resistant cells. The cultures were rinsed and placed in media containing the tritium-labeled actinomycin D, and, as in the previous experiment, again only the sensitive cells were labeled in spite of the fact that marked alterations had occurred to the surface of the resistant cells.

Bacterial and mammalian cells which become resistant to purine analogs develop alternate pathways for the synthesis of essential purine metabolites. Development of resistance to amethopterin, an analog of folic acid, is in part due to the increase in folic acid reductase within the cell which then binds amethopterin (*3*). In both of these cases the development of resistance does not appear to alter the manner in which these analogs enter the cell. Actinomycin D was not incorporated into the nuclei of drug-resistant cells after damage to the cell membranes by treatment with hypertonic or hypotonic media. This may indicate that an active mechanism is necessary for the incorporation of the drug and that this mechanism is absent from the membranes of resistant cells. Another alternative is that the drug can enter the cytoplasm but is unable to enter the nuclei because of a change in permeability of the nuclear membranes of resistant cells. Subak-Sharpe (*4*) has reported that hamster cells which were resistant to actinomycin D were also more resistant to puromycin. Since actinomycin D is used as an antitumor agent, investigators should be aware of the change it can induce in the permeability of the limiting or nuclear membranes of surviving tumor cells, and of its effect on further chemotherapy by other antitumor agents.

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Chromosome Changes Induced by Infections in Tissues of Rhynchosciara angelae

Abstract. *The main effects of two infections, one by a protozoan and the other by a virus, in cells of* Rhynchosciara angelae (*Diptera, Sciaridae*) *are an increase in cell size and changes in the size, shape, and behavior of the chromosomes. The X chromosome of some cells reacts differently from the autosome to the protozoan infection. Some chromosomes show specific, easily traceable points after infection by the virus. Some of the effects of these infections may be similar to the effects of infective agents in other organisms.*

Diaz and Pavan have described two infections in the larvae of *Rhynchosciara angelae* (Diptera, Sciaridae) (*1*); these infections, one by a protozoan and the other by a DNA virus, induce hypertrophy of the affected cells and their chromosomes. We now describe two infections (probably the same as those described by Diaz and Pavan) recently found in several cultures of *R. angelae*.

In *R. angelae* most of the cells of the larvae and of the adults have polytene chromosomes. The increase in size of these multistranded elements after these infections is normally represented by an increase in the number of chromonemata that form each individual chromosome (*2*). In addition to this increase in diameter, some cells, after a certain stage of infection, show both an increase in the total length of the chromosome and specific changes in some of the chromosomal bands.

The polytene chromosomes of Diptera function as though they are in a permanent interphase, since they are normally very active and have a great part of their DNA in a distended phase. The relatively great size, the polytene structure, and the fact that these chromosomes occur in various tissues of the larva and of the adult of many species of Diptera permit observations on the behavior of these chromosomes and of specific chromosomal regions in different physiological stages of the organism as well as in different stages of development (*2, 3*). When either of the two infections occurs in cells of different tissues, with an accompanying increase in chromosome size, it is possible to observe the reaction of the whole chromosome or of individual bands to the infective agents.

The protozoan (probably a microsporidian) infects the cytoplasm and was found in cells of such tissues of the larvae and adults of *R. angelae* as salivary gland, intestine, muscle, fat body, and other glands (Fig. 1). This infection, when affecting only a few tissues, is not lethal to the larva or the adult. Frequently an infected larva is transformed into an adult whose morphology is apparently normal. Some infected larvae, however, give rise to phenocopies, many of which do not pass the beginning of metamorphosis. These abnormal developments may be due to an effect of the infected cells on the development of the embryonic cells or to an infection in some of the cells which would normally give rise to the organs.

In some infected cells (Fig. 1) the polytene chromosomes grow so that they are very similar to the salivary-gland chromosomes, which are frequently the largest polytene chromosomes of normal larvae. In other cases, however, the hypertrophied chromosomes induced by the infection (Fig. 2) are very different in shape from normal polytene chromosomes and resemble instead the peculiar chromosomes found in some cells of supposedly normal larvae of a Cecidomydae, *Lestodiplosis* sp. (*4*). In some cells infected by the protozoan, the X chromosome shows a greater increase in size than the other chromosomes of the same nucleus (Fig. 2). We still do not have measurements of the DNA content of these enlarged X chromosomes, but the pale pink color they show after Feulgen staining, when compared with the autosomes, suggests that the larger volume attained by the X chromosomes is due to a higher activity (production of RNA) of this chromosome induced by the infection.

In heavily infected cells, the surface of the chromosomes frequently has a coat of RNA, which shows strong incorporation of H^3-uridine from 20 to 40 minutes after the larva is injected. A rather disperse multibranched nucleolus can frequently be found in the base of the X chromosomes of in-

fected cells. Another peculiarity commonly found in the nucleus of infected cells is a partial or total asynapsis shown by some chromosomes. In some cases the abnormal pairings of the chromosomes in different cells of the same tissue are so alike that one is led to think that some of these unpairings may be related to the function of the chromosomes in these cells. In many cells these asynaptic chromosomes or regions of the chromosomes may have been present in the cells previous to the infection, as indicated by the unpairings of chromosomal sections