifex* ♀*F* (1·0 mm). Ends of the left arms of bivalent VIII showing terminal granules and single fused subterminal 'sphere'.

FIGURE 64. HP *danubialis* ♀*A* (1·0 mm). Ends of the left arms of bivalent VIII showing single fused subterminal 'sphere' and the conspicuous, more proximally located 'pseudo-spheres' (*PS*) which sometimes tend to confuse the identification of bivalents V and VIII; compare this photograph with figure 57 above.

PLATE 21

FIGURE 65. LP *karelinii* ♀*L* (1·0 mm). Bivalent VII entire, showing lumpy objects about the centromeres, interhomologue fusion between the more distal of the two pairs of conspicuous axial granules in the left arms (*AGF*), possibly accompanied by a chiasma, two further chiasmata, and interhomologue fusion of right-arm terminal granules.

FIGURE 66. HP *karelinii* ♀*L* (1·0 mm). Region around the centromeres of the bivalent VII shown in figure 65. Notice the refractile bars from which the centromere granules protrude, and the neighbouring sites bearing lumpy objects.

FIGURE 67. HP *carnifex* ♀ anonymous (1·0 mm). Region around the centromeres of bivalent VII, to be compared with figure 66. Notice the presence of similar lumpy objects, but absence of any axial bars adjacent to the centromeres.

FIGURE 68. LP *karelinii* ♀*G* (1·1 mm). Bivalent IX entire, with two chiasmata.

FIGURE 69. LP *carnifex* ♀*J* (1·1 mm). Bivalent IX entire, with two chiasmata.

FIGURE 70. HP *karelinii* ♀*F* (1·0 mm). Region around the centromeres of bivalent IX, showing axial bars and protruding centromere granules.

FIGURE 71. LP *carnifex* ♀*O* (1·2 mm). Bivalent X entire, with two chiasmata, and with inconspicuous objects at the giant loops sites of both homologues. An outline drawing of this bivalent is shown in figure 15*d*, p. 168, and a detailed drawing of the region around its centromeres in figure 14*a*, p. 166.

FIGURE 72. LP *carnifex* ♀*O* (1·0 mm). Bivalent X entire, with interhomologue fusion between the left-arm terminal granules, also between relatively inconspicuous objects at the giant loops sites (*GLF*), and with two chiasmata.

FIGURE 73. LP *carnifex* ♀*R* (0·9 mm). Bivalent X entire, showing non-homologous fusion between the terminal granule of one of the left arms and an axial granule in the heteromorphic region of chromosome I (*LEF*)—there is a large contorted loop and conspicuous axial granule of chromosome I, marked *HL* I, close to the non-homologous fusion—and interhomologue fusion between giant loops (*GLF*).

FIGURE 74. LP *carnifex* ♀*P* (1·0 mm). Bivalent X entire, homozygous for conspicuous giant loops (*GL*), and with interhomologue fusion of left-arm terminal granules.

FIGURE 75. LP *carnifex* ♀ anonymous (0·8 mm). Bivalent X entire, homozygous for conspicuous giant loops (*GL*).

FIGURE 76. HP *carnifex* ♀*O* (0·9 mm). This photograph includes fused, relatively inconspicuous giant loops of the bivalent X in figure 72. A detailed drawing of this region is shown in figure 14*e*, p. 166.

FIGURE 77. HP *carnifex* ♀*C* (0·7 mm). Like figure 76, this photograph includes fused, relatively inconspicuous giant loops of bivalent X. The region is drawn in detail in figure 14*d*, p. 166.

PLATE 22

FIGURE 78. LP *carnifex* ♀*X* (1·4 mm). Bivalent X entire, homozygous for conspicuous giant loops (*GL*), and with interhomologue fusion of left- and right-arm terminal granules.

FIGURE 79. LP *carnifex* ♀*E* (1·1 mm). Bivalent X entire, heterozygous for conspicuous giant loops, and with interhomologue fusion of left-arm terminal granules.

FIGURE 80. LP *carnifex* ♀*E* (1·6 mm). Bivalent X entire, heterozygous for conspicuous giant loops, and with interhomologue fusion of left-arm terminal granules.

FIGURE 81. LP *carnifex* ♀*E* (1·6 mm). Bivalent X entire, heterozygous for conspicuous giant loops, with identified free product (*FP*) nearby.

FIGURE 82. HP *carnifex* ♀*R* (1·3 mm). This photograph shows the left-arm ends, with homologous terminal granules fused, of a bivalent X homozygous for conspicuous giant loops (*GL*).

FIGURE 83. HP *carnifex* ♀*E* (1·6 mm). This photograph shows the left arm end of chromosome X, including conspicuous giant loops (*GL*) and identified free product (*FP*) nearby, from a bivalent heterozygous for giant loops. A detailed drawing of this region is shown in figure 14*h*, p. 166.

FIGURE 84. HP *carnifex* ♀*E* (1·6 mm). This photograph includes the giant loops site of a heterozygous bivalent X isolated in medium B. Due to partial solution of matrix the characteristic lateral loop form of the giant loops, normally concealed by matrix fusion, has been exposed.

FIGURE 85. LP *karelinii* ♀*B* (1·1 mm). Bivalent X entire, with two chiasmata, showing diverse morphologies of homologous giant loops (*GL*).

FIGURE 86. LP *karelinii* ♀*A* (1·4 mm). Bivalent X entire, with two chiasmata, showing diverse morphologies of homologous giant loops (*GL*).

FIGURE 87. HP *karelinii* ♀*G* (0·7 mm). This photograph shows the region around the centromeres of bivalent X. Notice the tiny centromere granules flanked by short axial bars, characteristic of small oocytes. To the left of (above) the centromeres and adjacent to a chiasma are the refractile objects located at 38 units, whilst to the right (below) the centromeres are those located at 44 units (regularly dimorphic for size in this female). At the bottom of the photograph are the refractile objects located at 51 units.

FIGURE 88. HP *karelinii* ♀*F* (1·0 mm). This photograph shows the region around the centromeres of bivalent X. Notice the larger centromere granules projecting laterally from longer axial bars, typical of a larger oocyte—compare with figure 87. Near the top and bottom of the photograph are the refractile objects located respectively at 35 and 51 units.

FIGURE 89. HP *karelinii* ♀*H* (1·2 mm). This photograph shows the region around the centromeres of bivalent X. Notice interhomologue fusion of the centromere granules, and still longer axial bars.

PLATE 23

FIGURE 90. LP *carnifex* ♀*F* (1·0 mm). Bivalent XI entire, isolated in medium B. Notice the giant loops (*GL*), one pair having formed a double bridge (*GLDB*) by axial breakage, and the subterminal 'currant buns' in the left arms.

FIGURE 91. LP *carnifex* ♀*E* (1·6 mm). Bivalent XI entire, showing giant loops (*GL*) and subterminal 'currant buns' in the left arms.

FIGURE 92. LP *carnifex* ♀*R* (0·8 mm). Bivalent XI entire, showing diverse morphologies of the giant loops (*GL*), with one pair fused and the other pair separate, characteristic of this individual.

FIGURE 93. LP *carnifex* ♀*R* (0·9 mm). Bivalent XI entire, showing diverse morphologies of the giant loops (*GL*), with one pair fused and the other pair separate, characteristic of this individual.

FIGURE 94. LP *carnifex* ♀*Q* (1·3 mm). Bivalents XI and XII entire. Notice the giant loops (*GL*) of bivalent XI and the multiple giant loops (*MGL*) of one homologue only of bivalent XII.

FIGURE 95. LP *cristatus* ♀*F* (1·0 mm). Bivalent XI entire, showing subterminal 'currant buns' in the left arms and terminal granules of the right arms fused together. Notice that there are relatively small refractile objects in the middle region of this bivalent, but no giant loops.

FIGURE 96. HP *carnifex* ♀*O* (0·9 mm). This photograph shows part of bivalent XI including the giant loops located at 24 units. As is generally the case in small oocytes, the loop form is obscured by sister and interhomologue matrix fusion. A detailed drawing of this example is shown in figure 16*a*, p. 169.

FIGURE 97. HP *carnifex* ♀*M* (1·3 mm). Part of bivalent XI including the giant loops.

FIGURE 98. HP *carnifex* ♀P (0·9 mm). Part of bivalent XI including the giant loops.

FIGURE 99. HP *carnifex* ♀*O* (0·8 mm). End of left arm of chromosome XI, showing terminal granule and subterminal 'currant bun'.

FIGURE 100. HP *carnifex* ♀*O* (1·2 mm). End of left arm of chromosome XI, showing terminal granule and subterminal 'currant buns'.

FIGURE 101. LP *carnifex* ♀*E* (0·6 mm). Bivalent XII entire, with two chiasmata and left arm terminal granule fusion, from an individual lacking multiple giant loops on both homologues.

FIGURE 102. LP *carnifex* ♀*E* (1·1 mm). Bivalent XII entire, with two chiasmata, and lacking multiple giant loops.

FIGURE 103. LP *carnifex* ♀*Y* (1·1 mm). Bivalent XII entire, with two chiasmata, and with multiple giant loops on both homologues.

PLATE 24

FIGURE 104. LP *carnifex* ♀*J* (1·1 mm). Bivalent XII entire, with two chiasmata, one lying in the intercalary double-axis region (*IDA*), from an individual with multiple giant loops (*MGL*) on one homologue only.

FIGURE 105. LP *carnifex* ♀*J* (1·2 mm). Bivalent XII entire, with two chiasmata, and multiple giant loops (*MGL*) on one homologue only.

FIGURE 106. LP *carnifex* ♀*M* (1·3 mm). Bivalent XII entire, with two chiasmata, and multiple giant loops (*MGL*) on one homologue only.

FIGURE 107. HP *carnifex* ♀*J* (1·1 mm). Part of the bivalent XII shown in figure 104, including a chiasma in the double-axis region (*IDA*), multiple giant loops (*MGL*) on one homologue only, and the axial granules lying to the right of (below) the centromeres.

FIGURE 108. HP *carnifex* ♀ anonymous (1·0 mm). Parts of bivalents XI and XII showing fusion between the giant loops of one homologue of XI and the multiple giant loops of one homologue of XII. A detailed drawing of this example is shown in figure 18*d*, p. 172.

FIGURE 109. HP *cristatus* ♀*D* (1·2 mm). This photograph shows threefold fusion betwen the terminal granules of the left arms of bivalent XII and the terminal granule of one of the right arms of bivalent VIII.

FIGURE 110. LP *cristatus* ♀*A* (1·0 mm). Bivalent XII entire, with three chiasmata, showing the giant granular loops (*GRAN*), giant loops (*GL*) and neighbouring free products, and axial granules (*AG*) lying to the right of (below) the centromeres.

FIGURE 111. HP *cristatus* ♀ anonymous (0·8 mm). End of left arm of chromosome XII including the pair of giant granular loops.

FIGURE 112. HP *cristatus* ♀*B* (0·8 mm). End of left arm of chromosome XII including the pair of giant granular loops.

FIGURE 113. HP *cristatus* ♀*C* (1·3 mm). Ends of the left arms of bivalent XII, with fused terminal granules, and one pair of giant granular loops.

FIGURE 114. HP *cristatus* ♀ anonymous (1·2 mm). Part of bivalent XII including the giant loop pair located at 25·5 units, showing the characteristic, naturally occurring axial breakage at this site. The photograph only shows one pair of giant loops as the homologous region of the partner chromosome was accidentally detached during preparation. A detailed drawing of this region is shown in figure 23*c*, p. 188.

PLATE 25

FIGURE 115. LP *karelinii* ♀*G* (1·1 mm). Bivalent XII entire, with one chiasma and fused right-arm terminal granules, showing giant loops (*GL*) in low state of development. Notice the conspicuous refractile objects located just to the left of (above) the centromeres, and the axial granules (*AG*) located just to the right of (below) the centromeres.

FIGURE 116. LP *karelinii* ♀*B* (1·5 mm). Bivalent XII entire, with two chiasmata, one lying in the intercalary double-axis region (*IDA*), and with centromeric fusion. Notice the conspicuous giant loops (*GL*) and the terminal double-axis regions in the right arms.

FIGURE 117. LP *karelinii* ♀*B* (1·5 mm). Bivalent XII entire, with two chiasmata and very conspicuous giant loops (*GL*).

FIGURE 118. HP *karelinii* ♀*G* (0·8 mm). Part of bivalent XII including the centromeres, intercalary double-axis region (*IDA*) at about 31 units, refractile objects to the left of (above) the centromere bars at 32 units, and axial granules (*AG*) to the right of (below) the centromere bars at 36·5 units. In the region between the centromere granules and the axial granules of the two homologues, notice the different degrees of incorporation of chromomeres within the centromere bars.

FIGURE 119. HP *karelinii* ♀*B* (1·5 mm). Part of the bivalent XII shown in figure 116, including a chiasma in the intercalary double axis region (*IDA*) and fused centromeres.

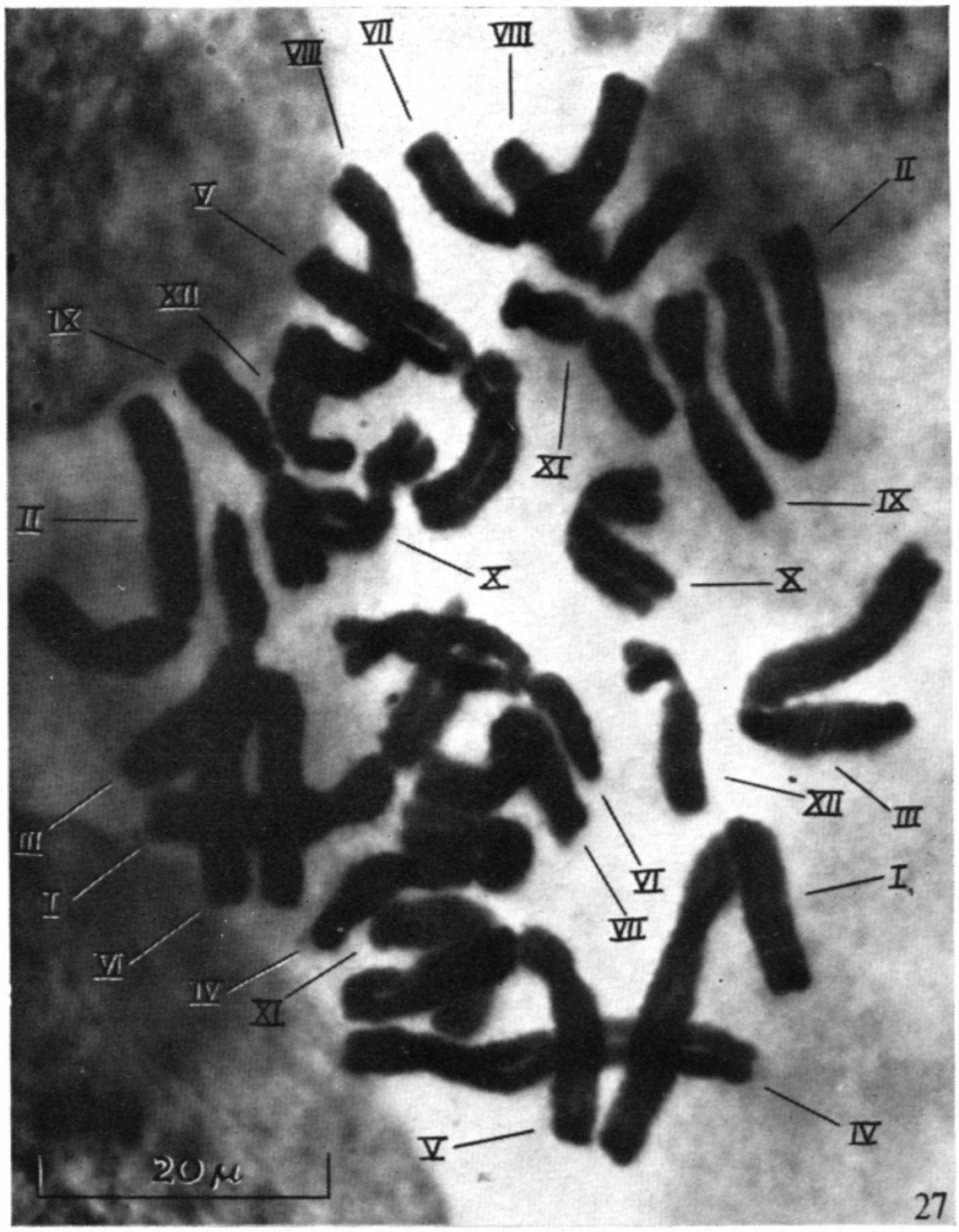
FIGURE 120. HP *karelinii* ♀*G* (1·3 mm). Part of bivalent XII including the centromeres, largely obscured by haloes from the refractile objects located at 32 units, to show the series of axial granules lying between the intercalary double-axis region (*IDA*) and the giant loops (*GL*). Similar, though less conspicuous axial granules are visible in the homologous region of figure 118. Notice also the dense refractile objects to the right of (below) the centromere on one homologue only; this heterozygosity is referred to in the text, § VI, p. 202.

FIGURE 121. HP *danubialis* ♀*D* (1·3 mm). Part of bivalent XII including the intercalary double-axis region (*IDA*) and the axial granules (*AG*) located just to the right of (below) the centromeres. Although these landmarks pinpoint the centromeres with some precision, the centromeres cannot be seen in the photograph nor were they evident in the preparation; this is characteristic of *danubialis*.

FIGURE 122. HP *karelinii* ♀*F* (1·0 mm). Part of bivalent XII from the centromeres to the ends of the right arms, to show the terminal double-axis regions. Notice the dense objects carried on one homologue only lying half-way between an axial granule (*AG*) at 36·5 units and the right-arm end (*RE*), to be seen also in figure 120.

FIGURE 123. HP *carnifex* ♀*O* (1·0 mm). This photograph includes the ends of the right arms of bivalent XII and shows the terminal double-axis regions with sister axes relationally coiled.

FIGURE 124. HP *carnifex* ♀*H*(0·8 mm). This photograph includes the end of the right arm of a chromosome XII isolated in medium B and shows the chromomeres of the terminal double-axis region, with sister axes relationally coiled.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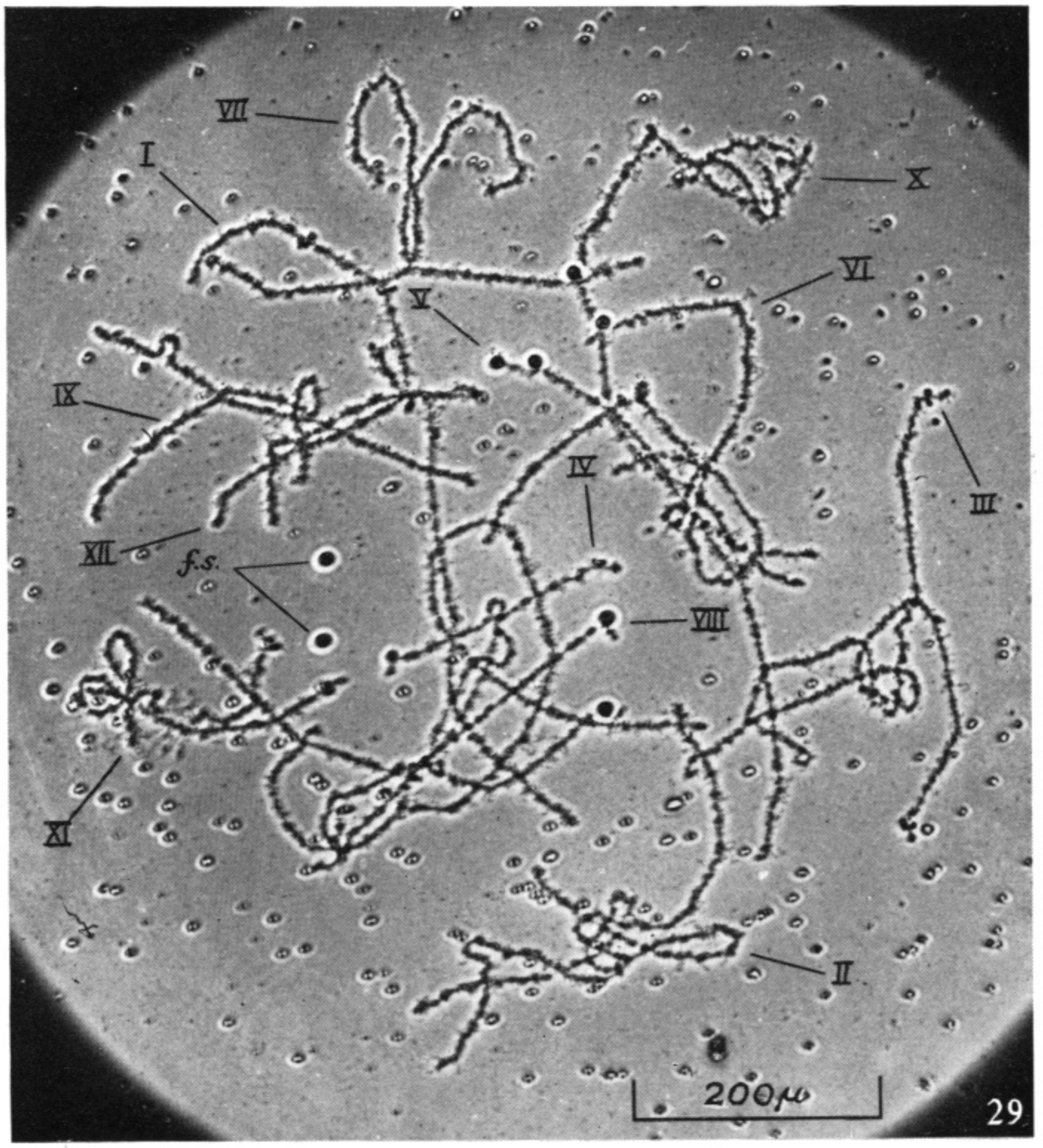
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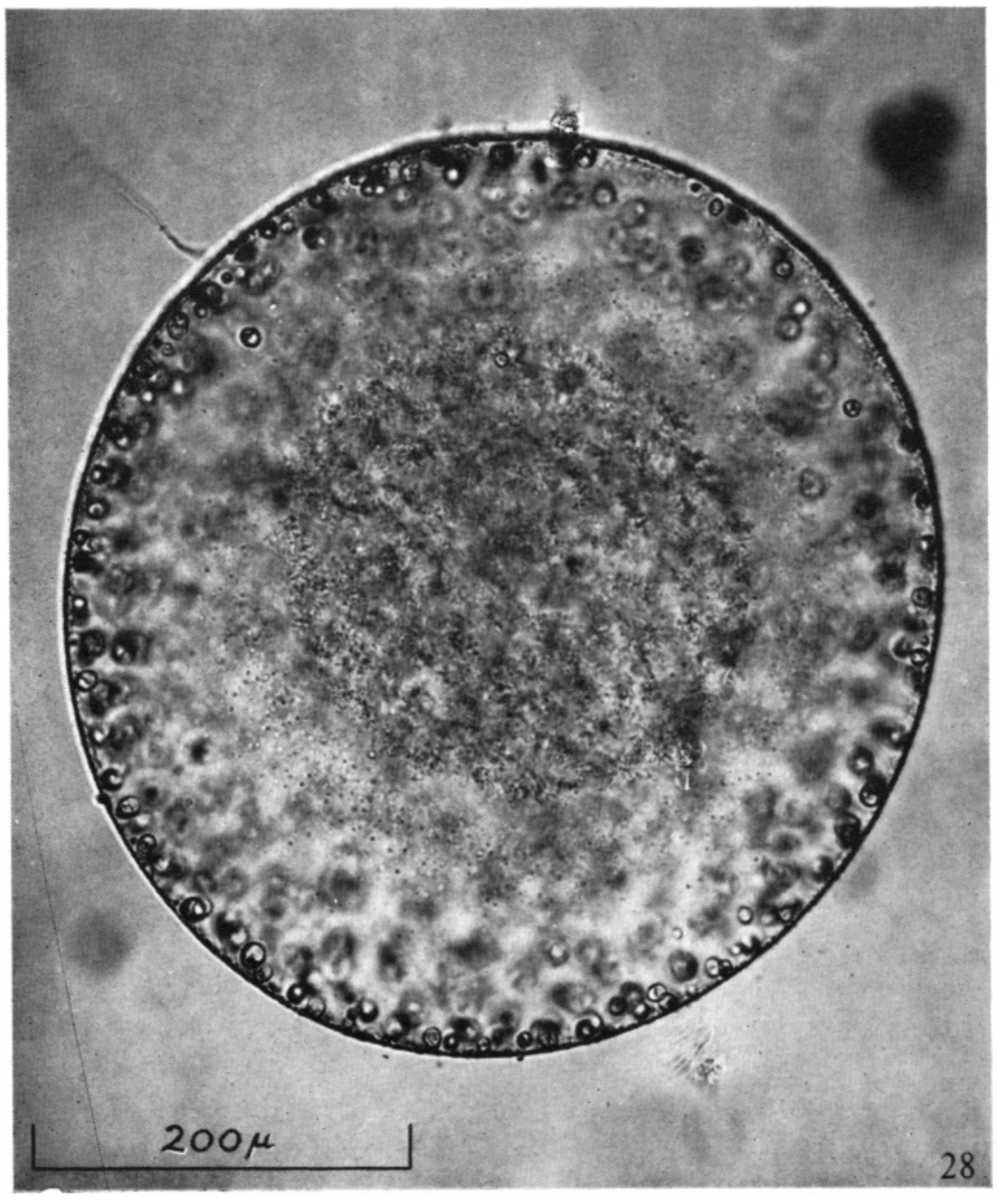
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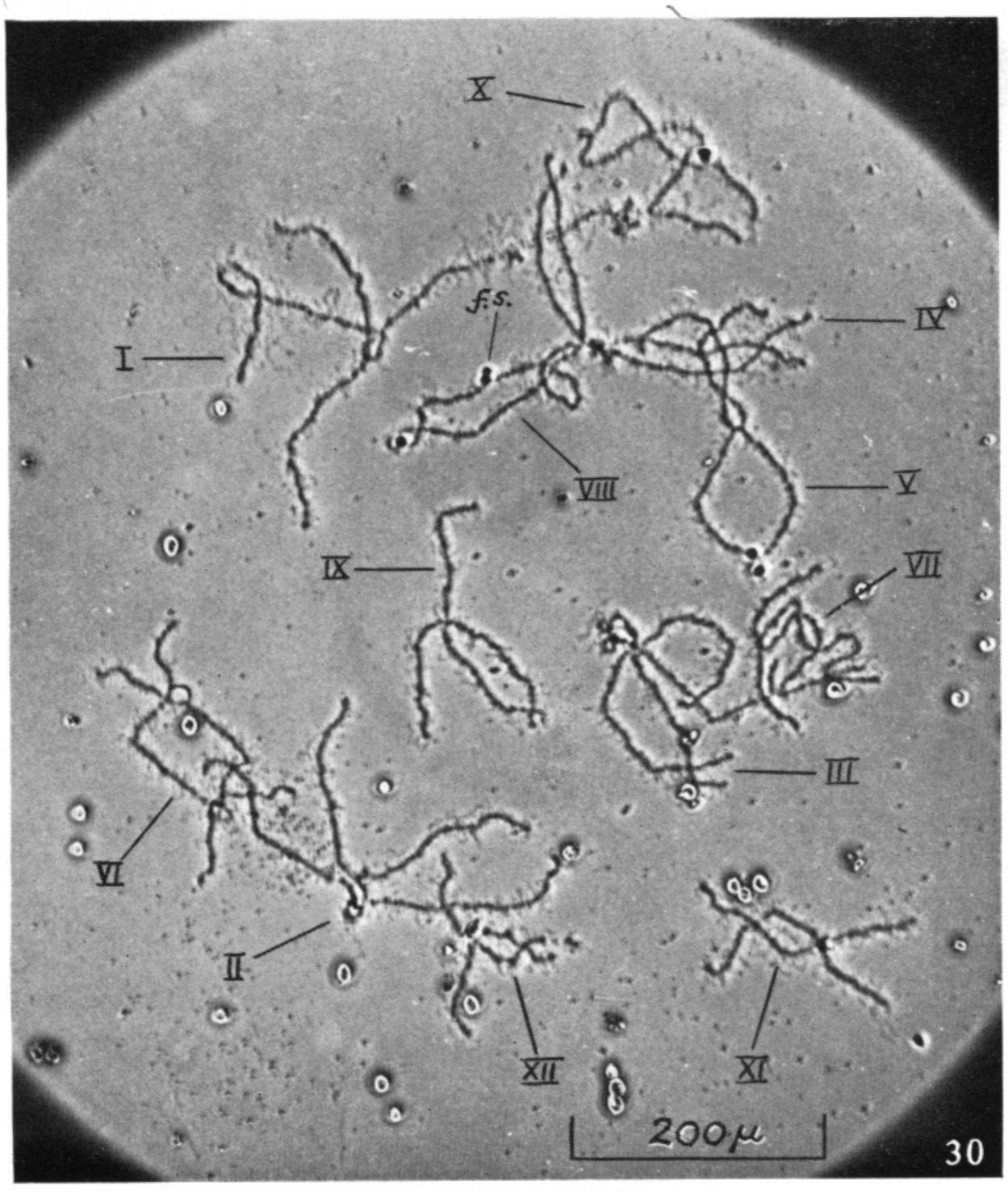
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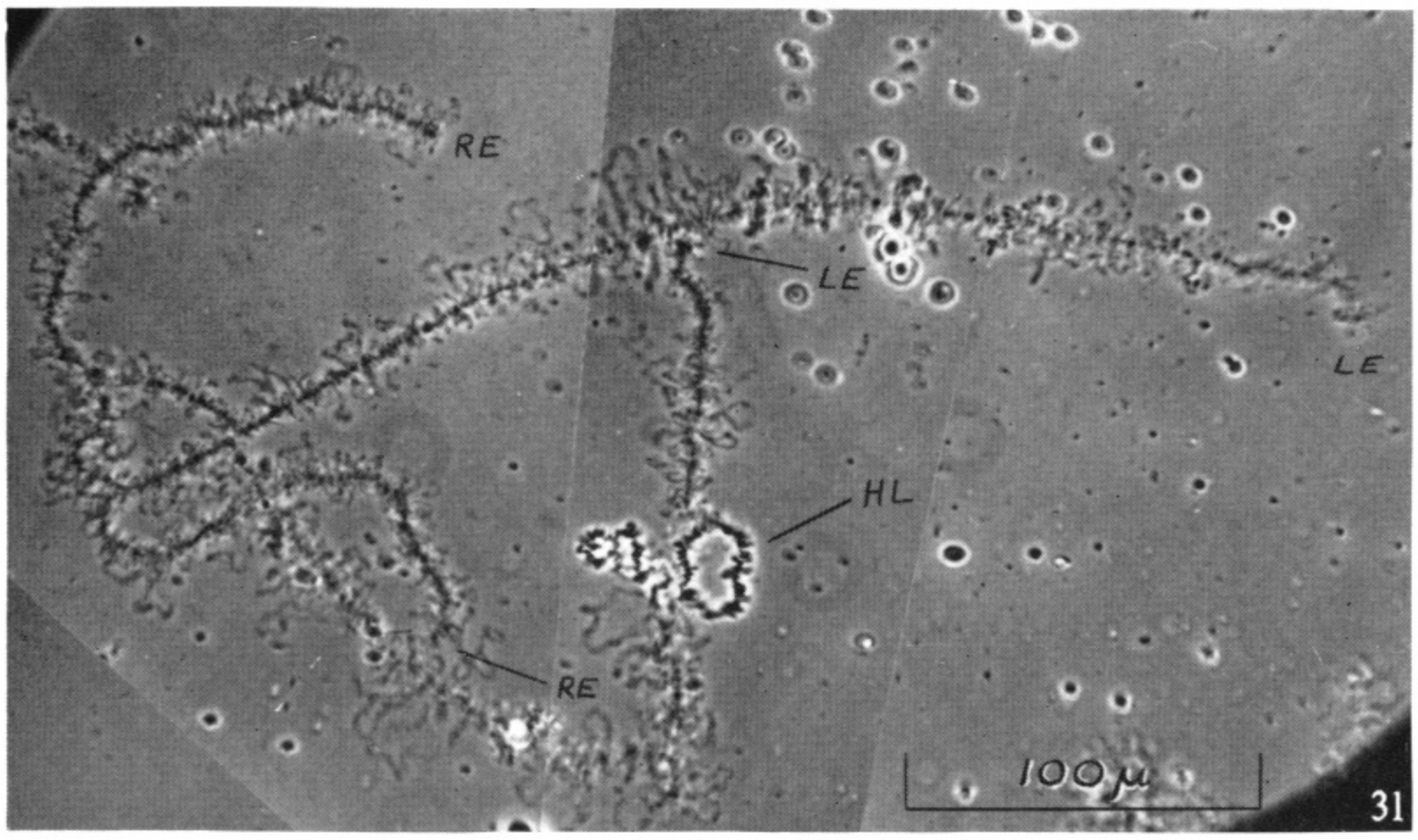

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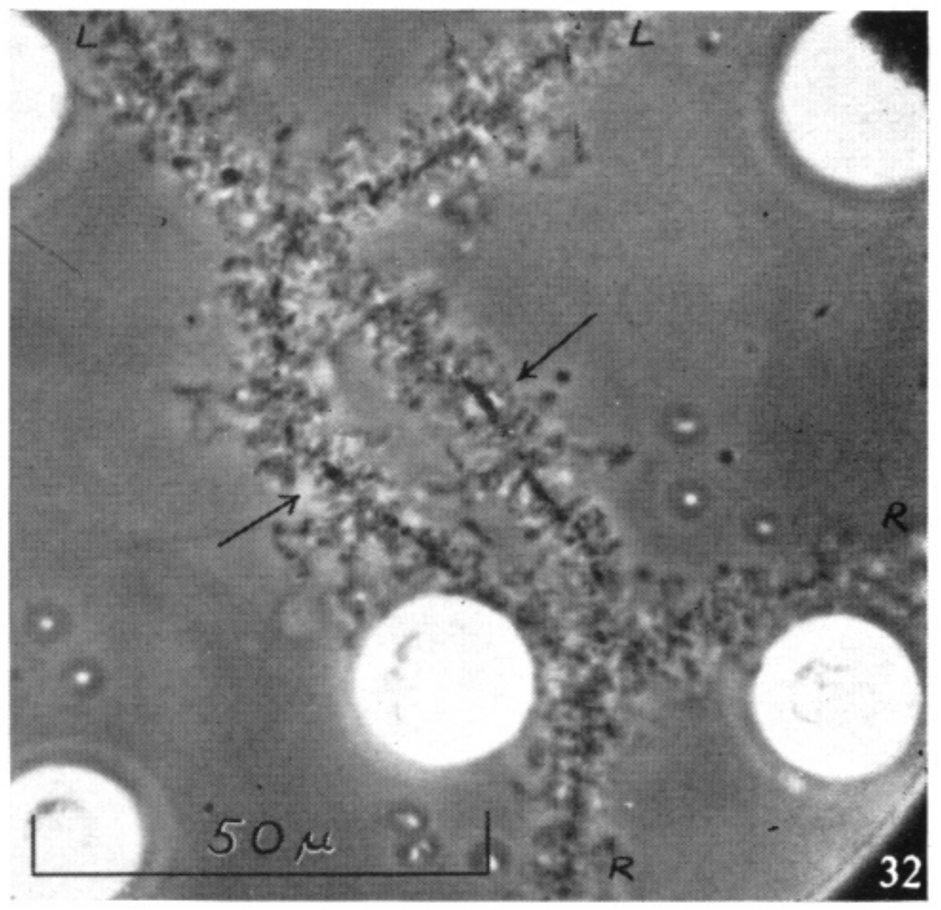

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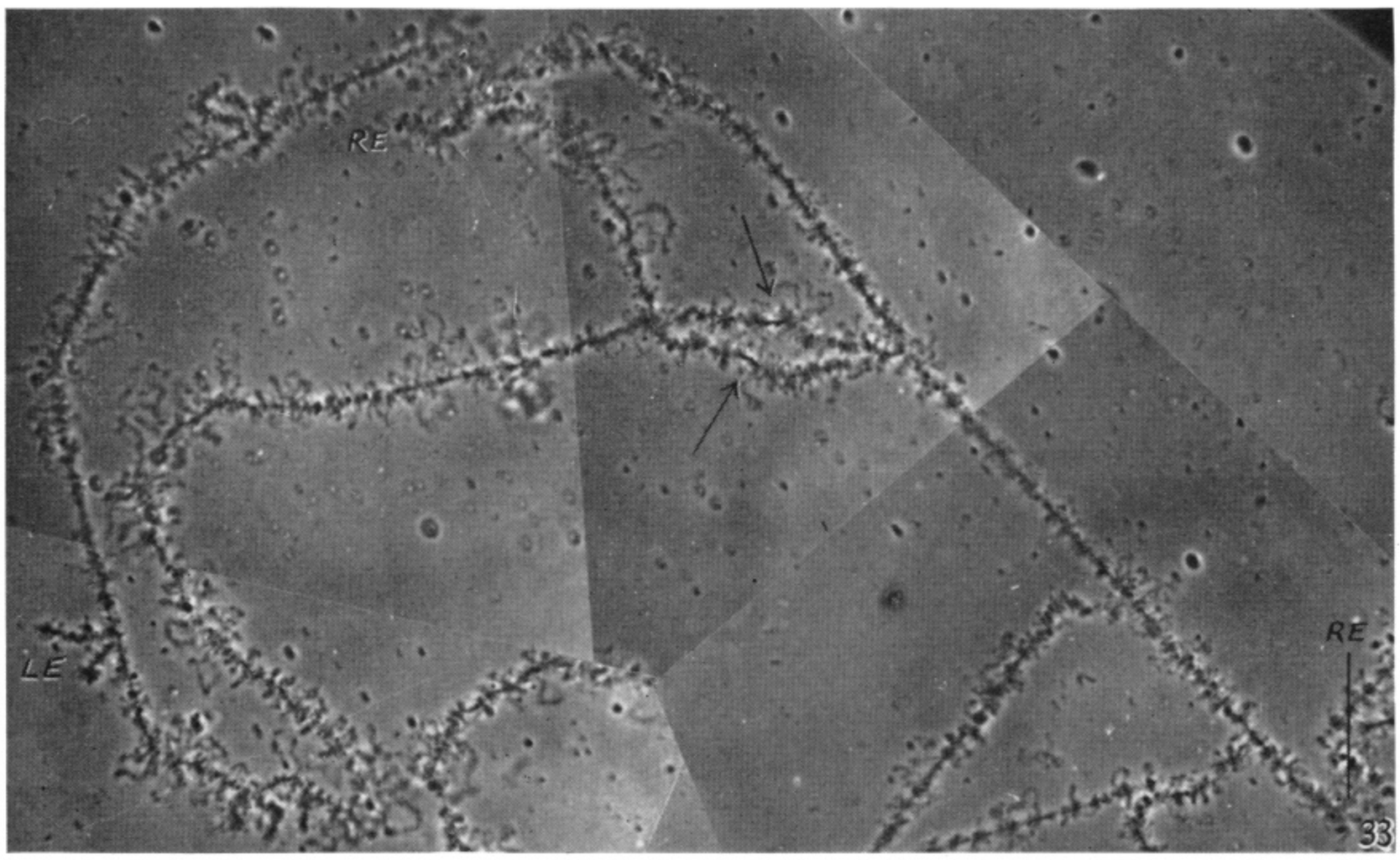

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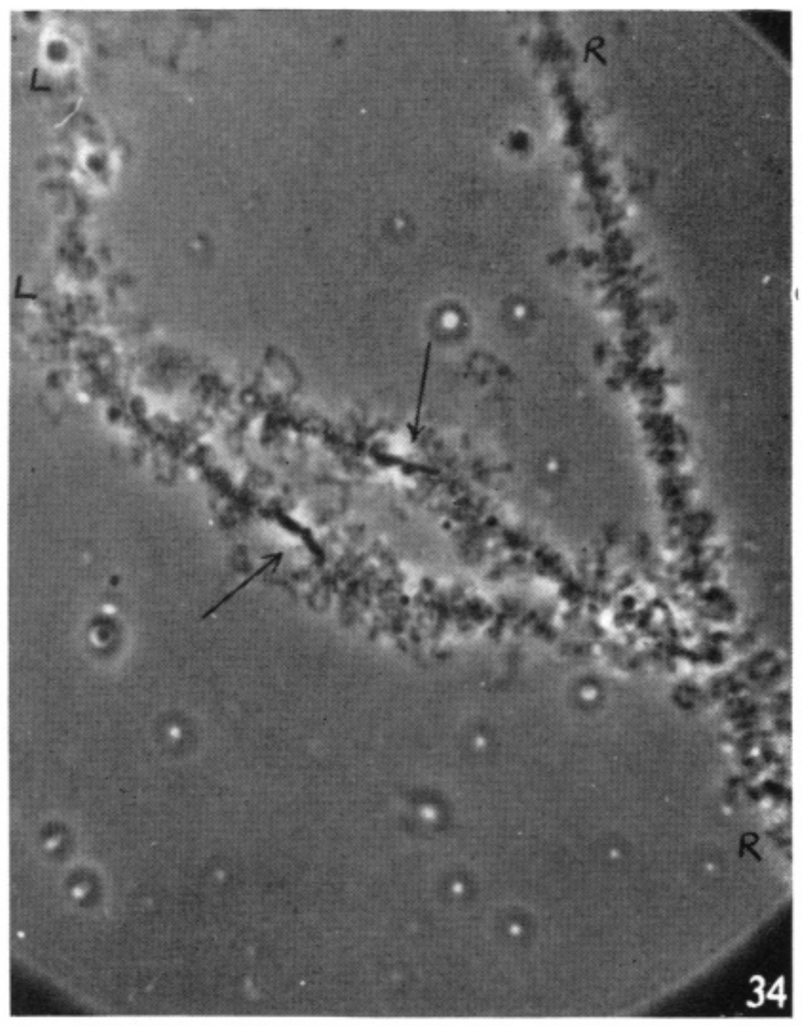

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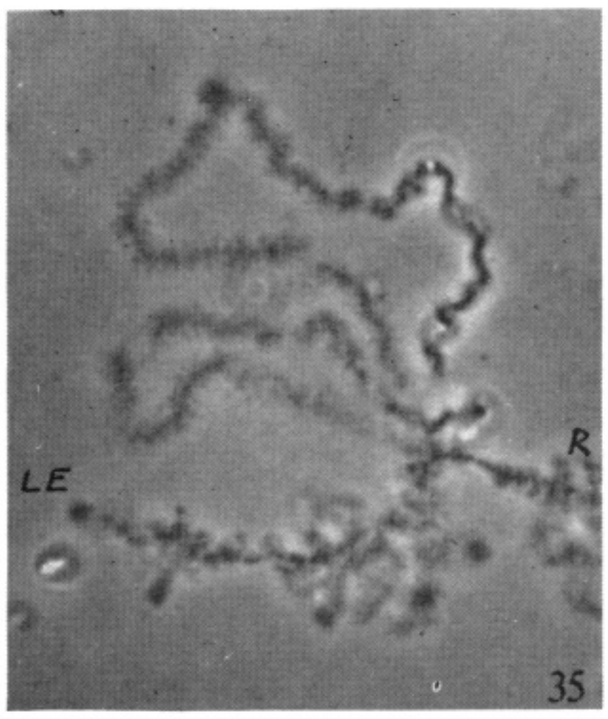

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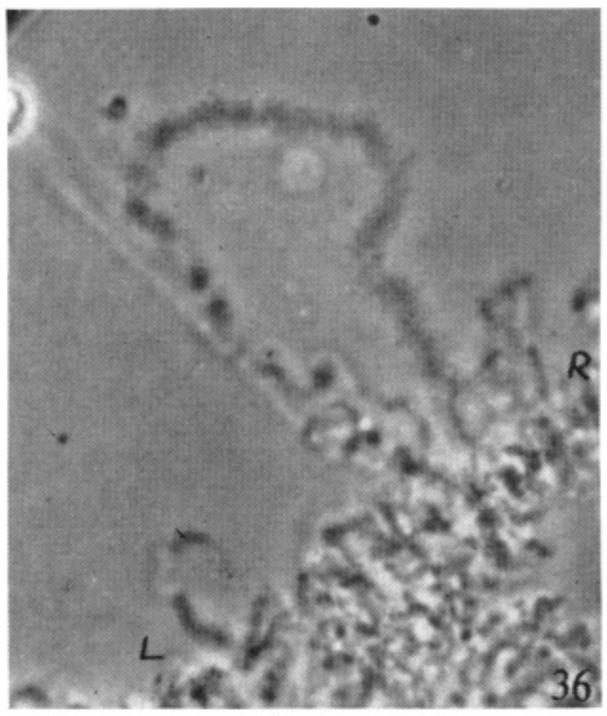

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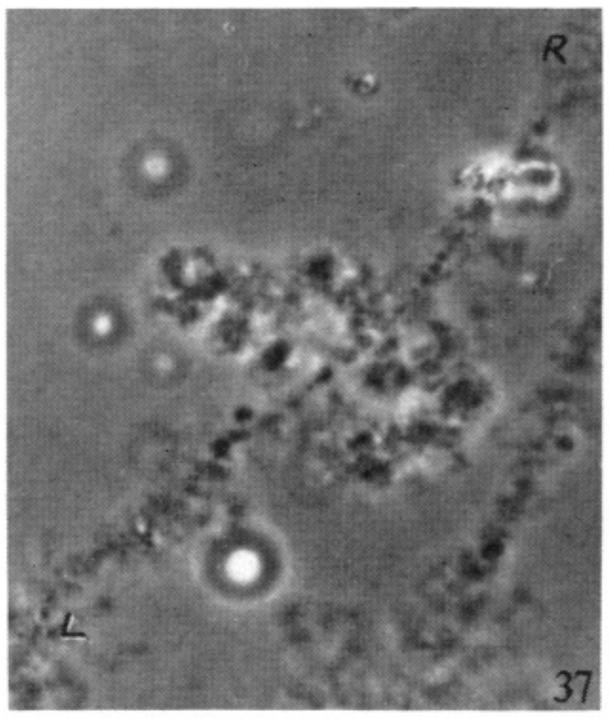

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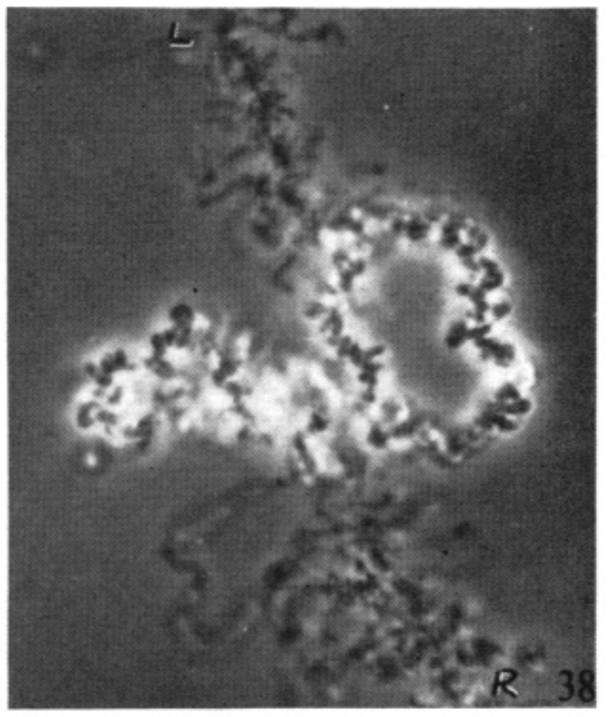

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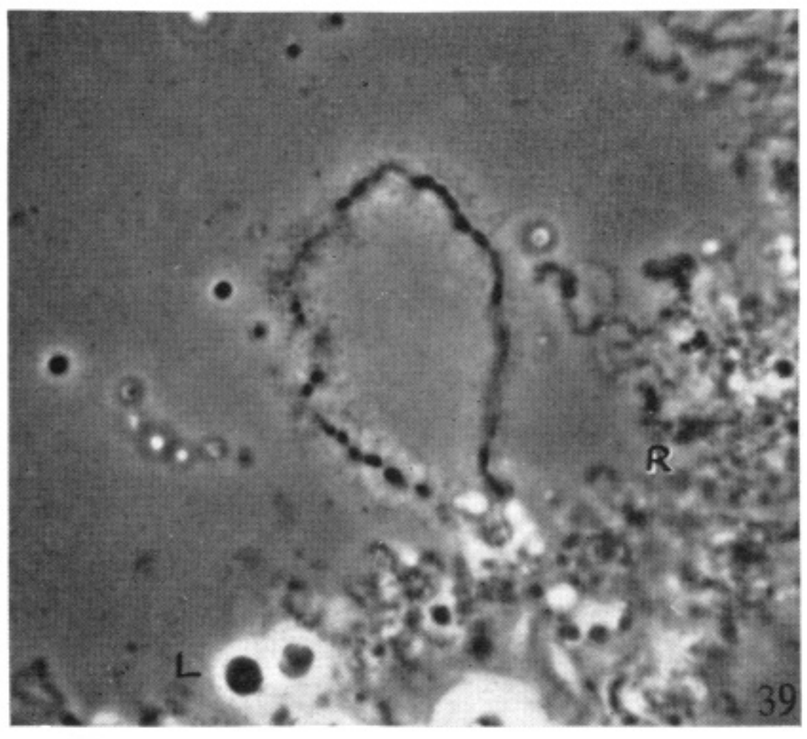

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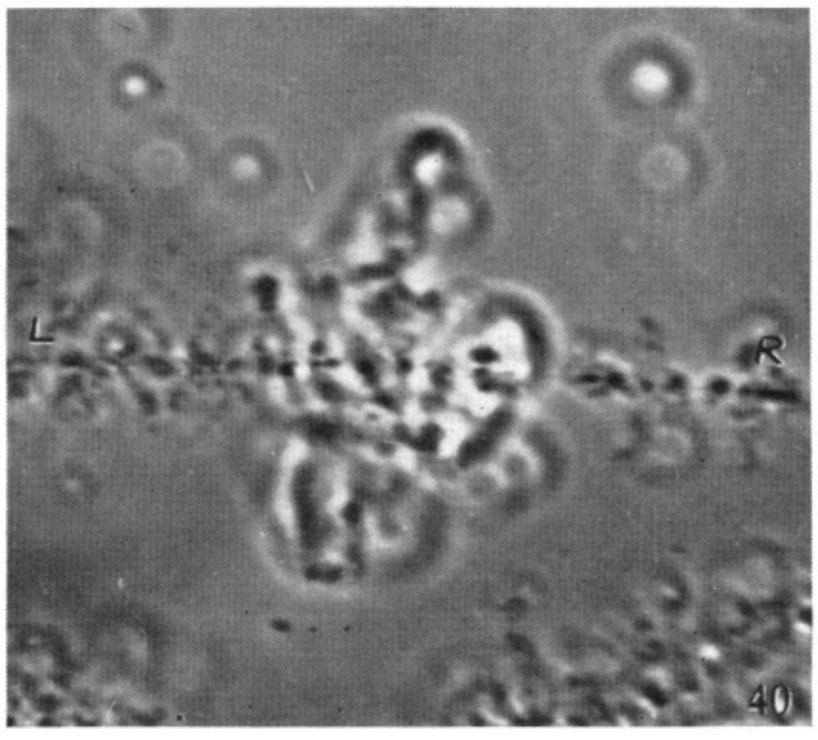

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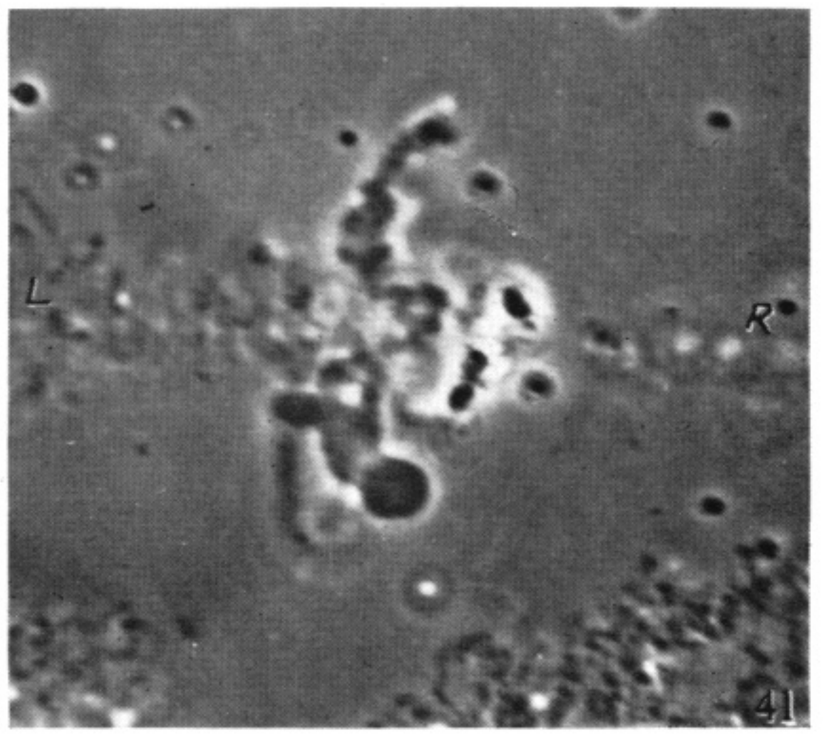

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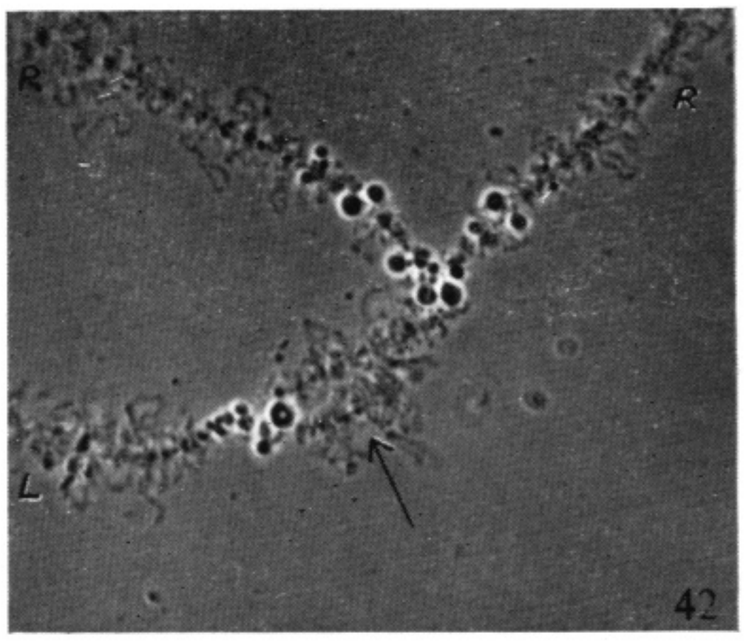
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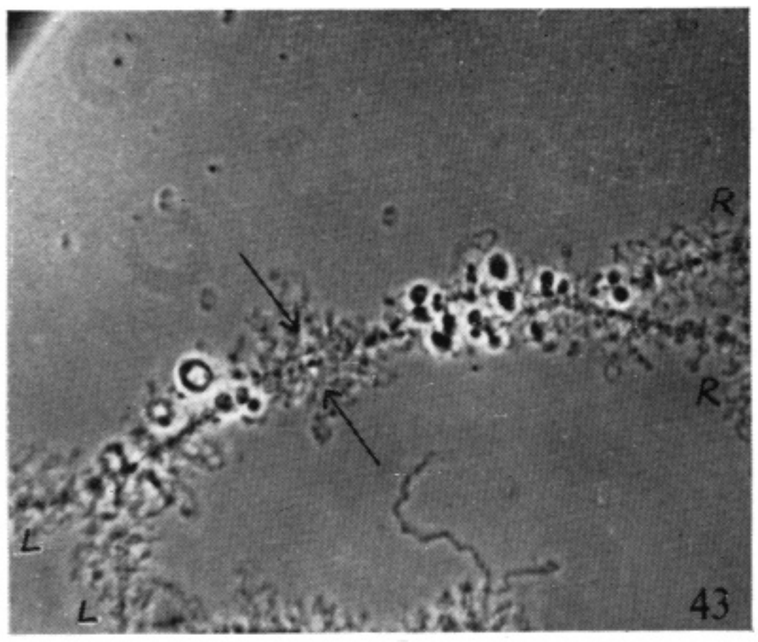
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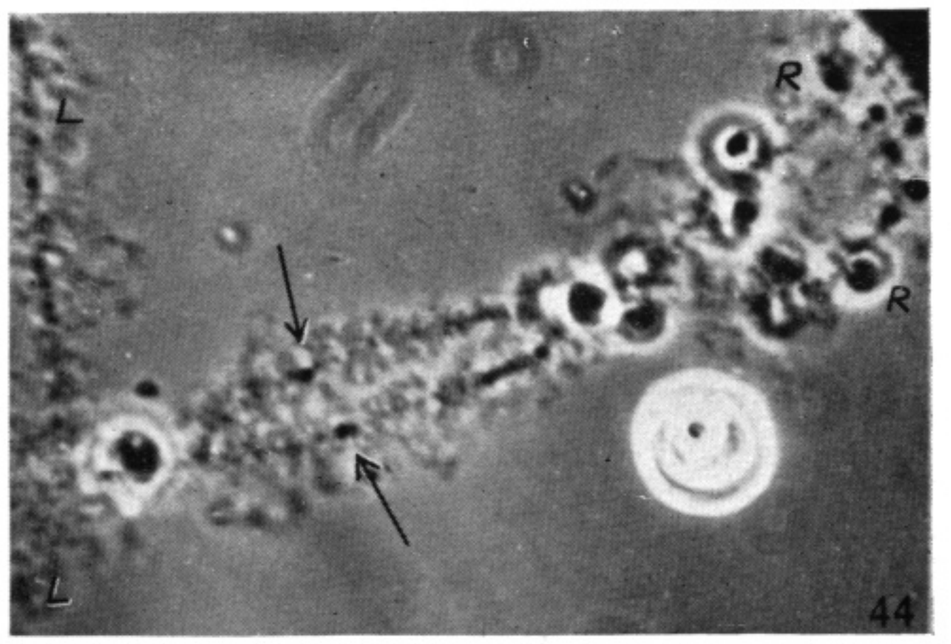
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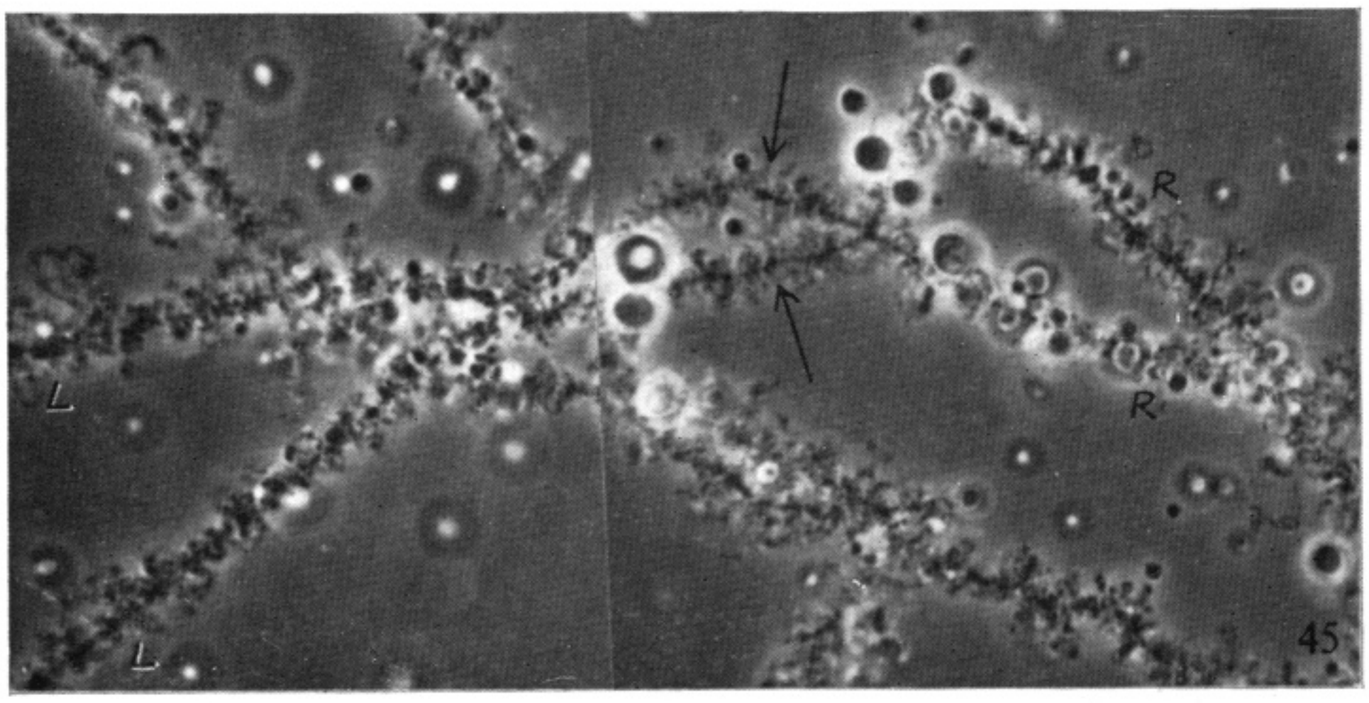
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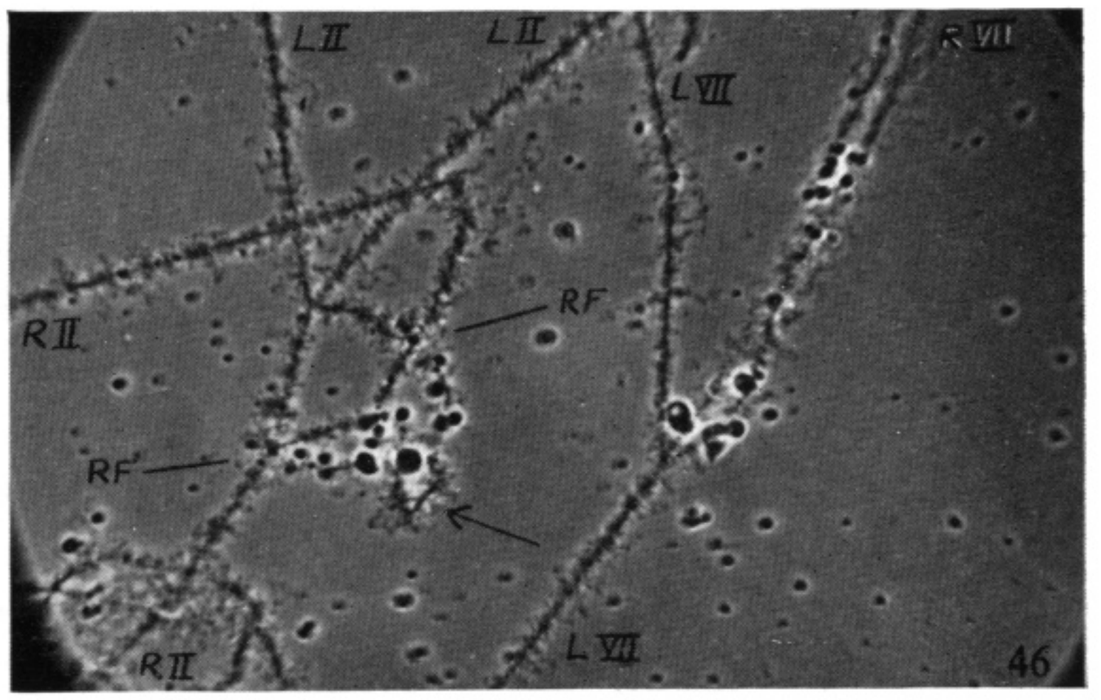
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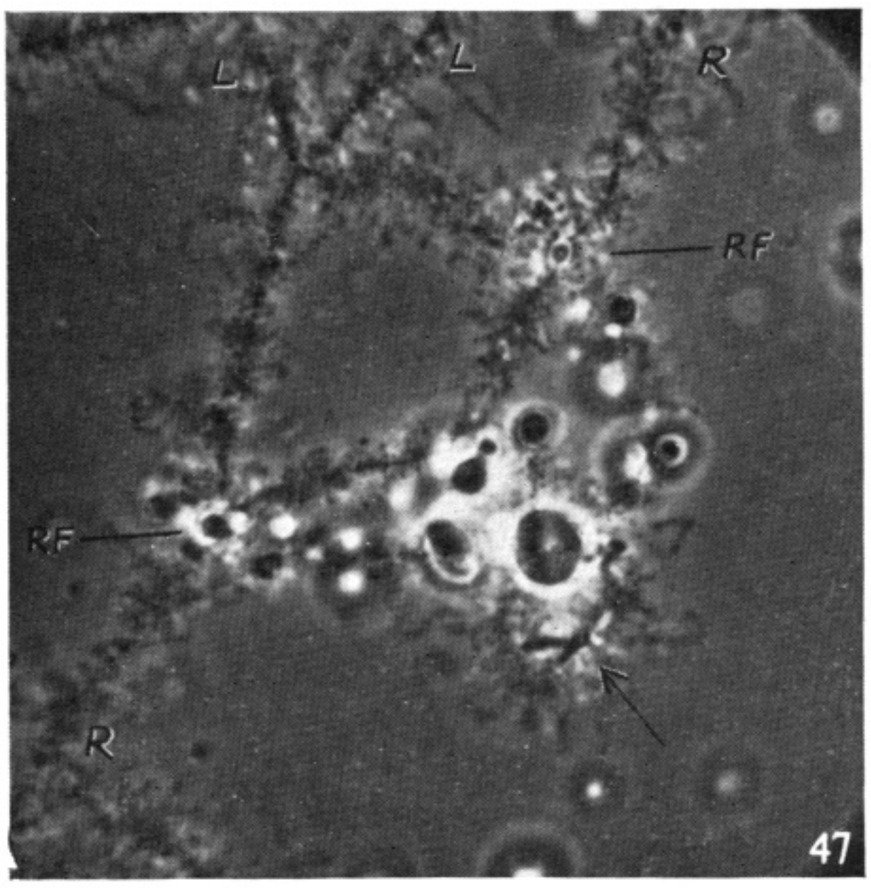
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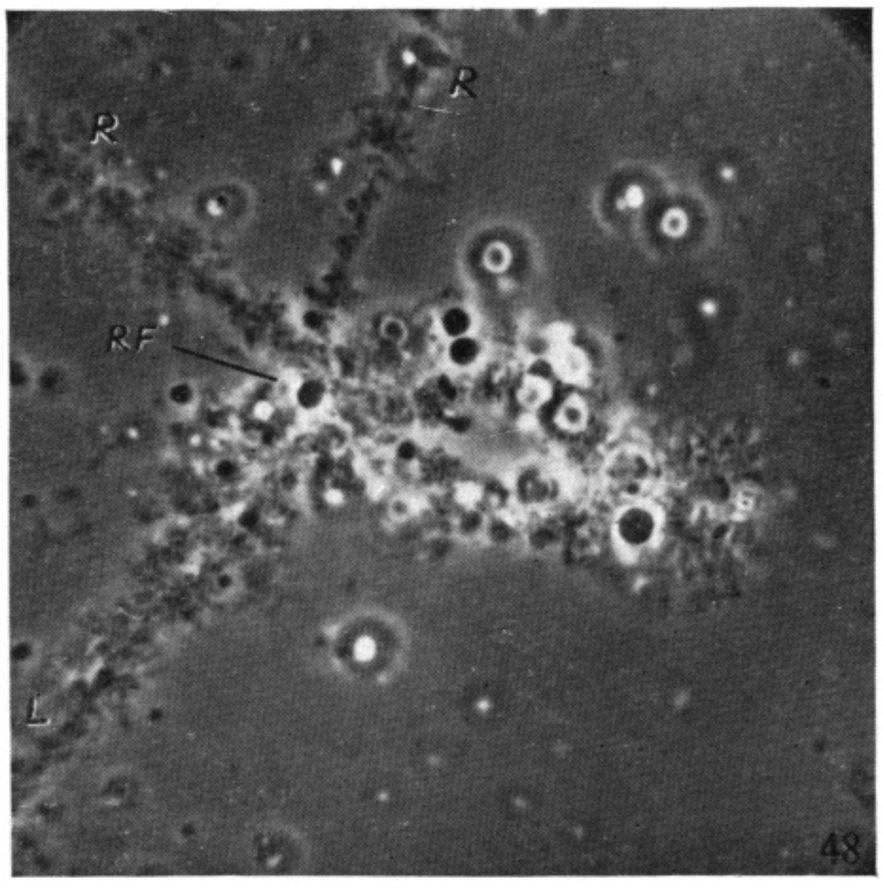
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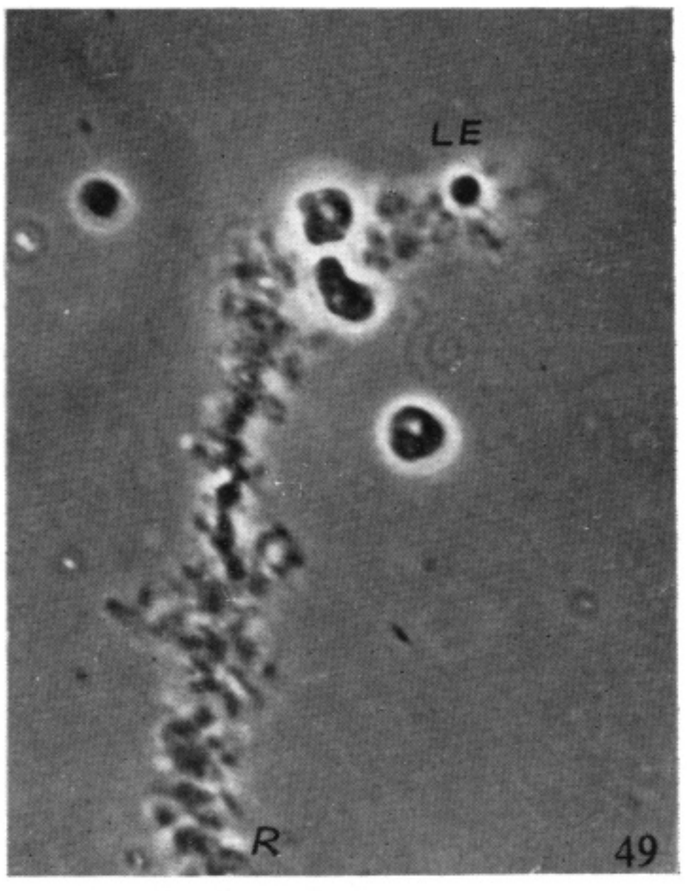
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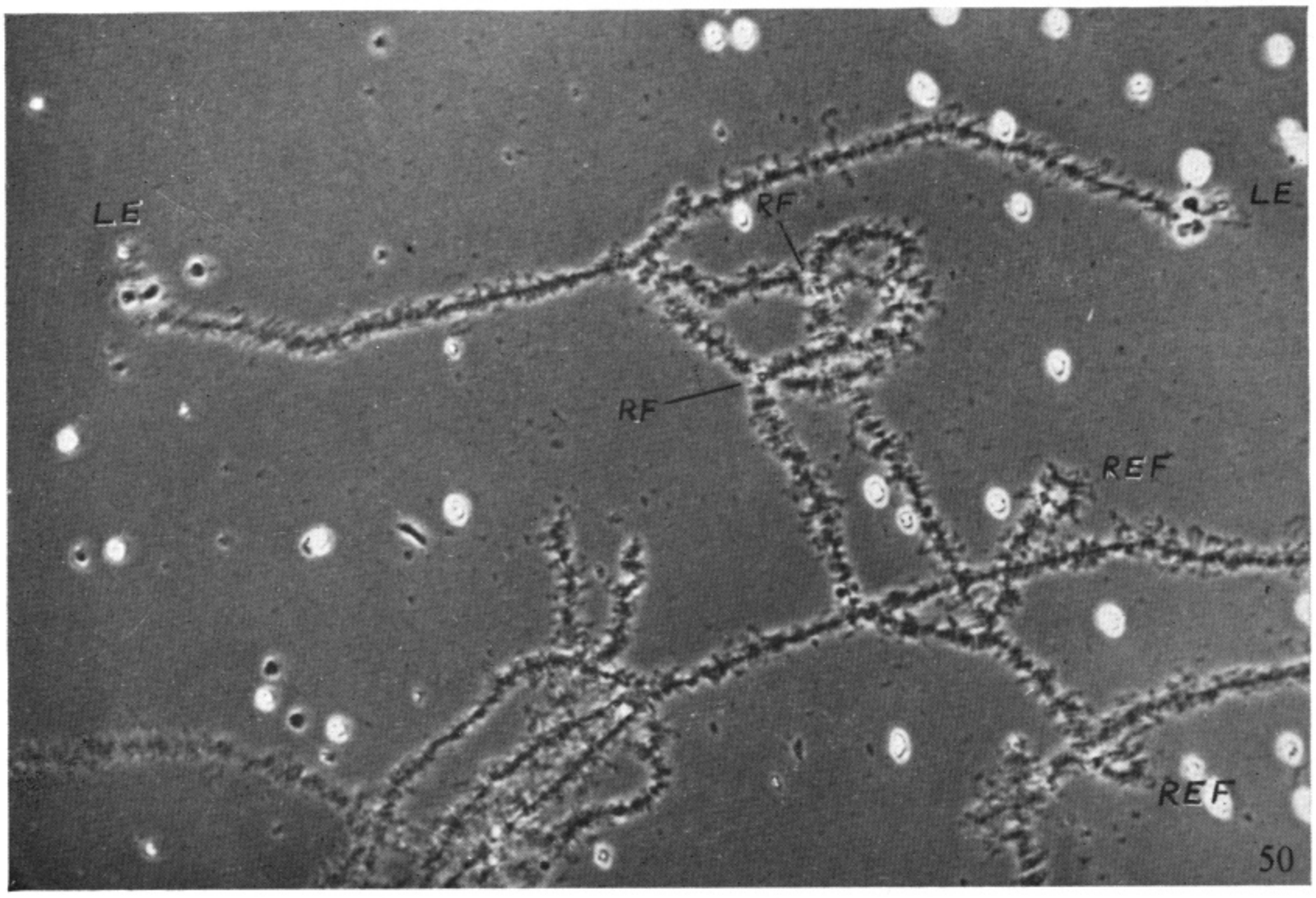
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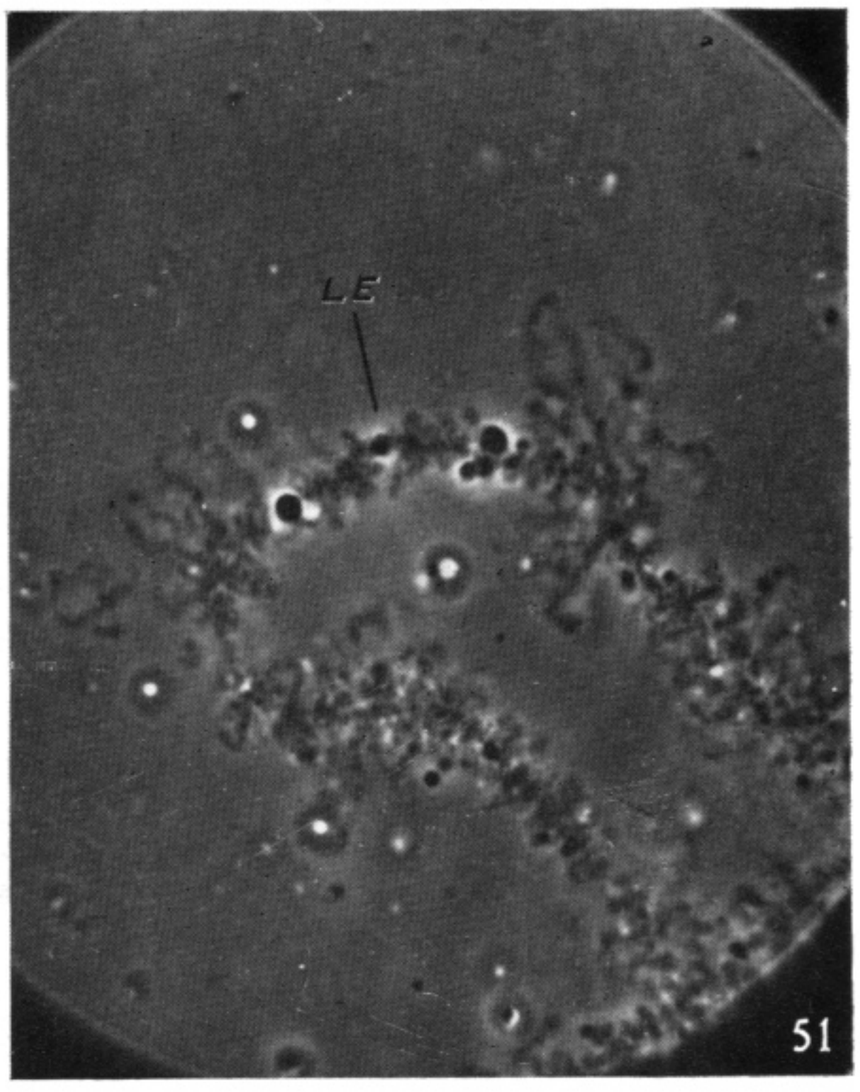
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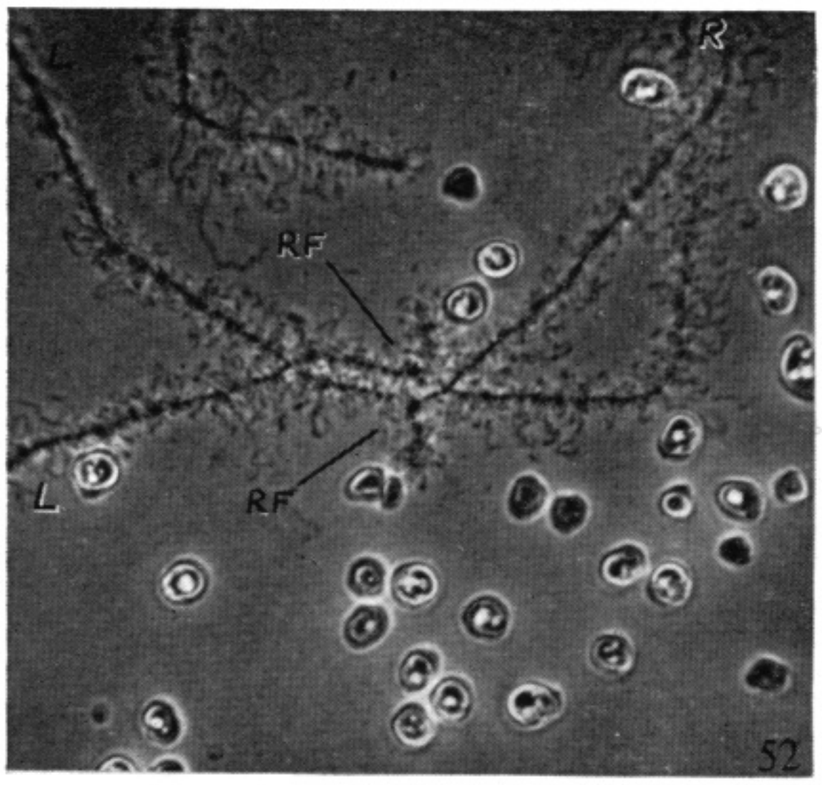
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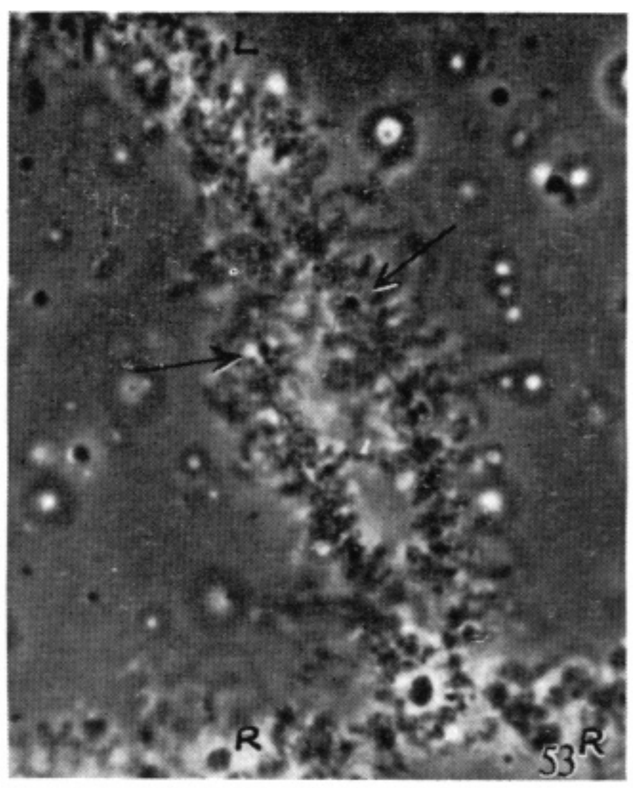
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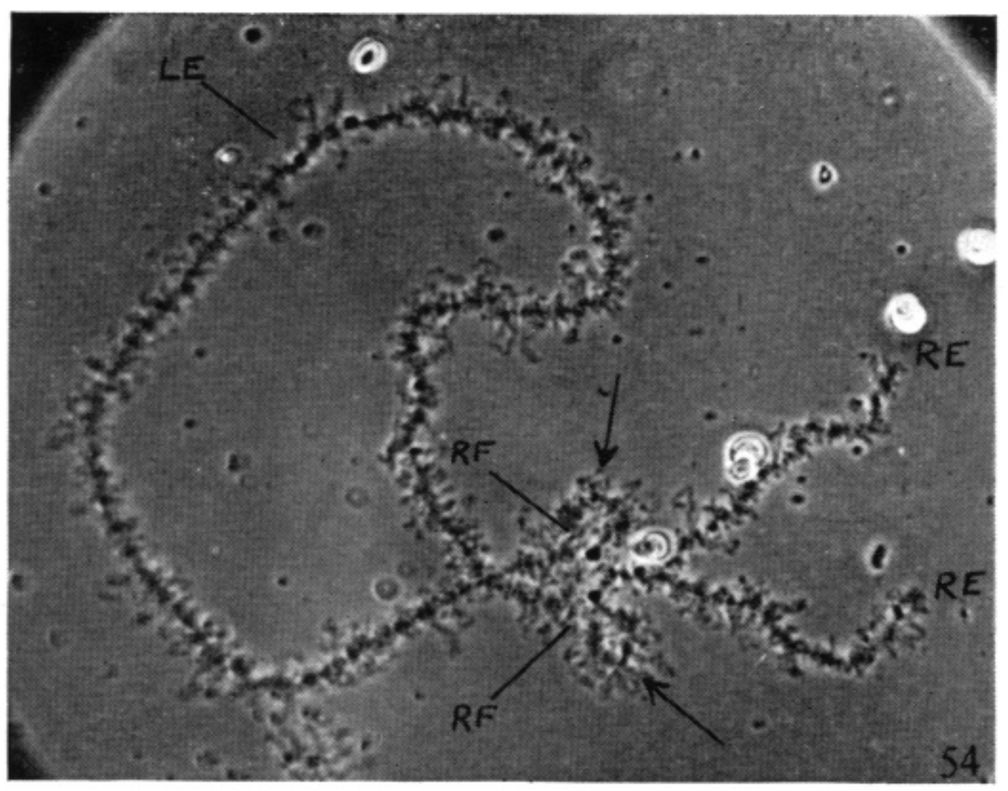
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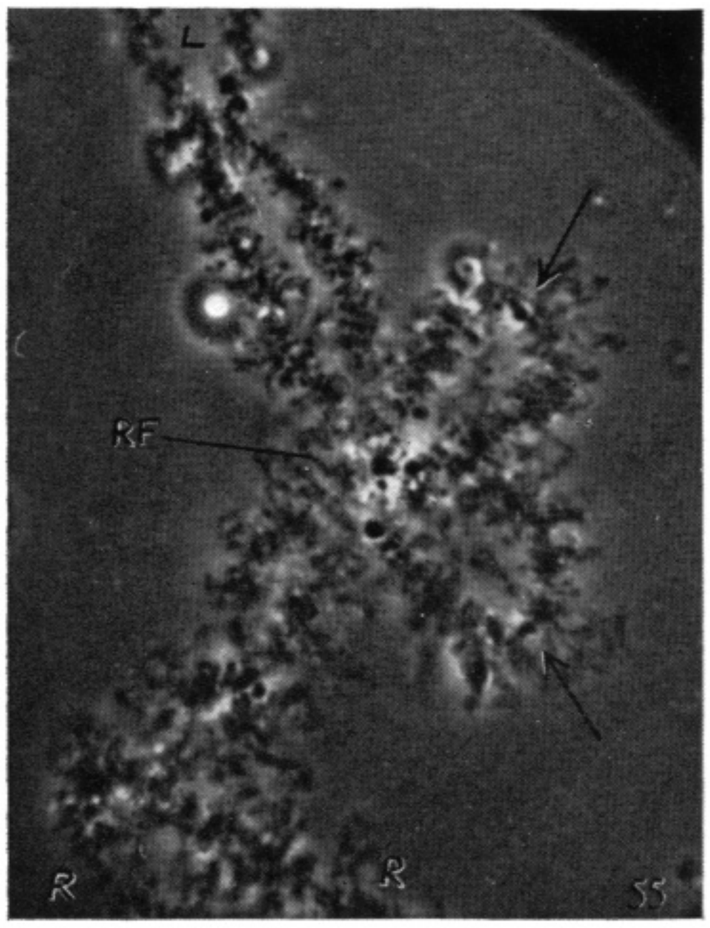
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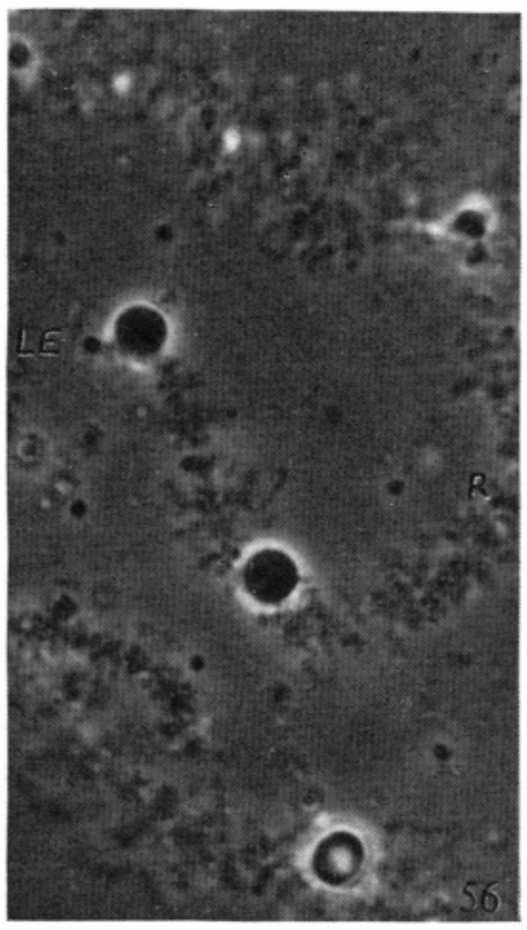
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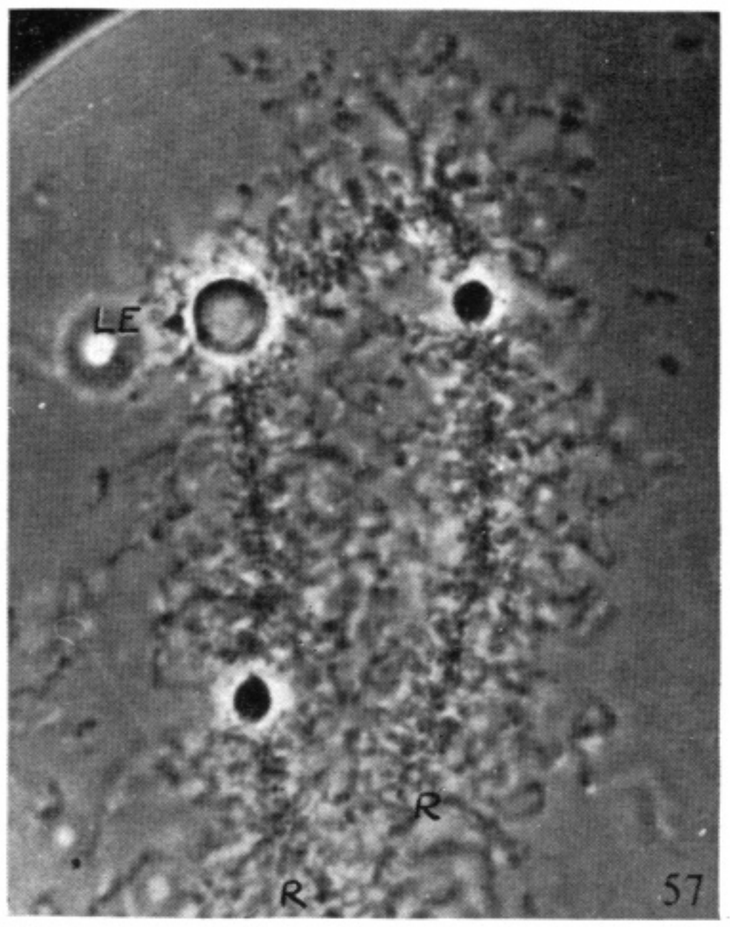
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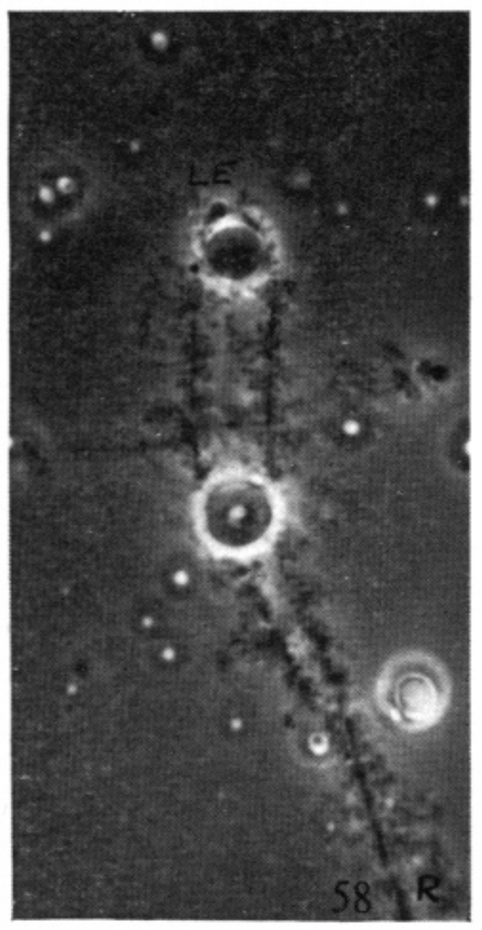
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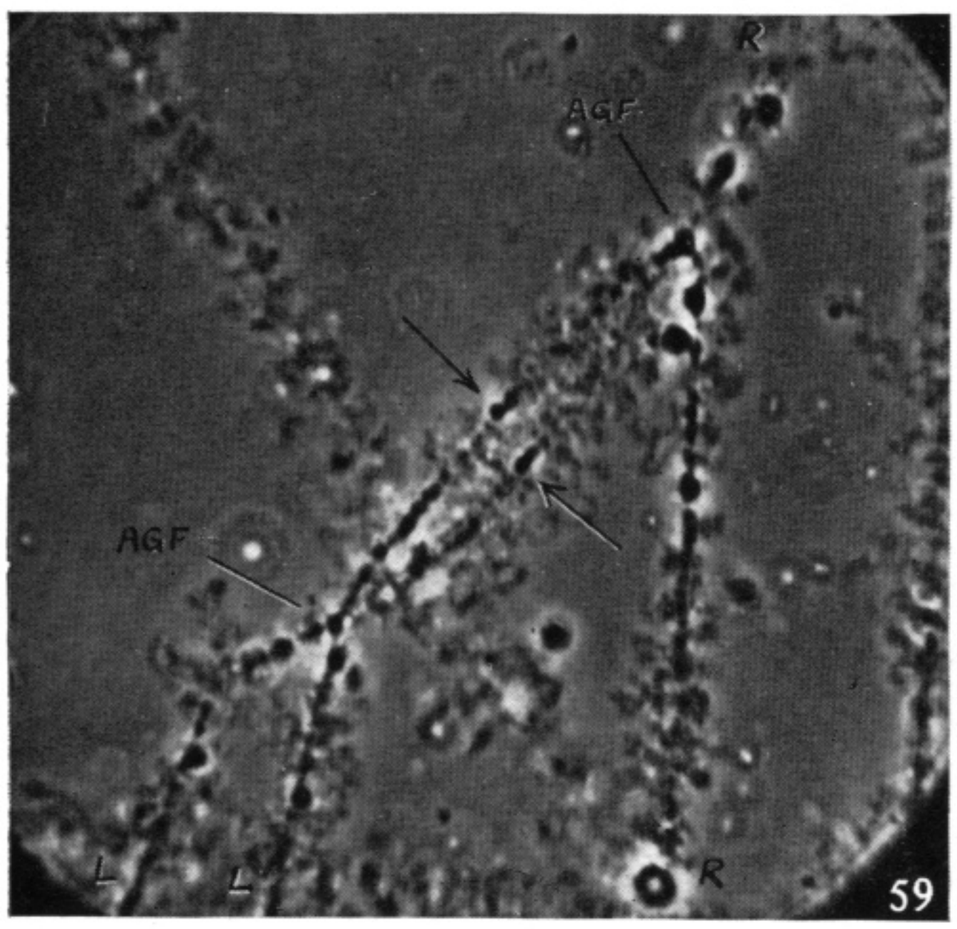
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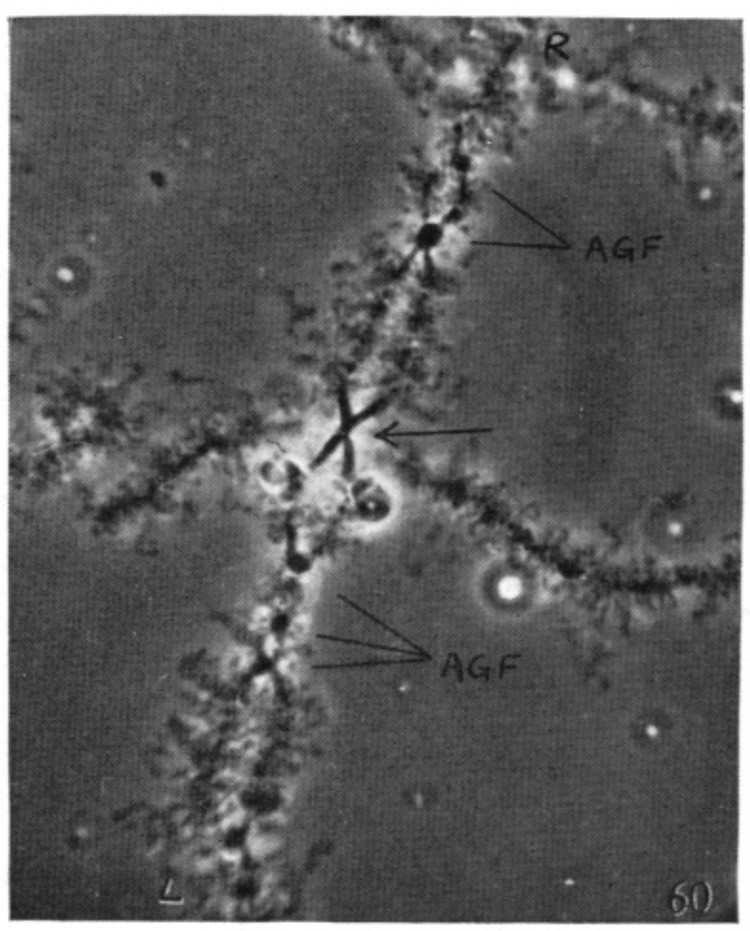
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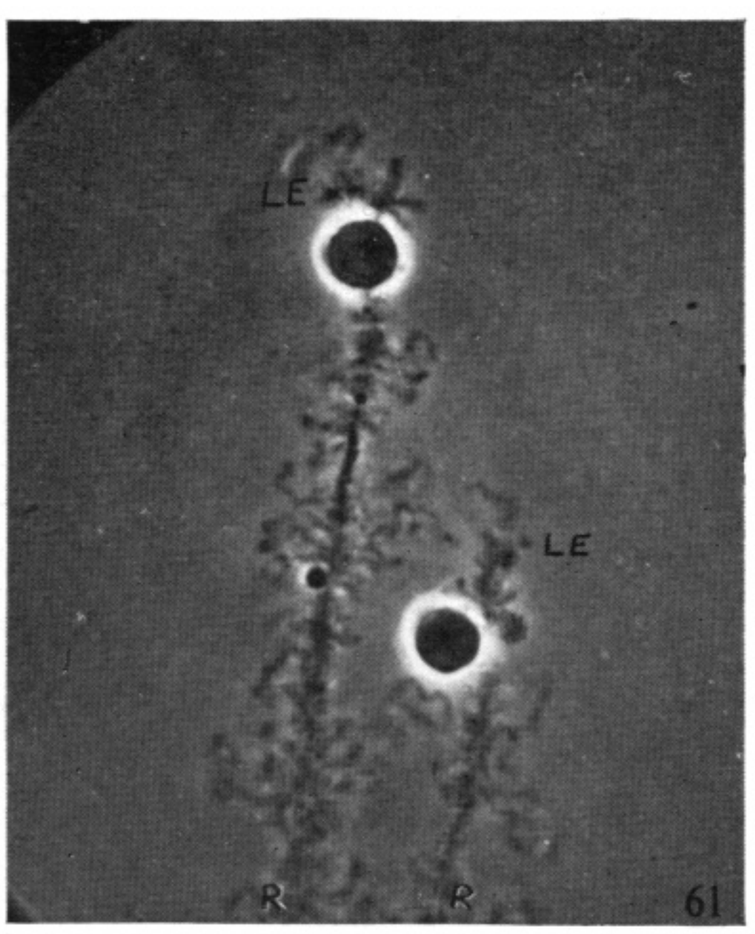
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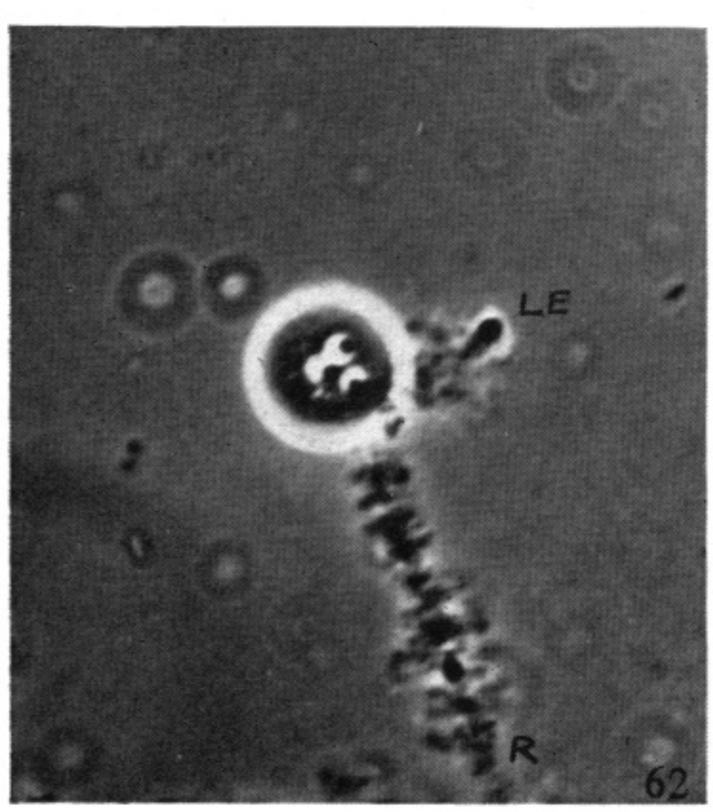
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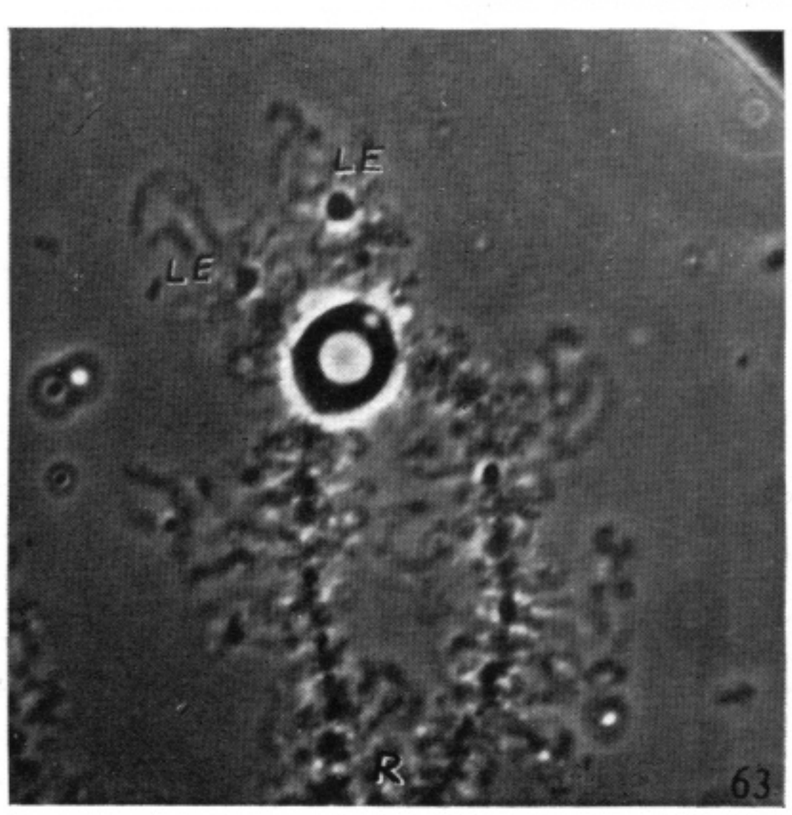
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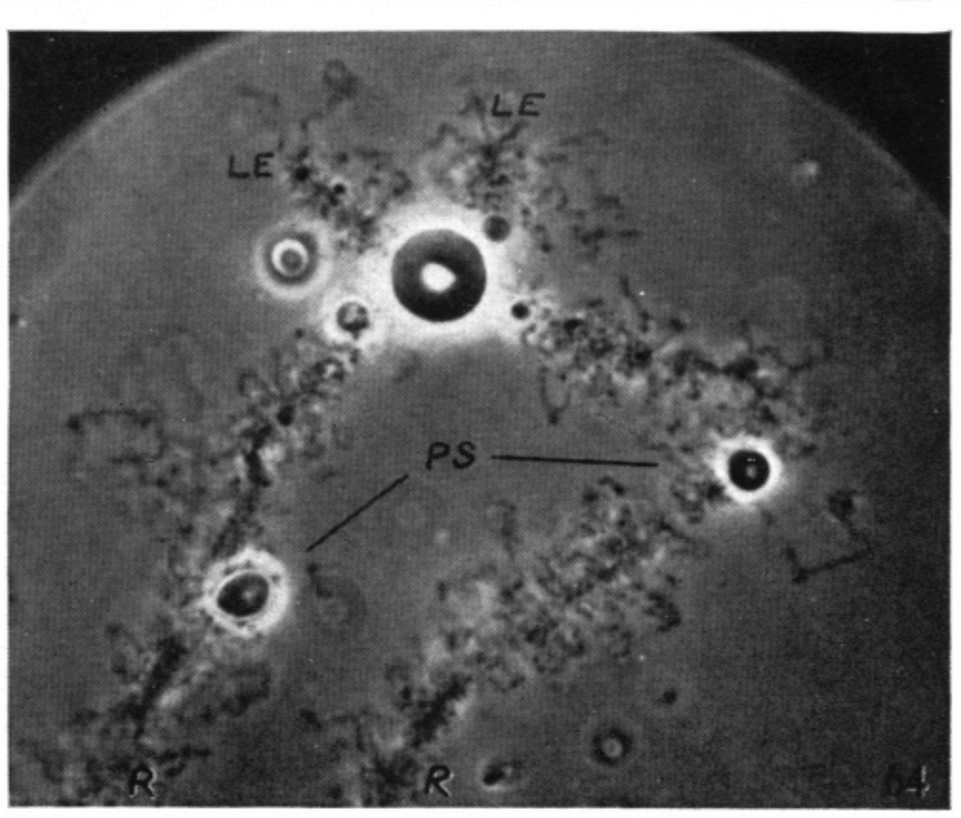
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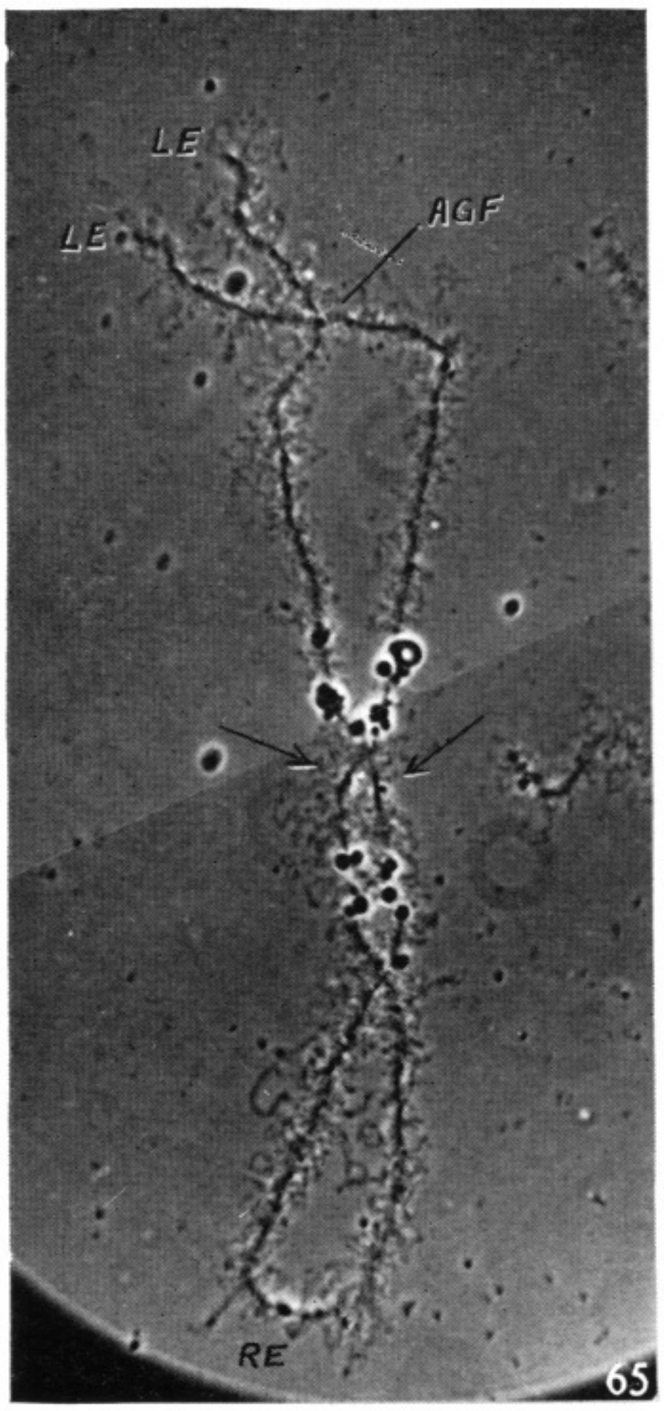
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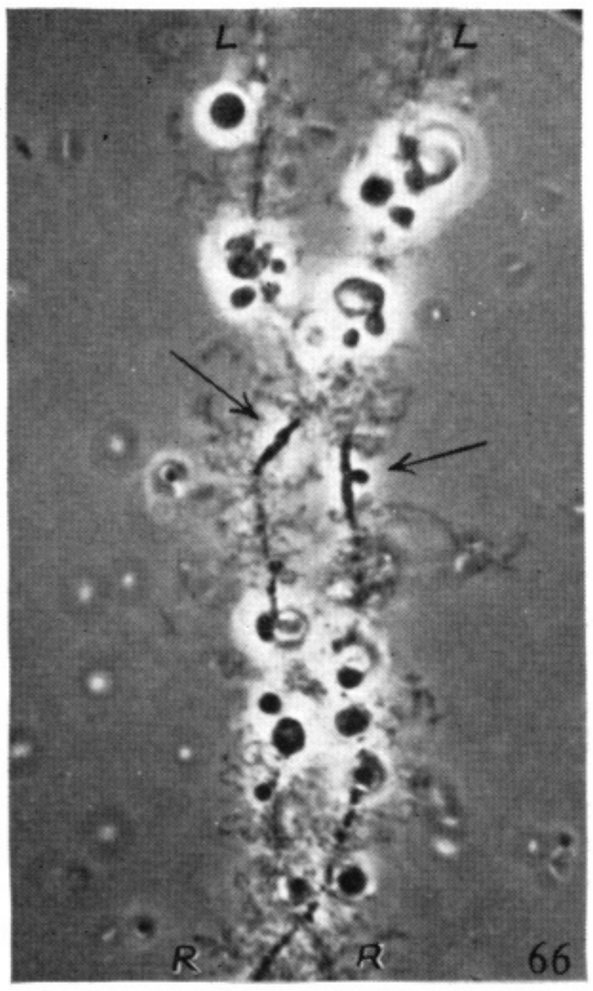
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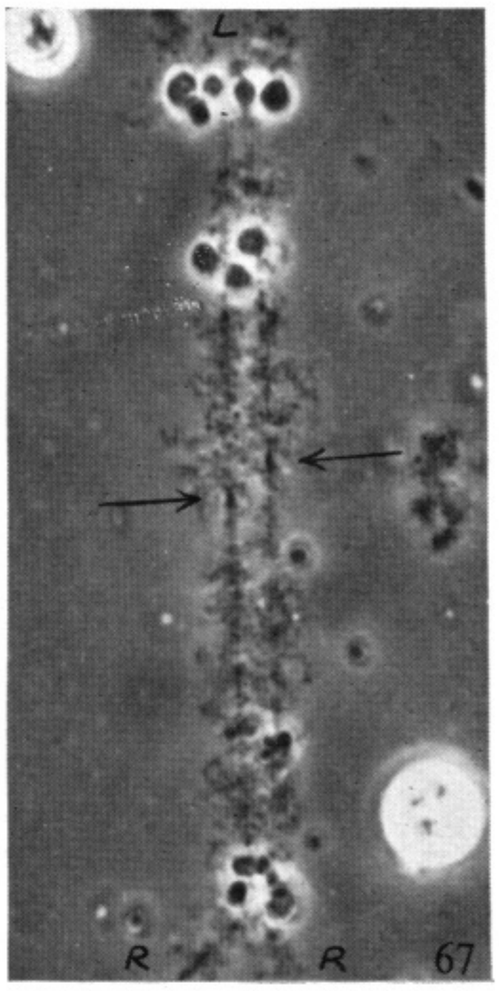
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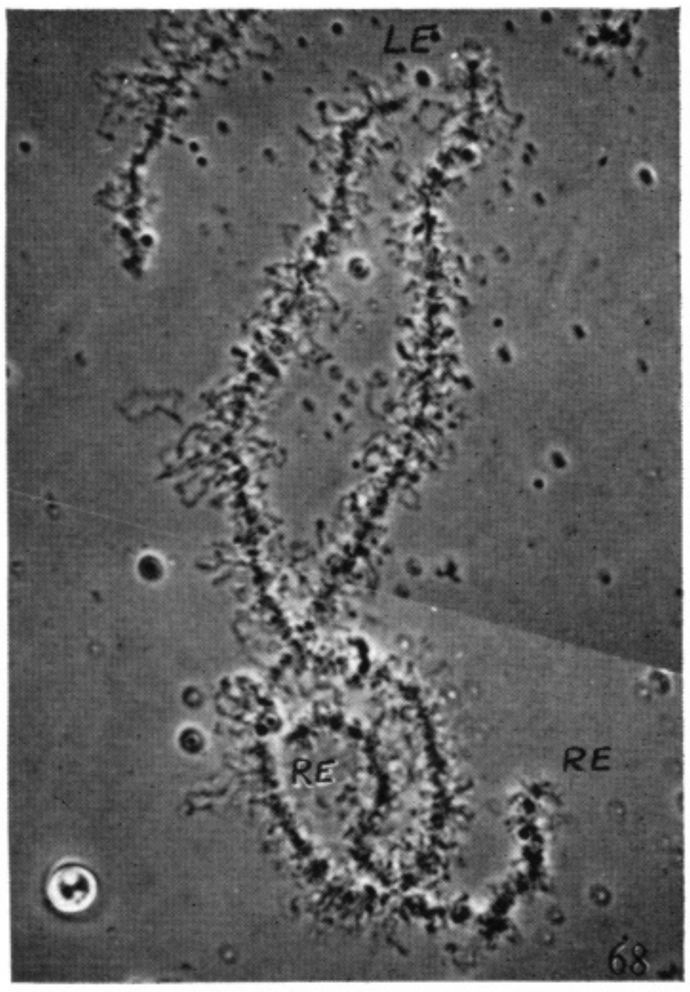
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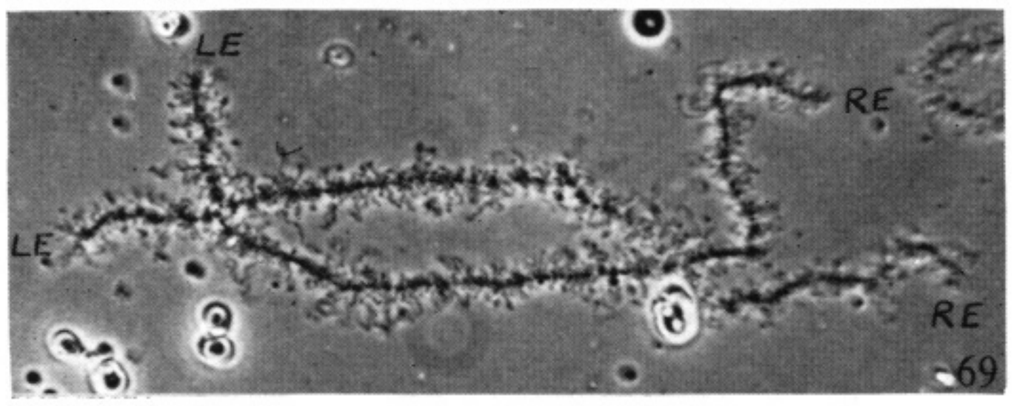
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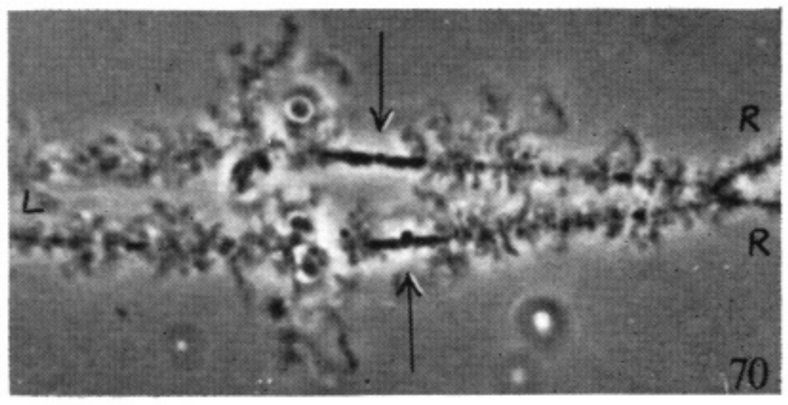
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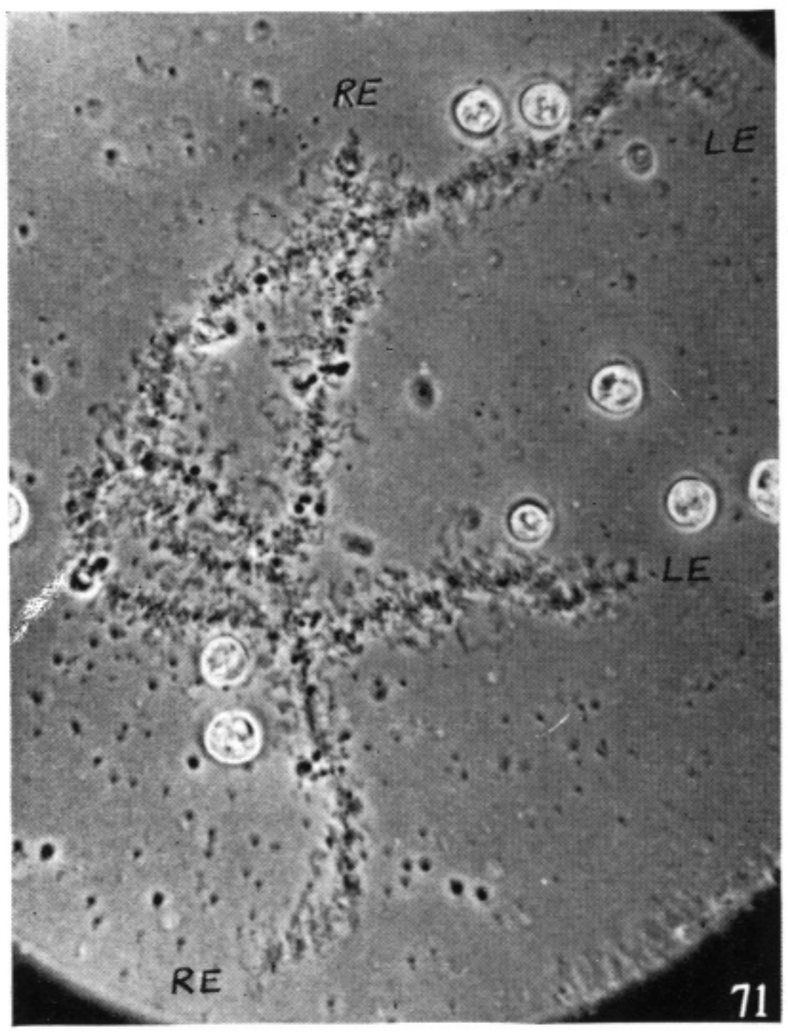
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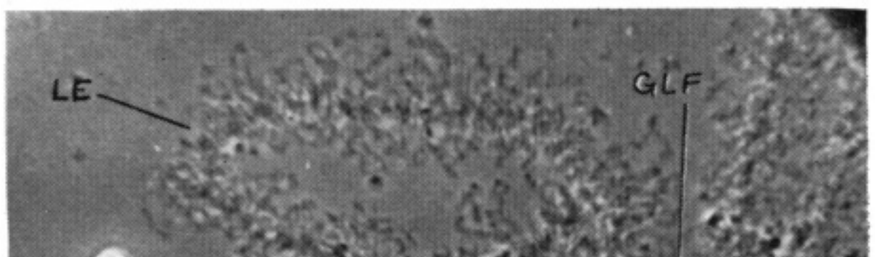
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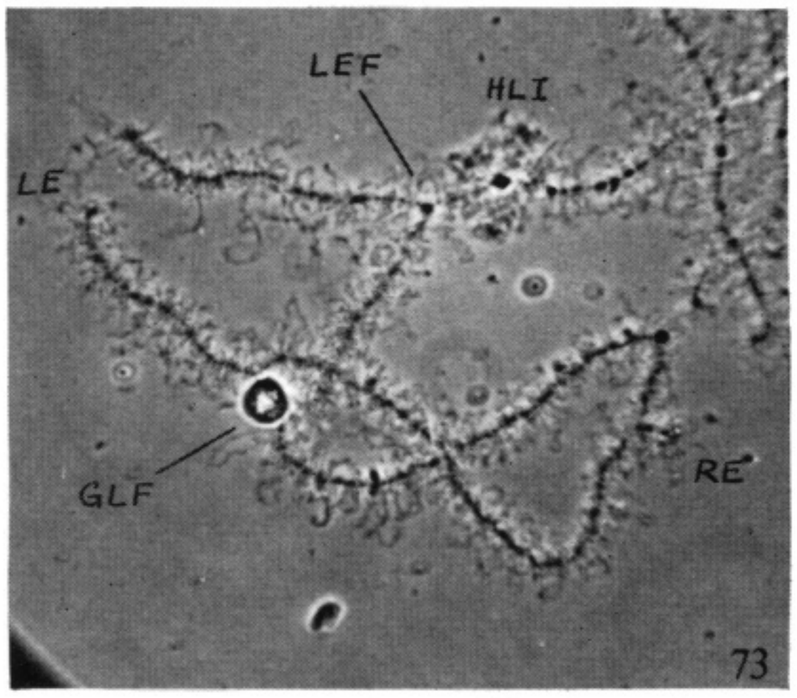
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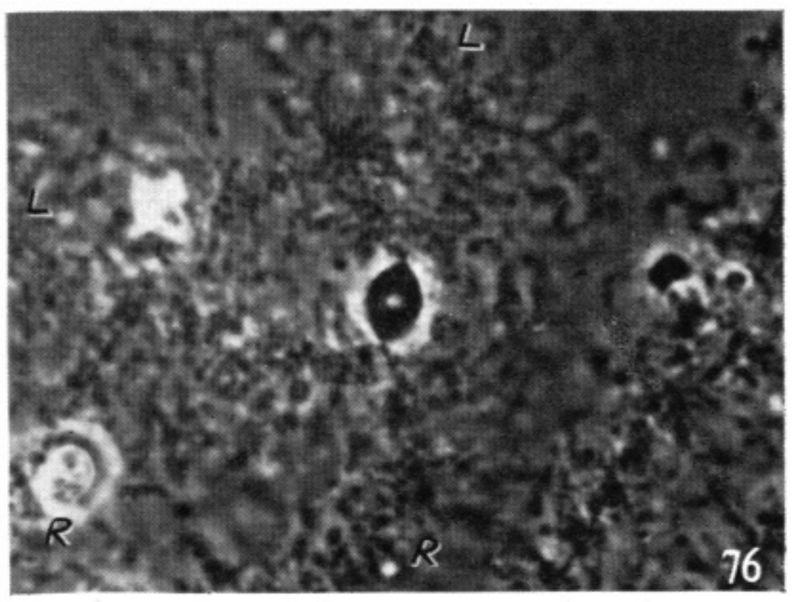
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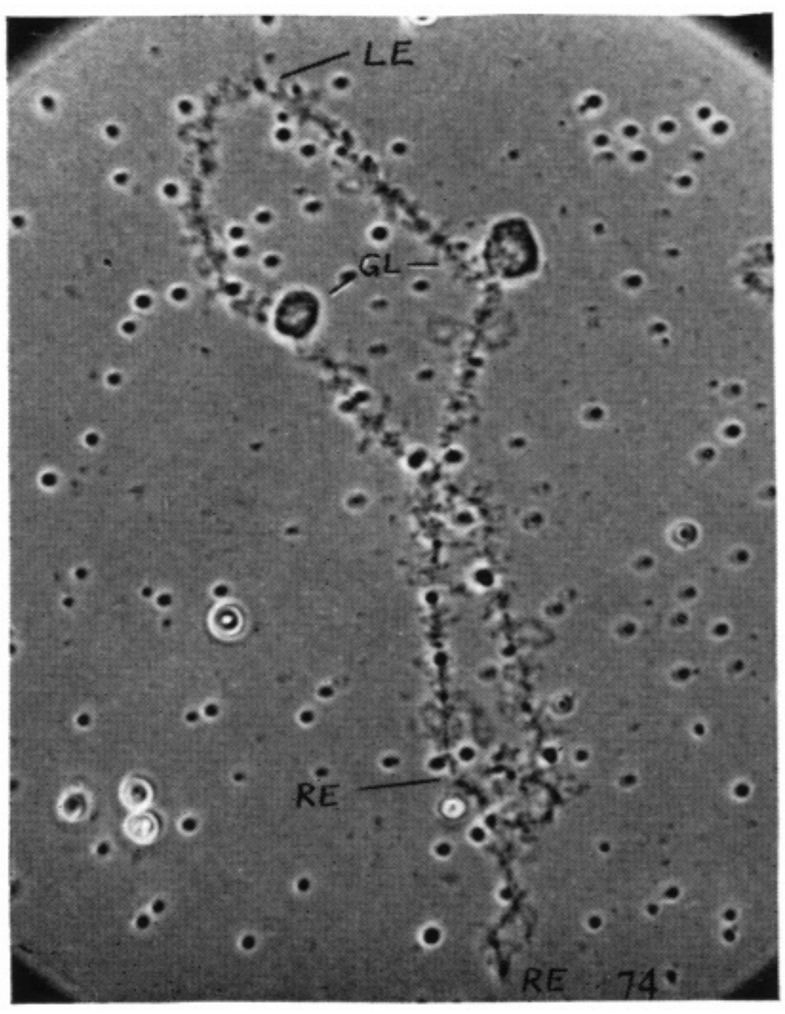
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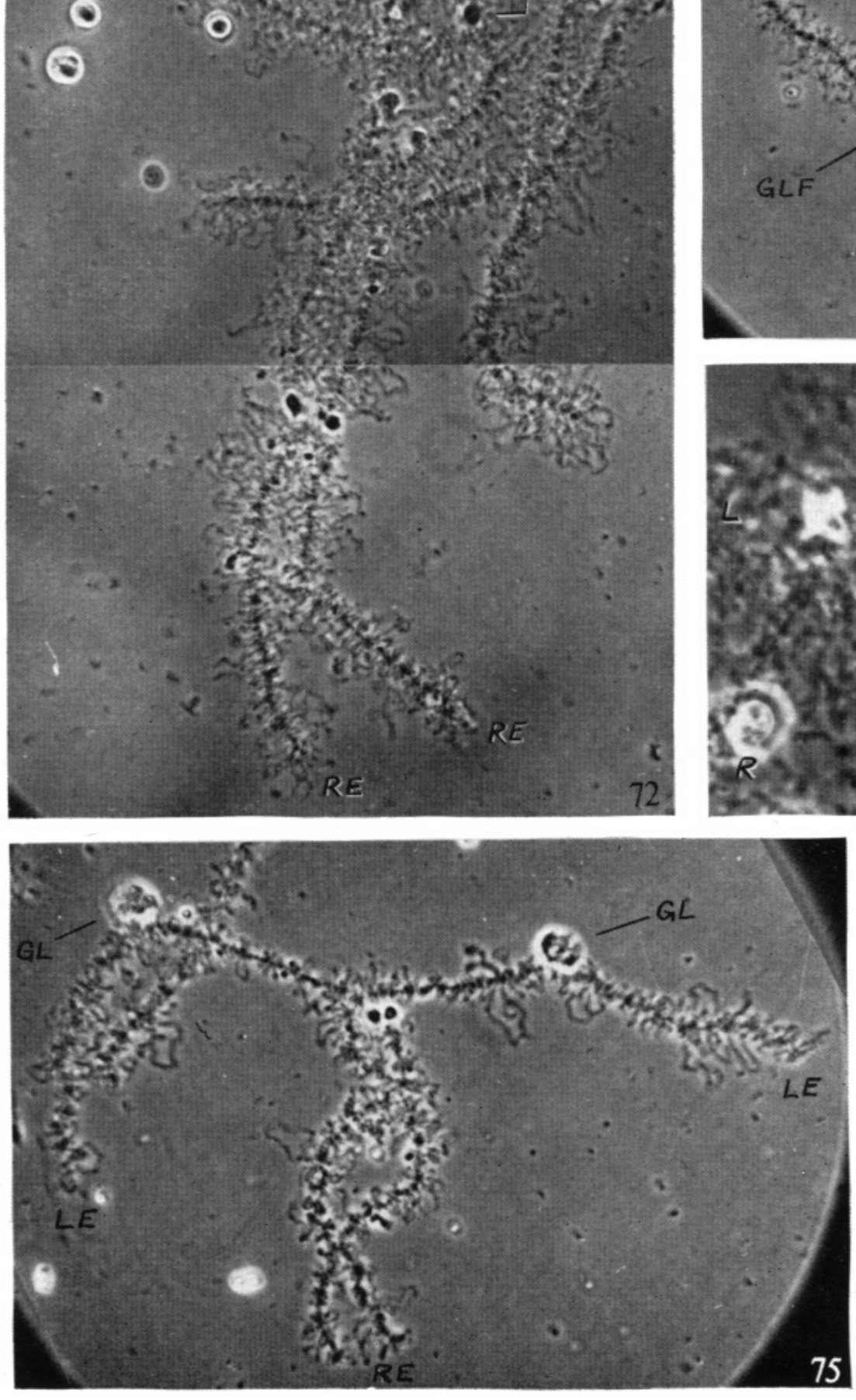
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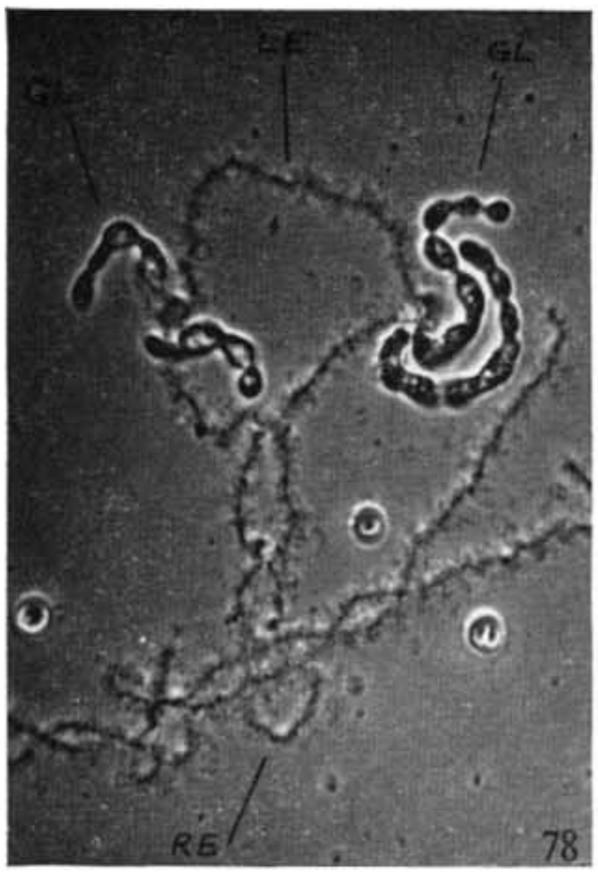
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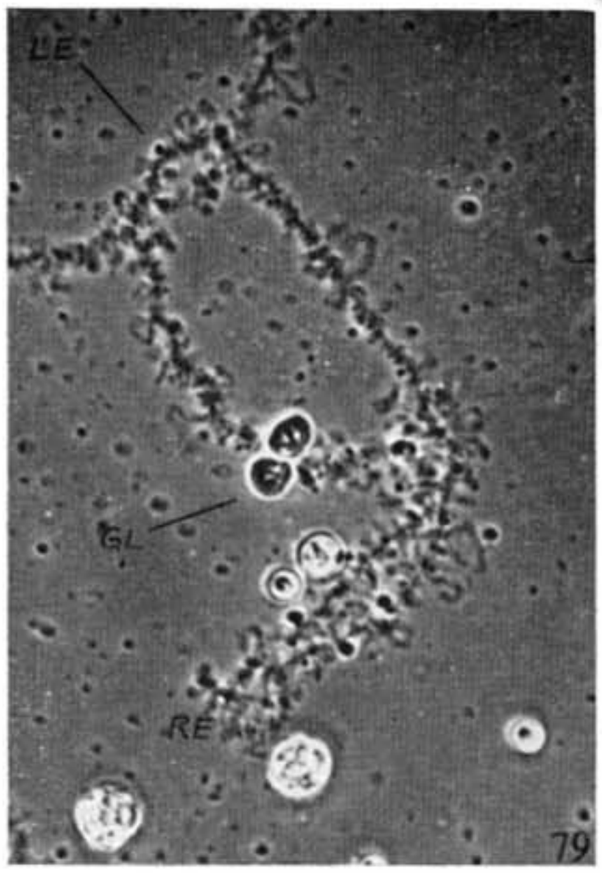
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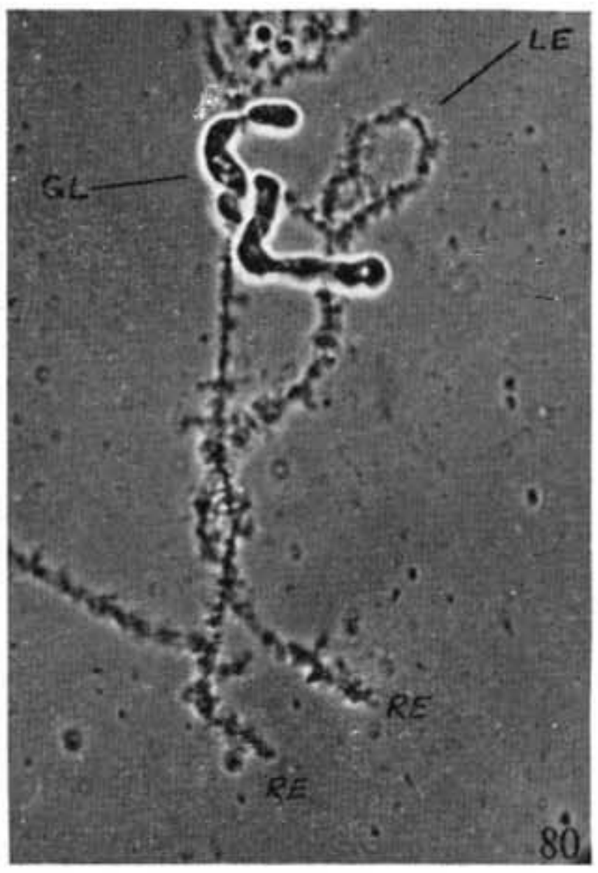
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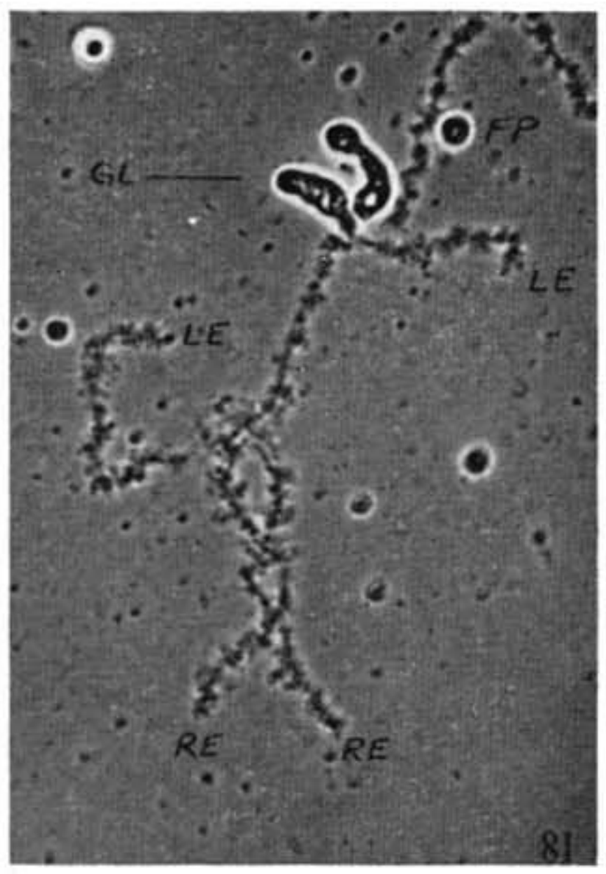
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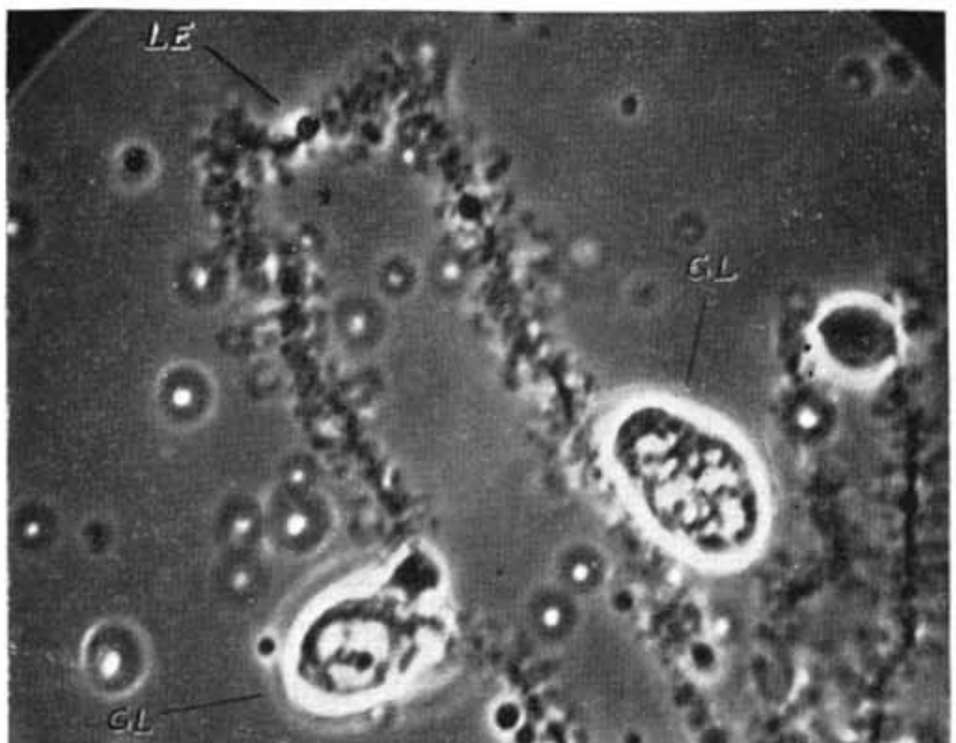
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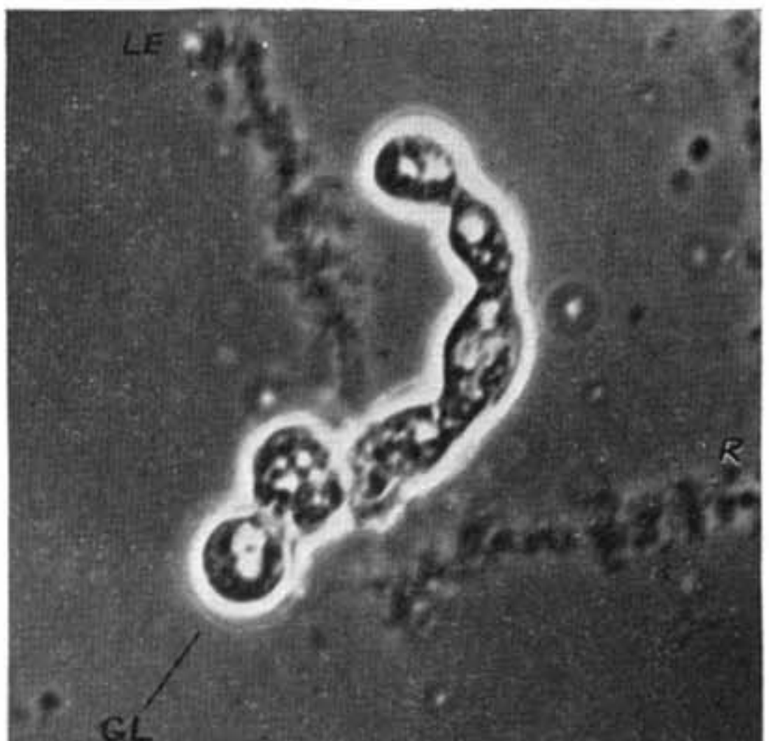
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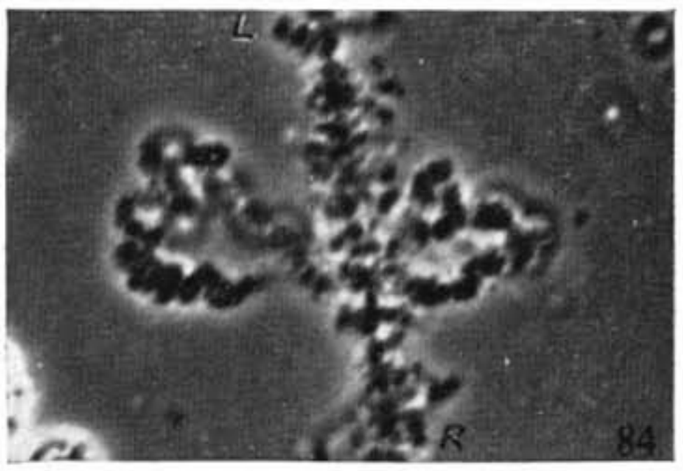
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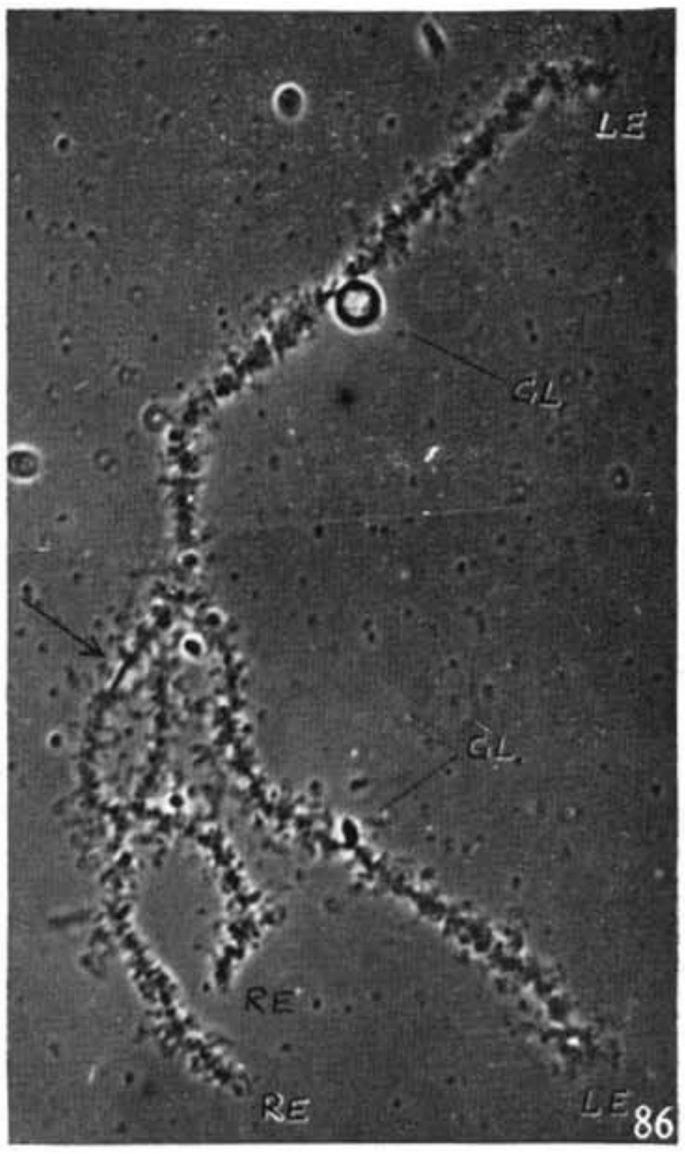
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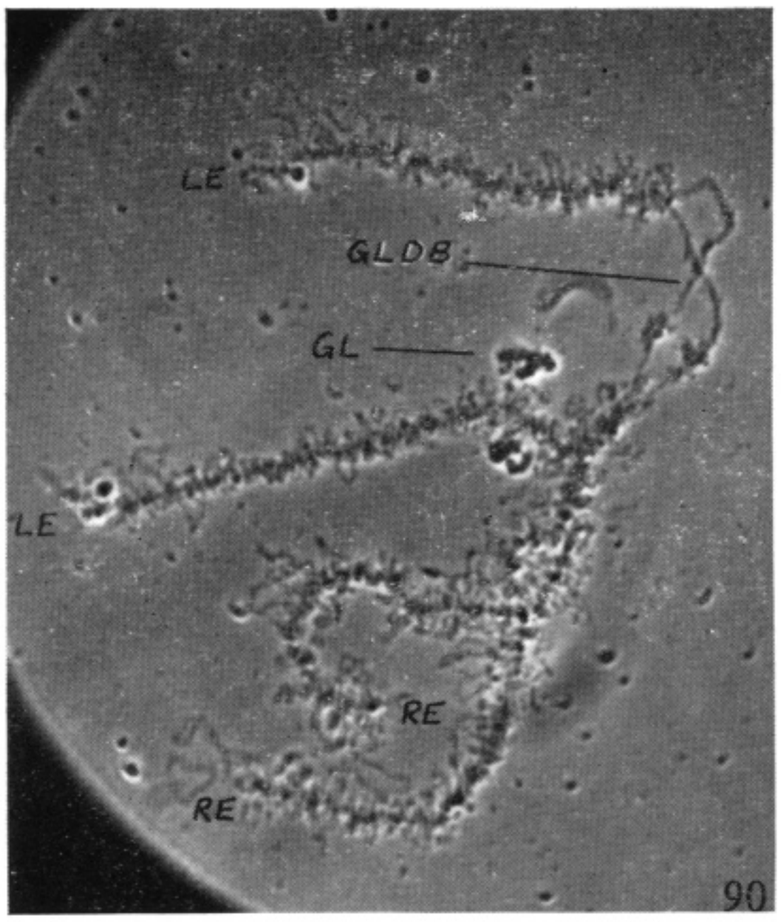
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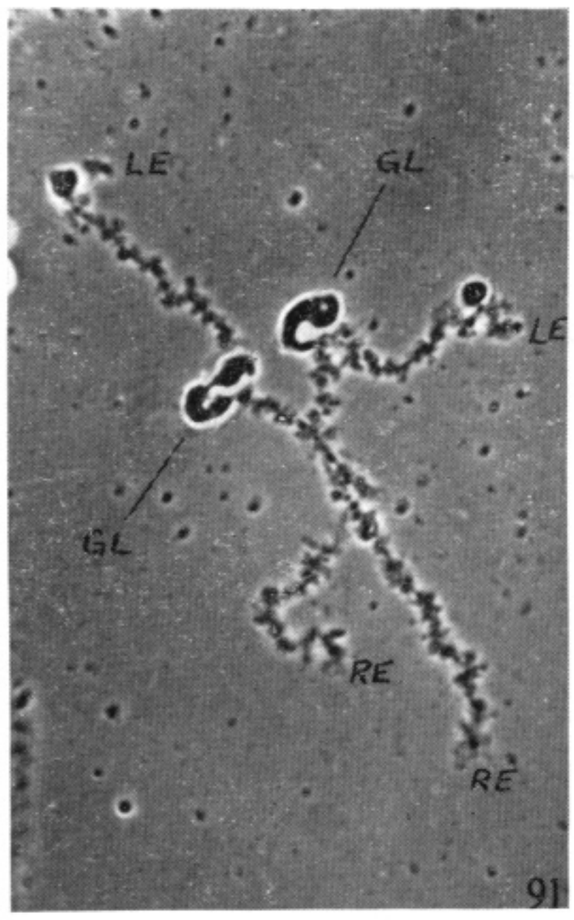
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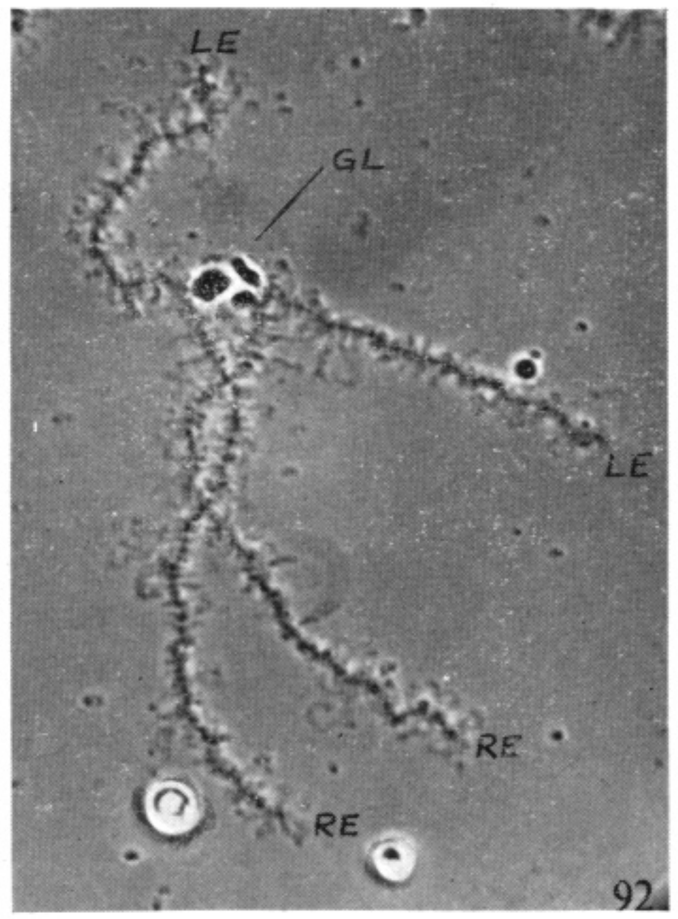
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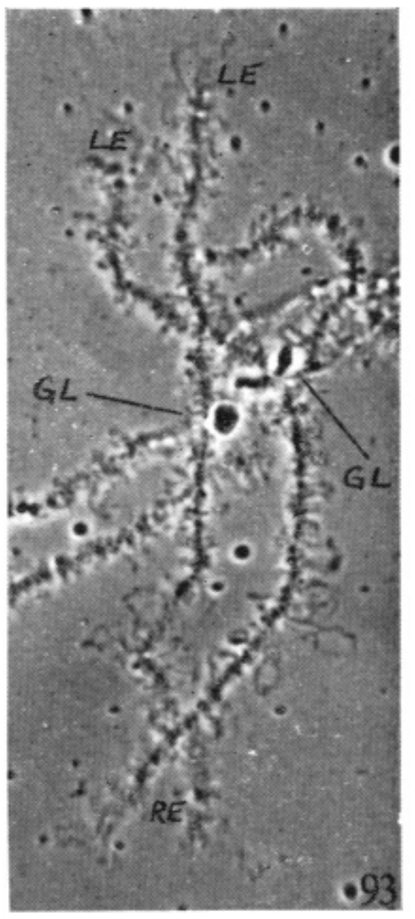
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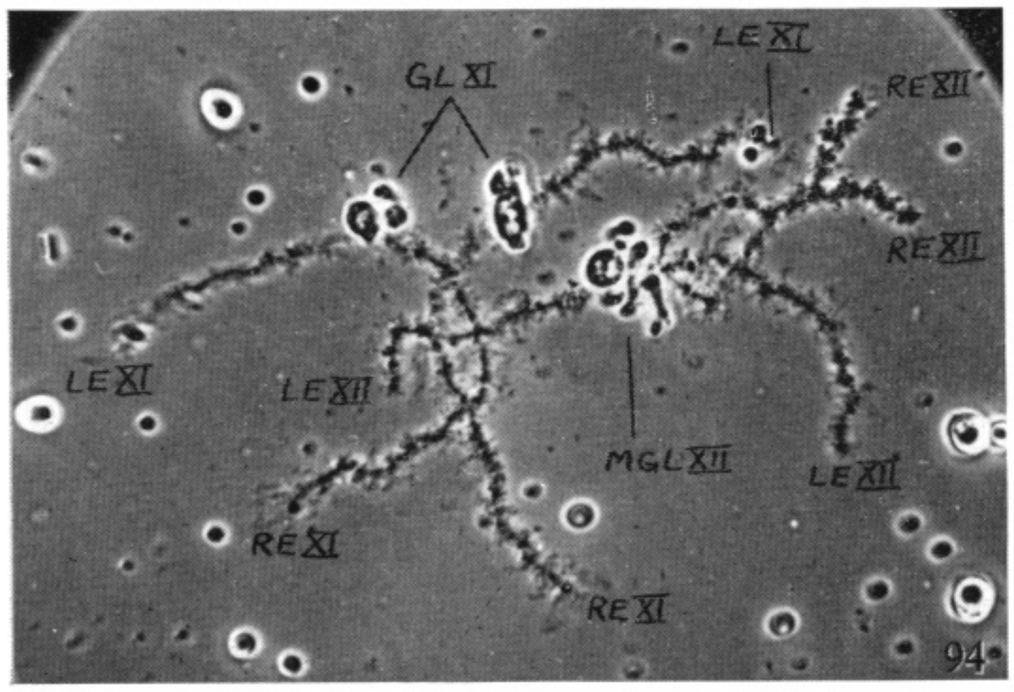
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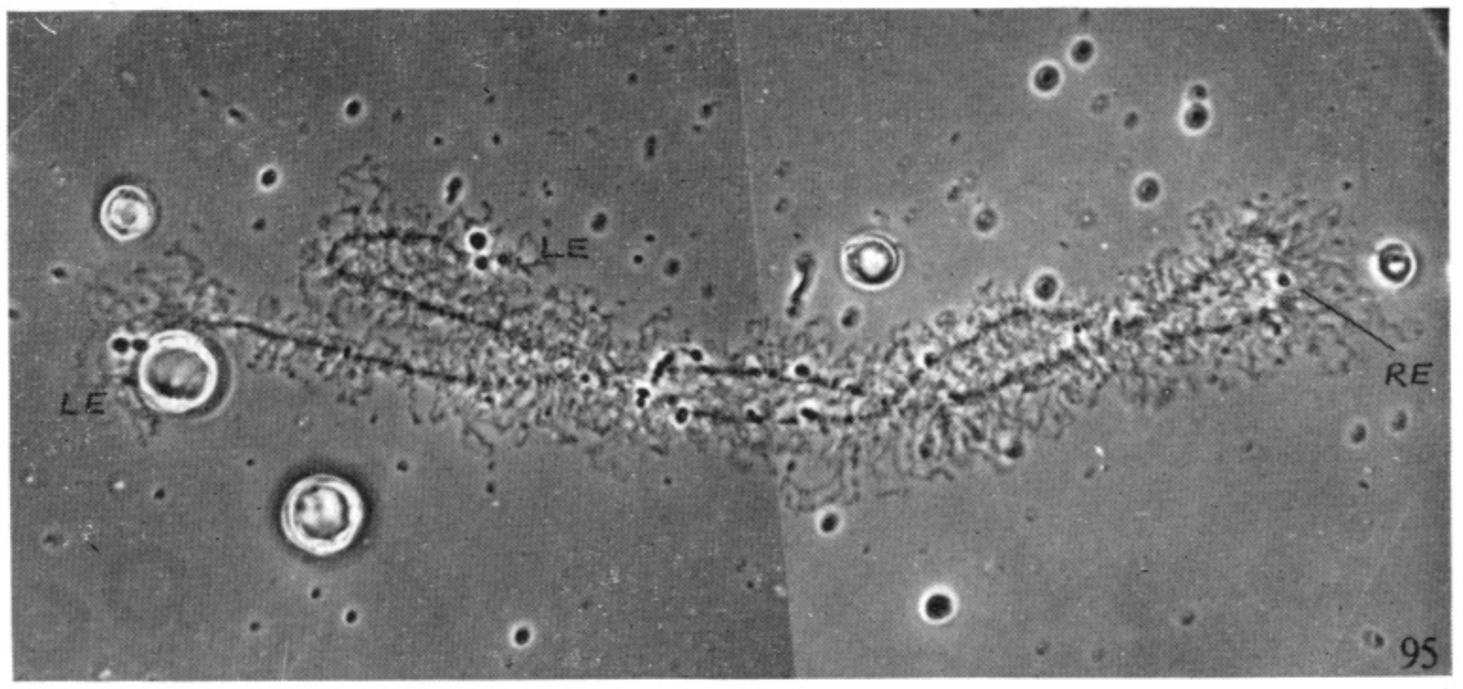
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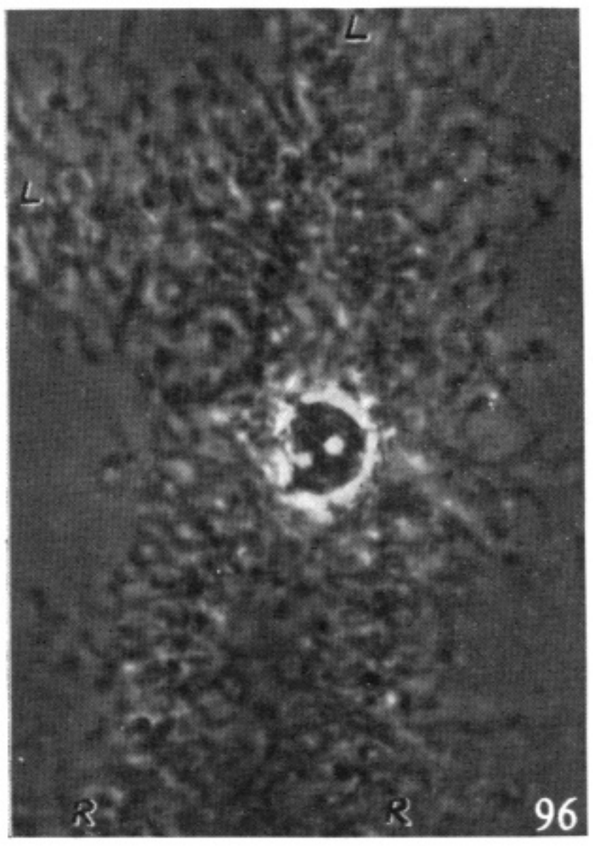
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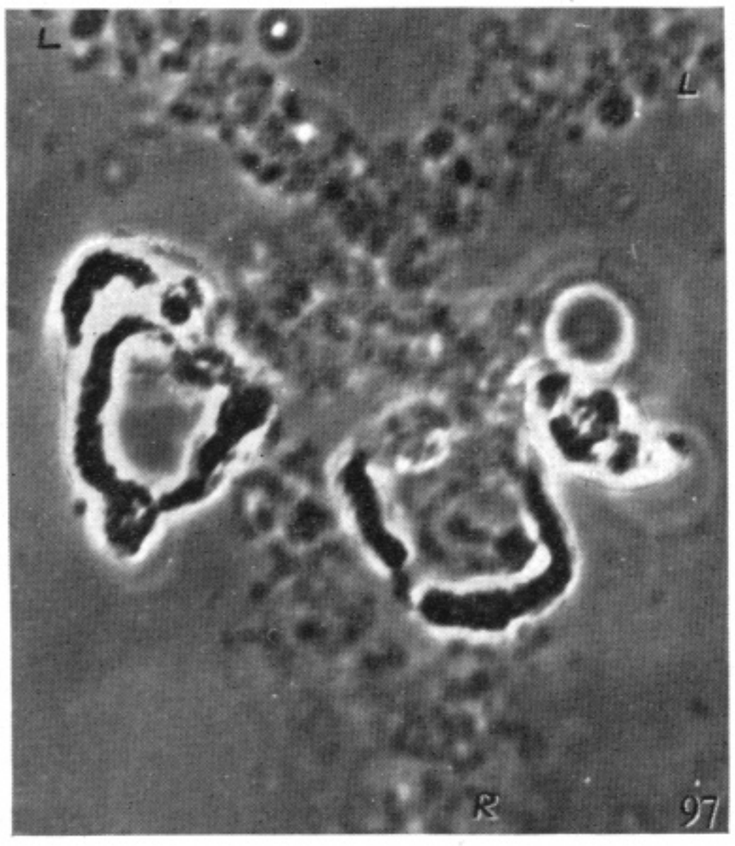
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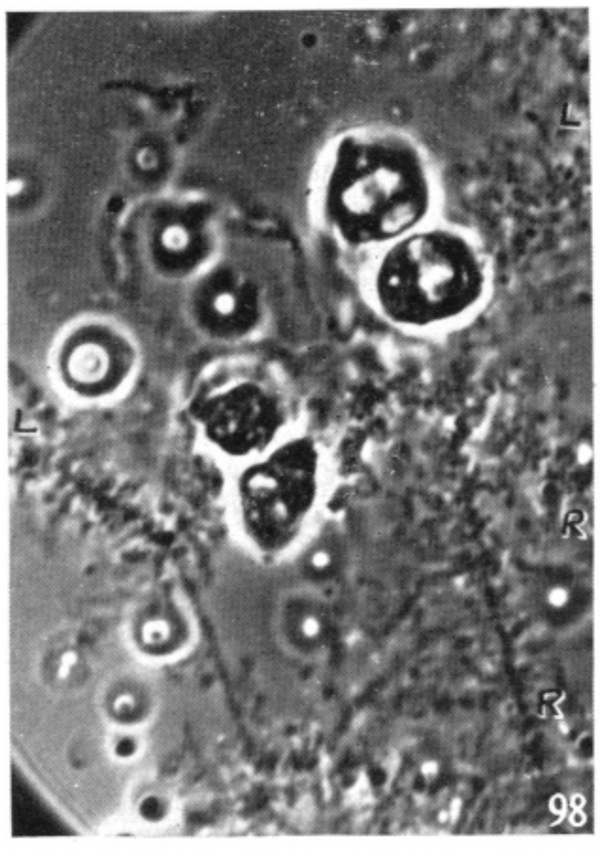
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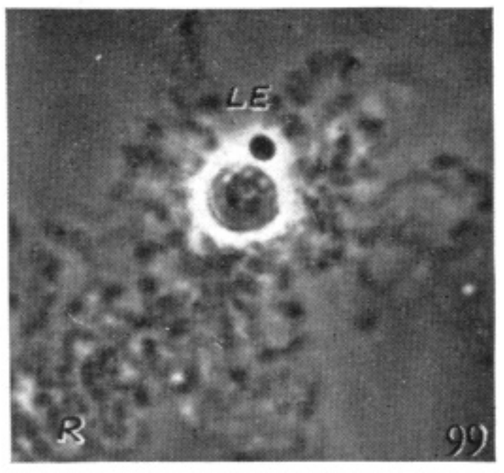
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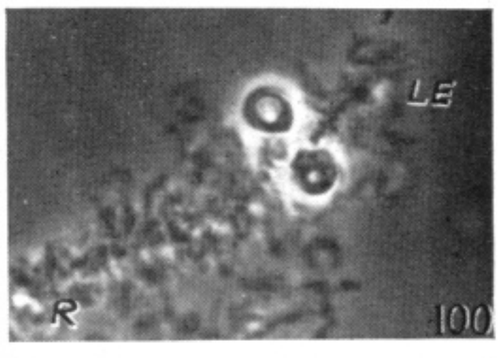
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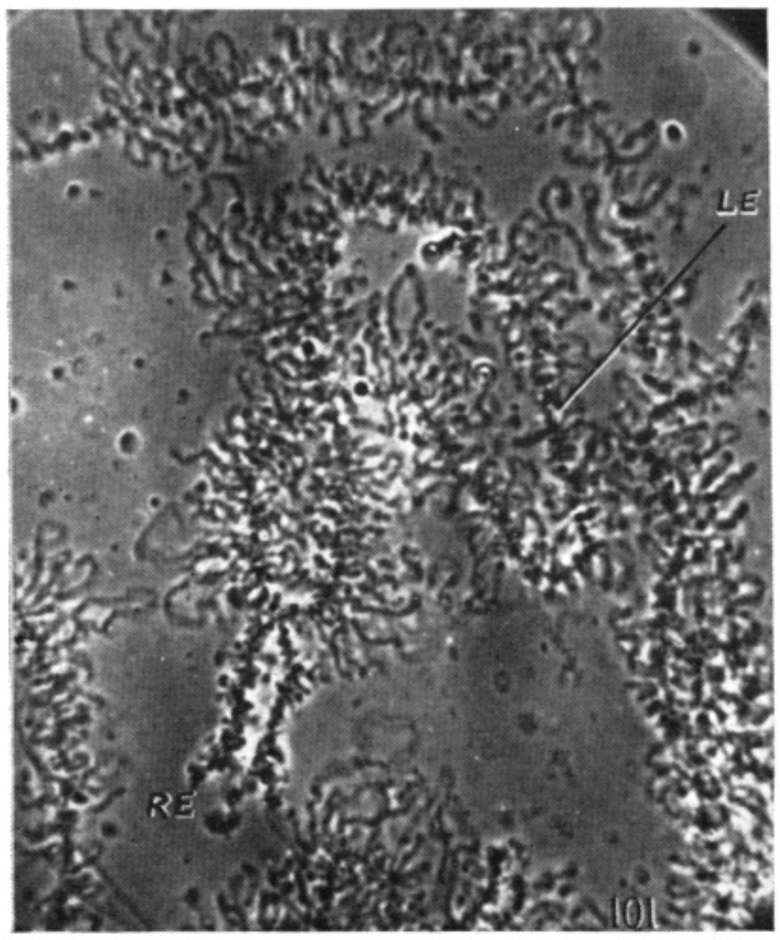
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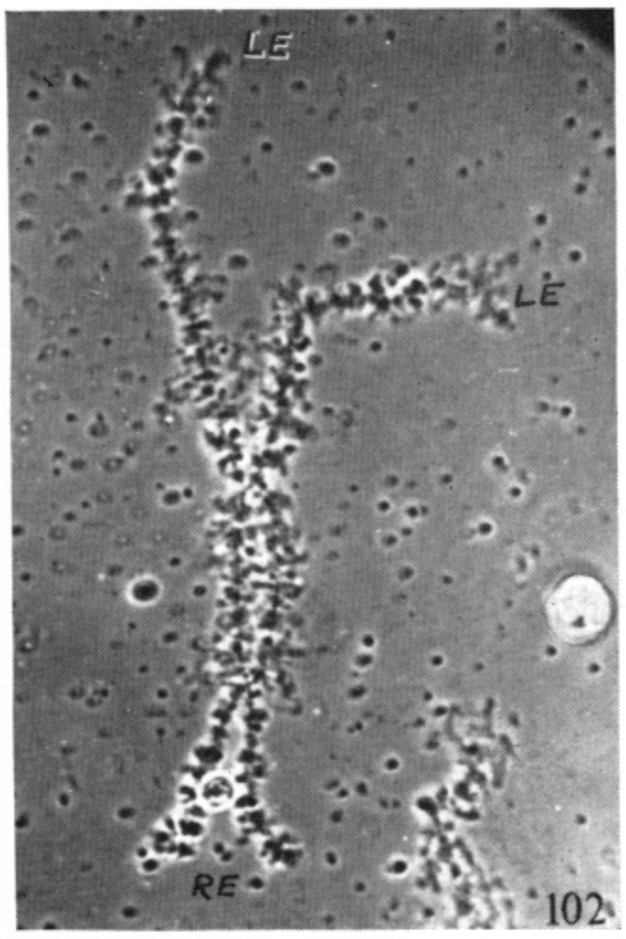
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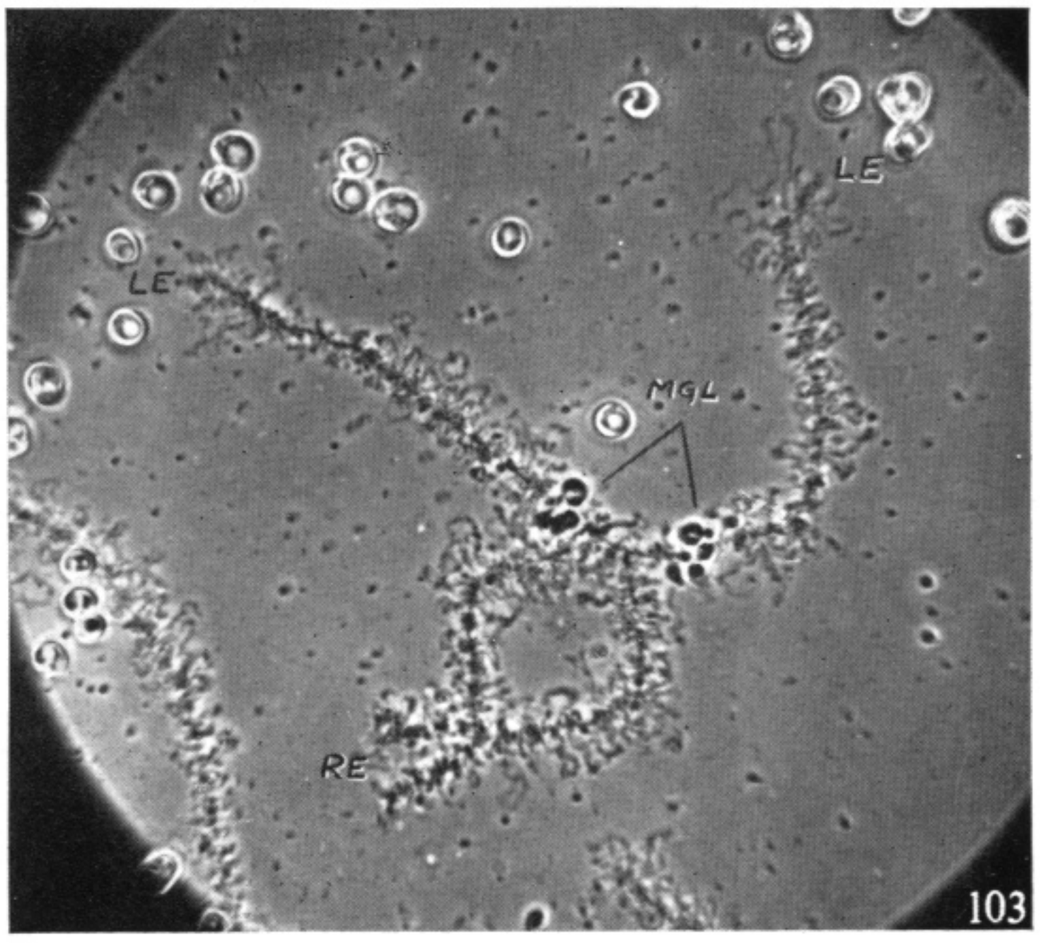
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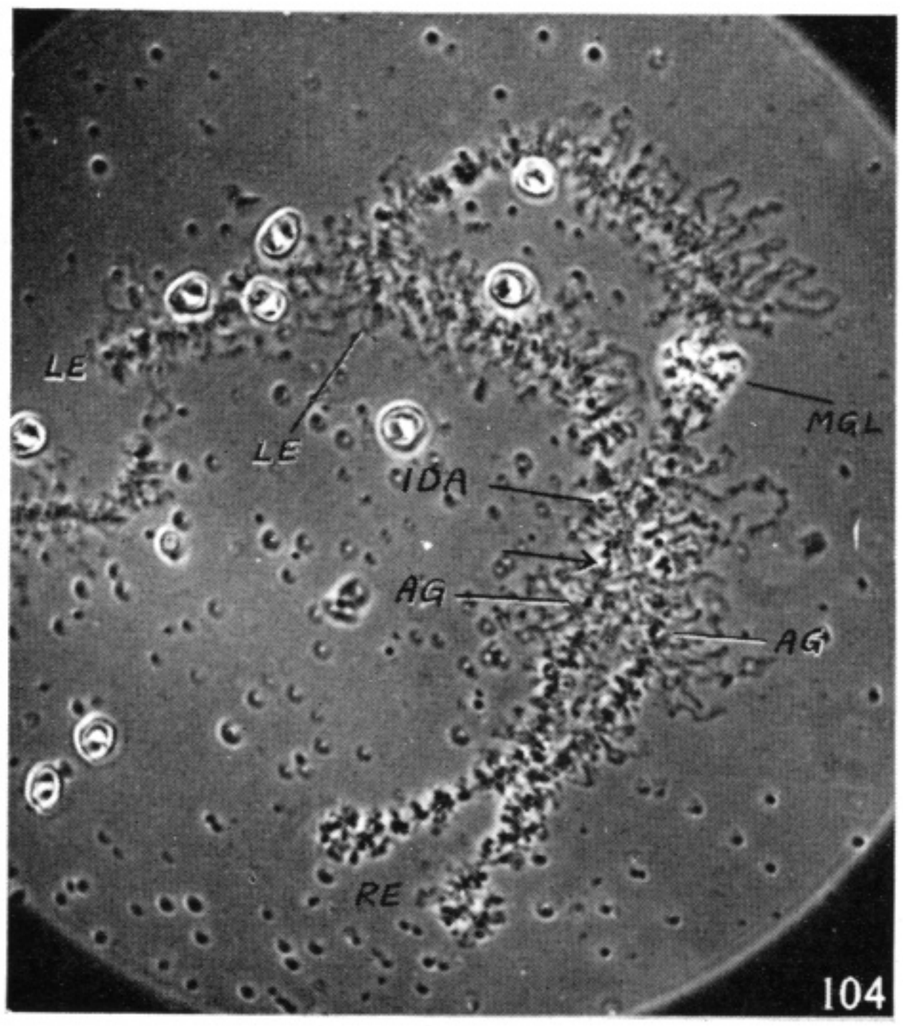
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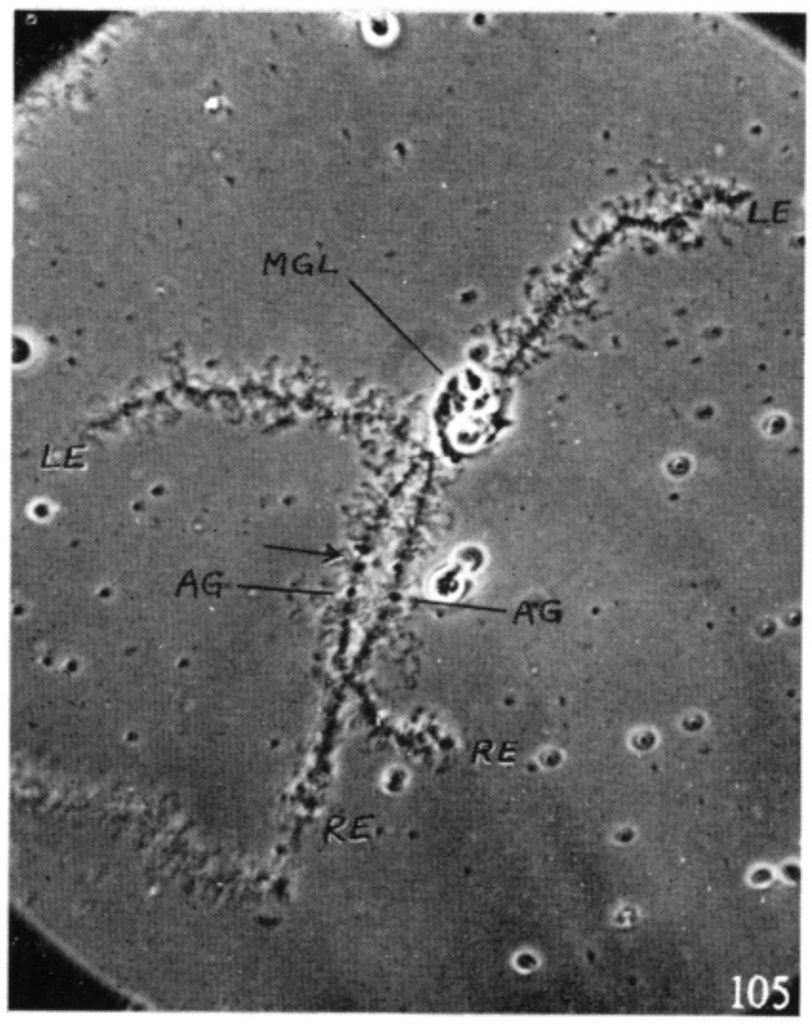
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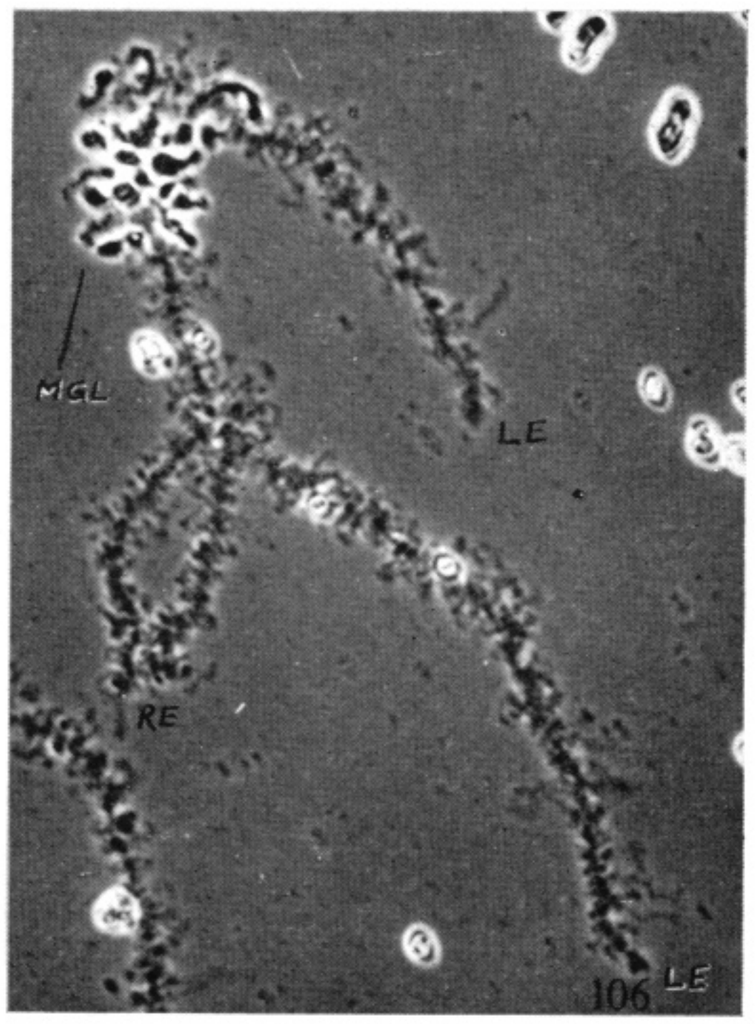
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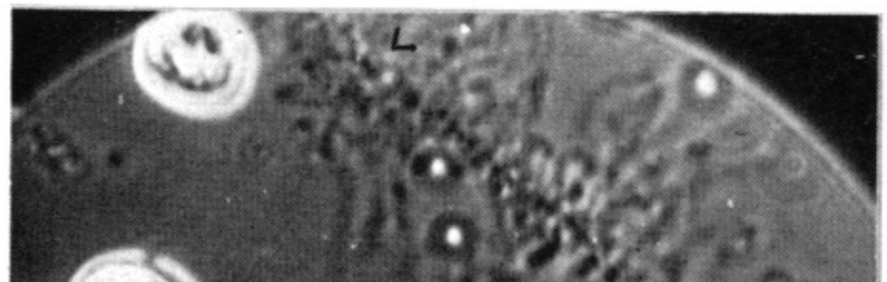
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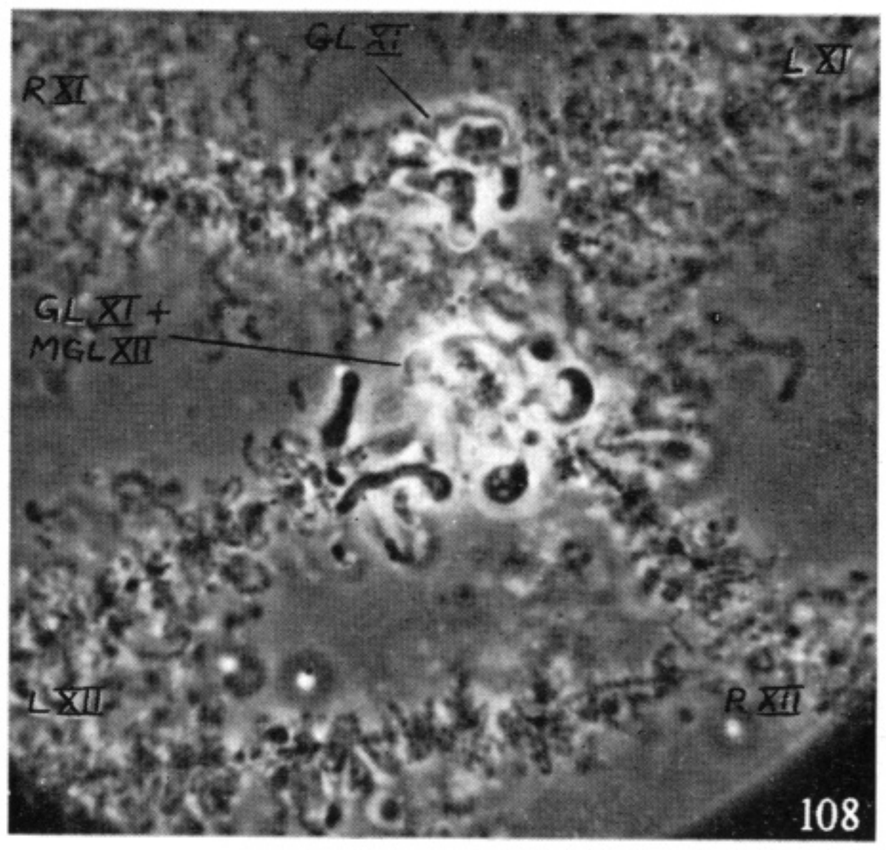
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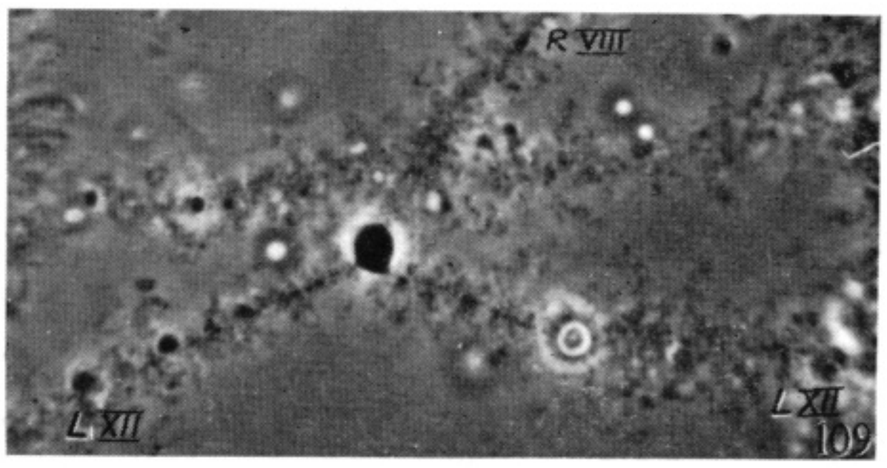
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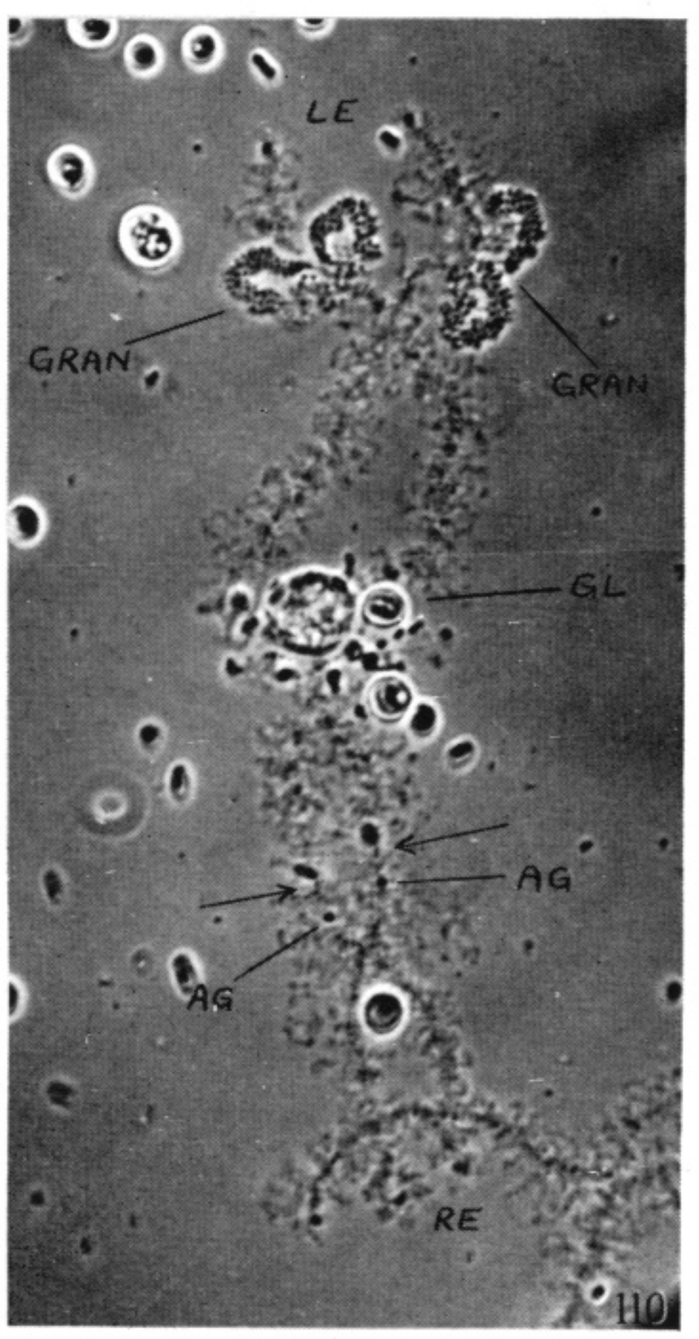
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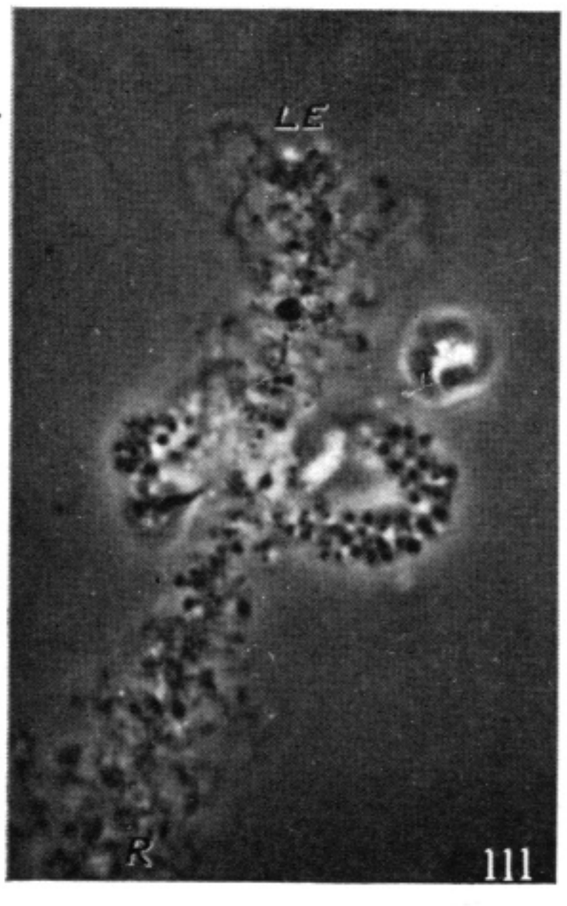
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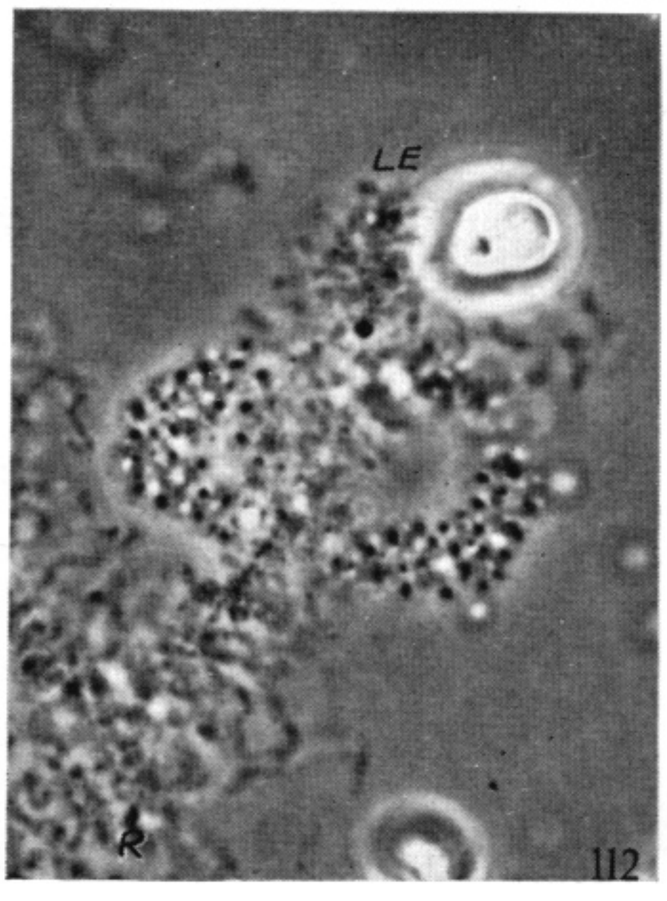
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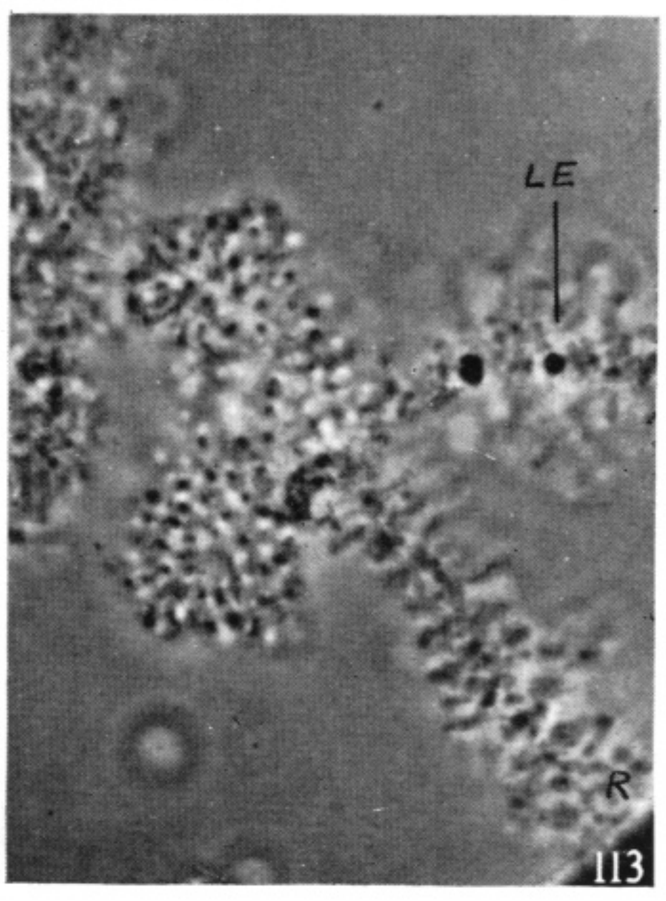
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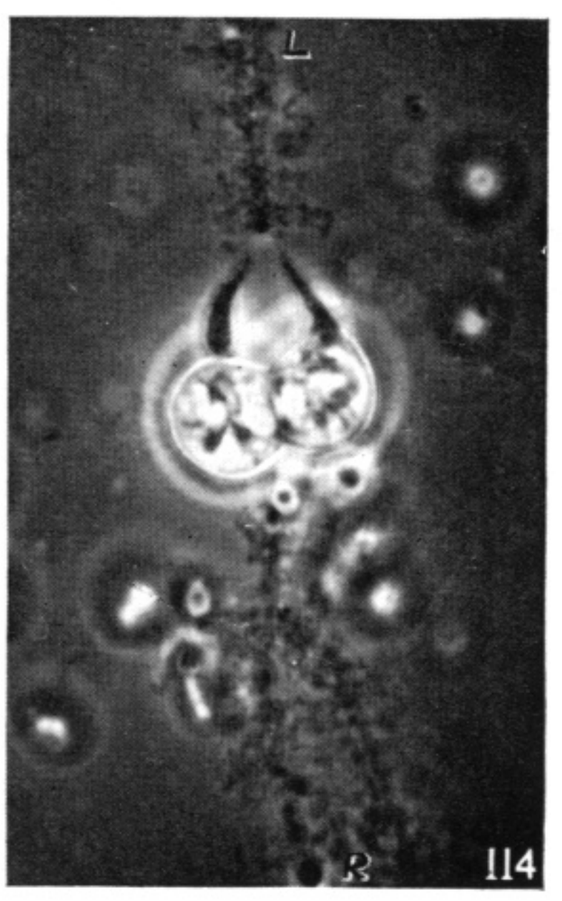
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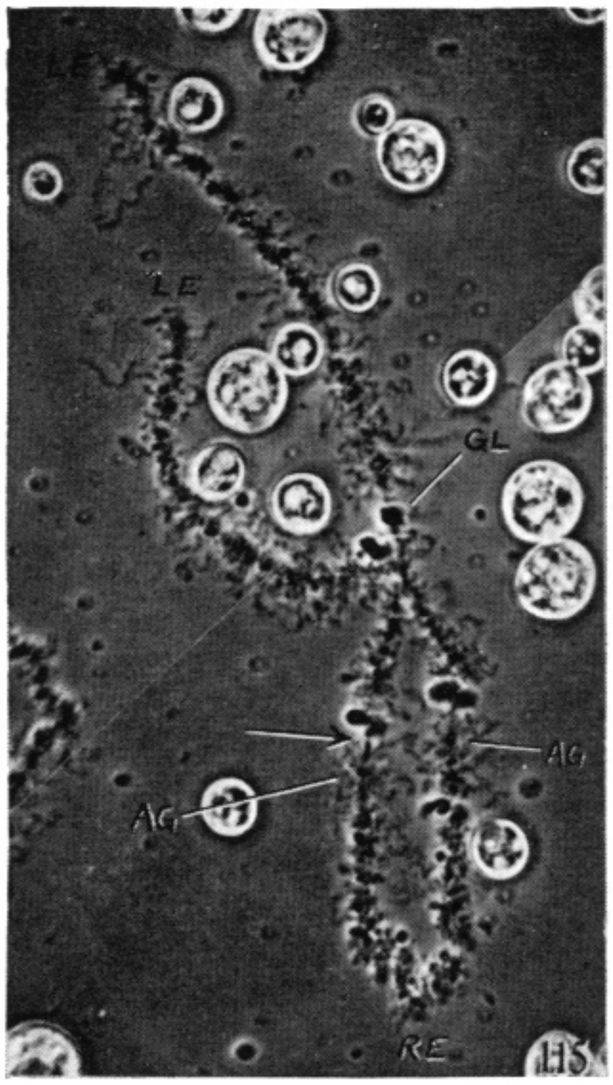
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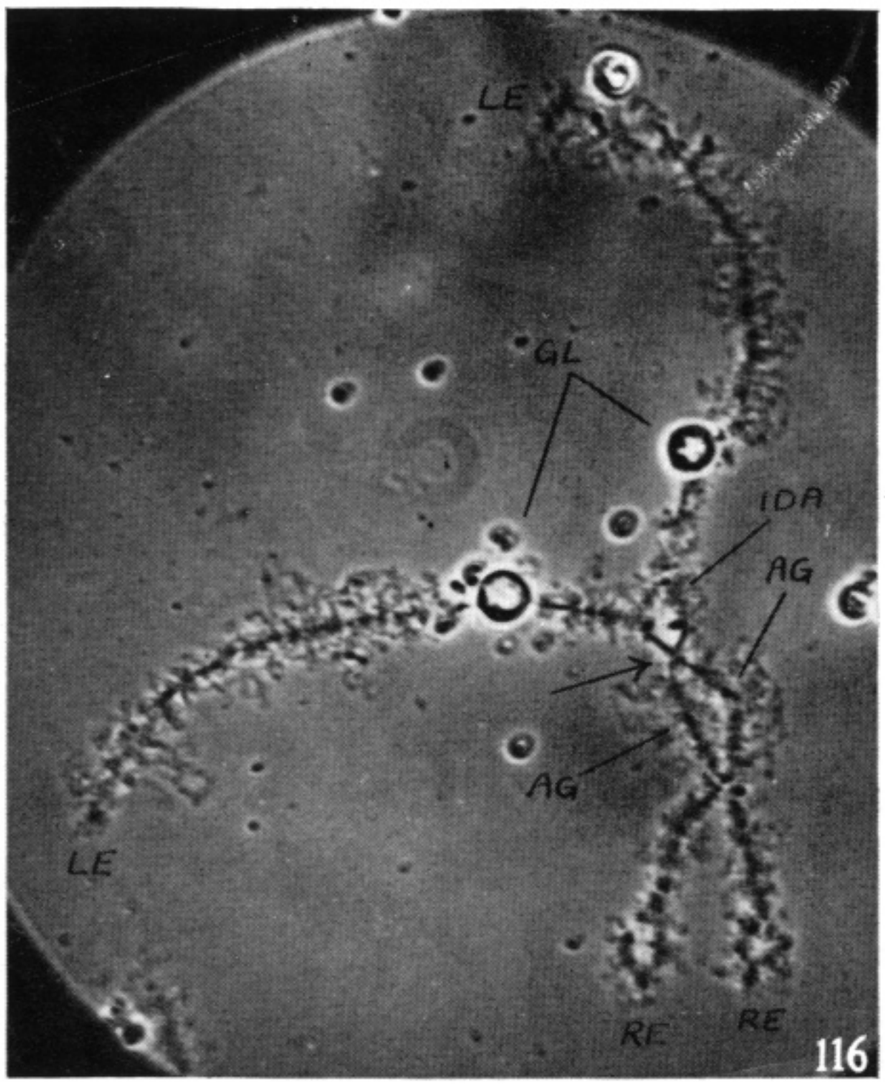
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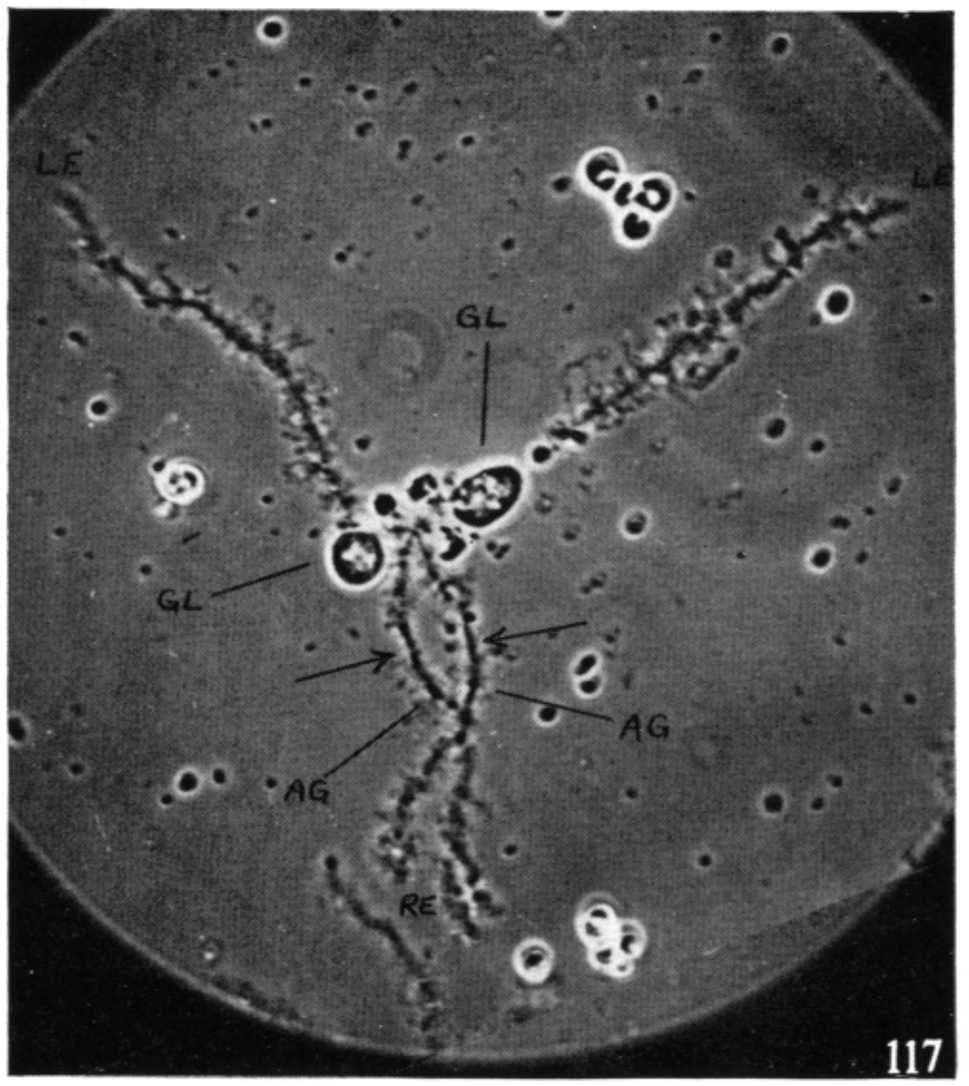
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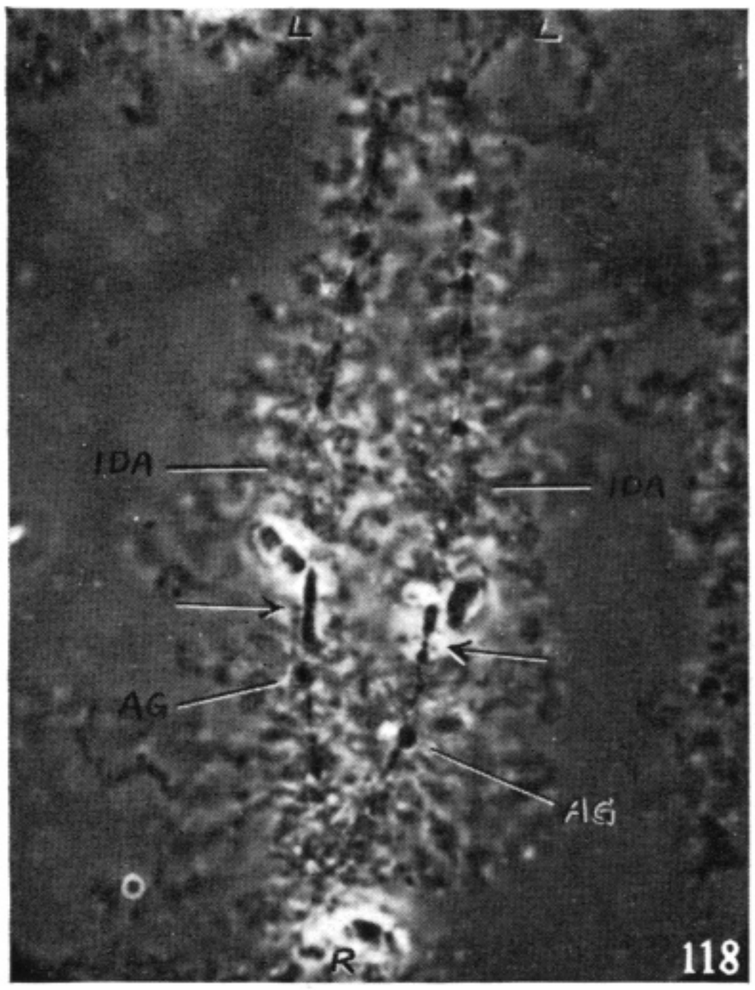
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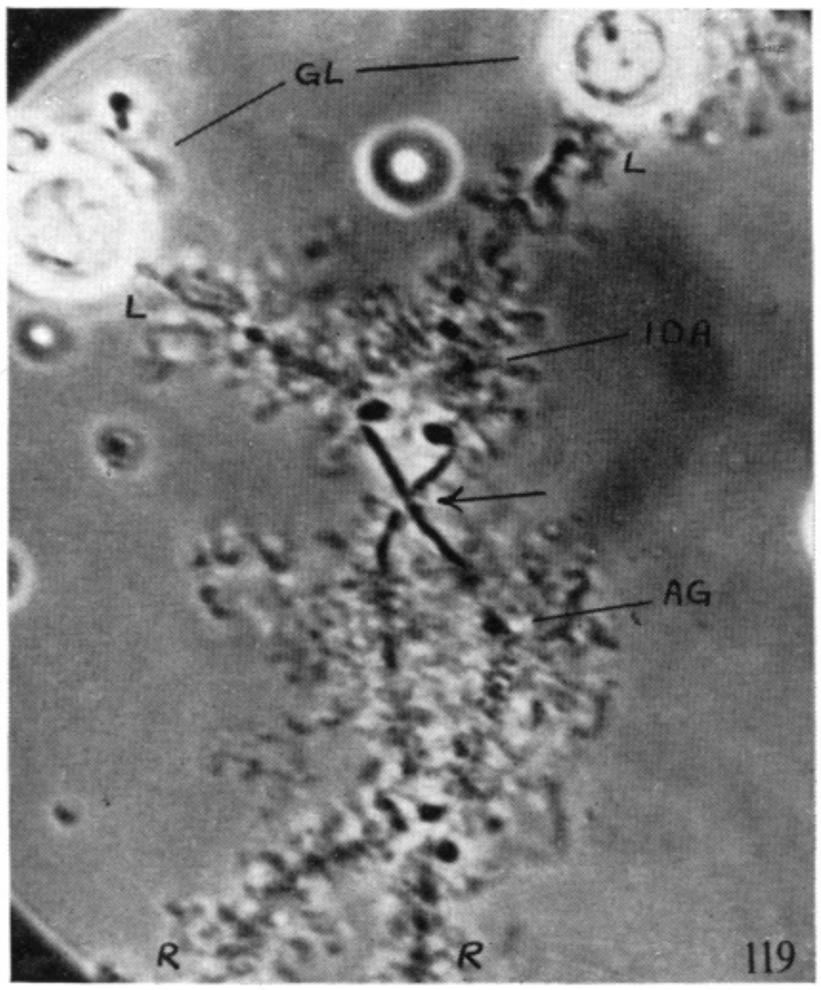
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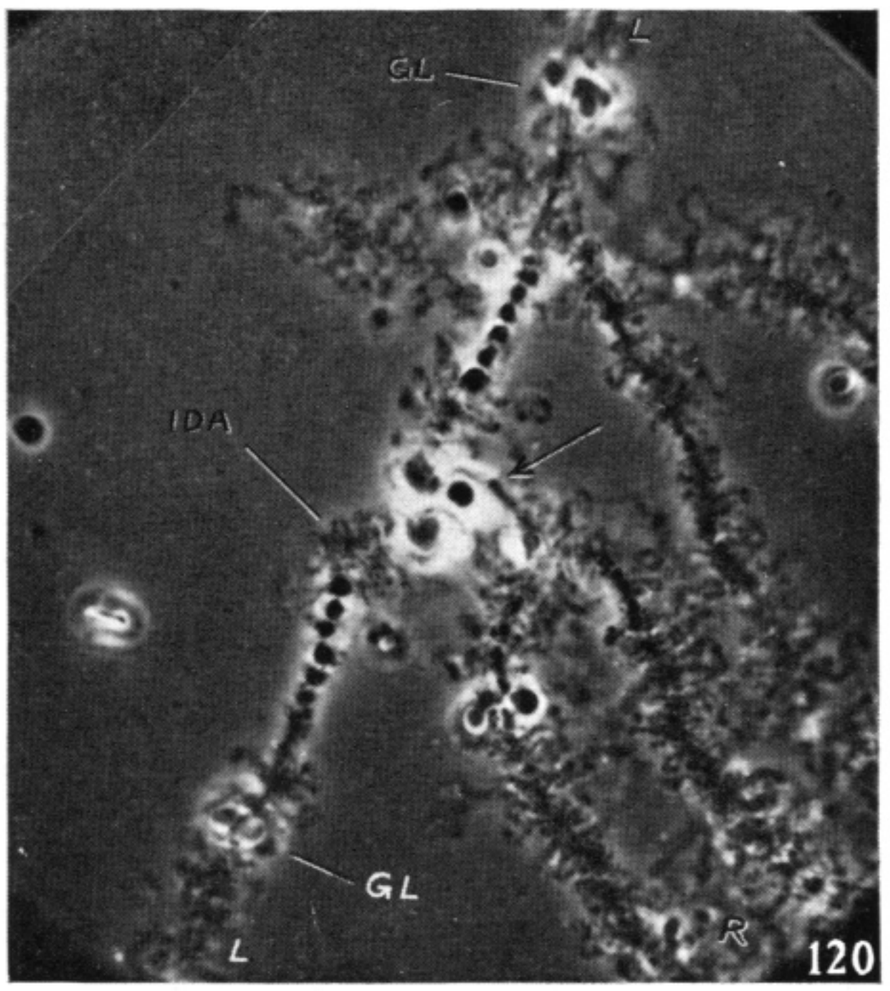
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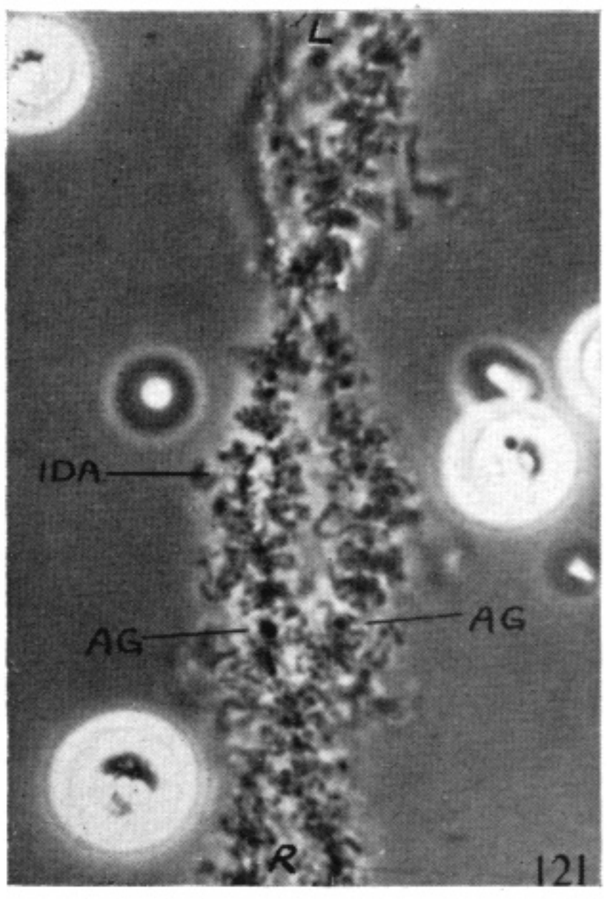
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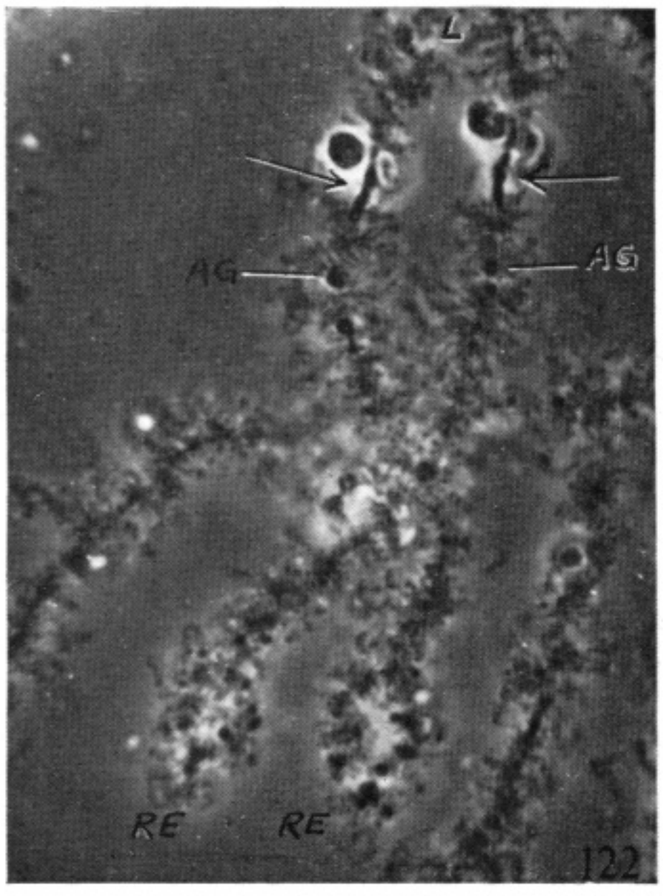
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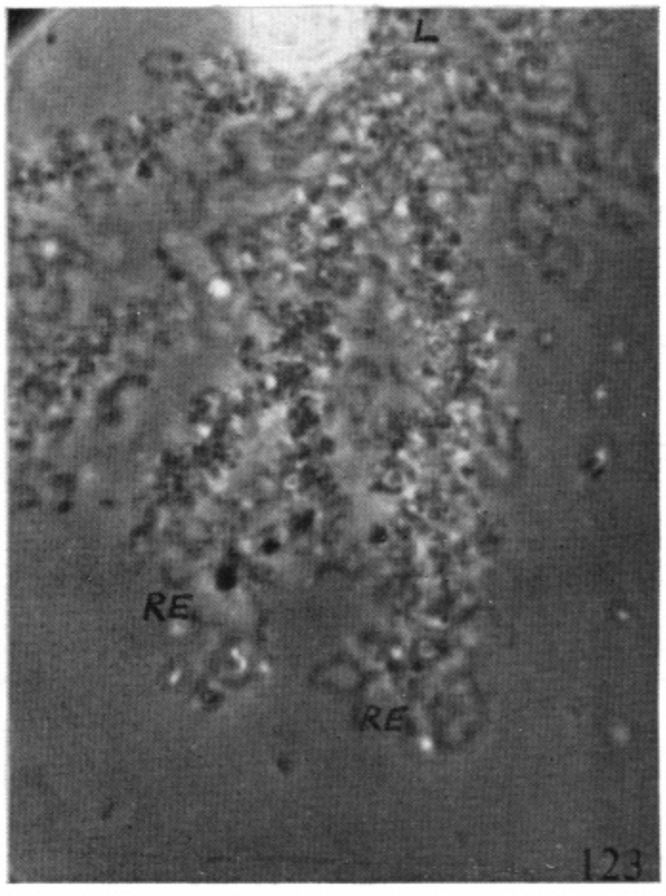
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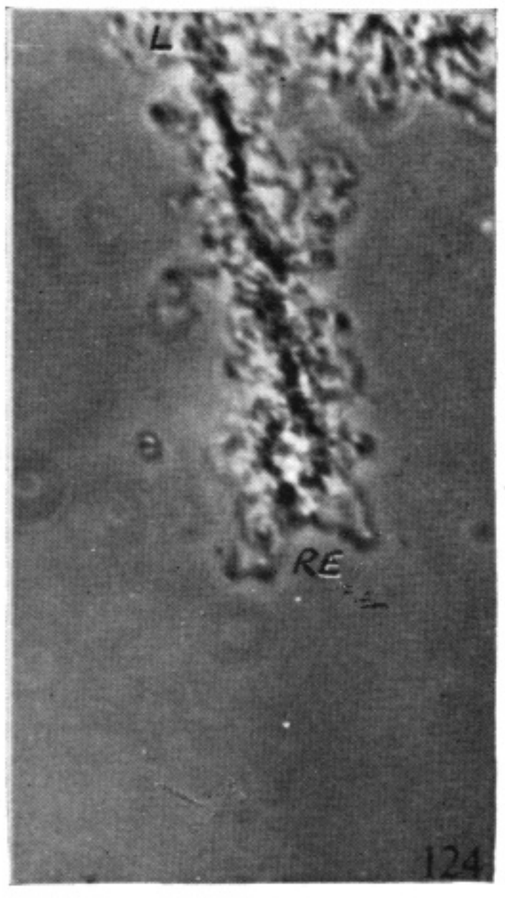
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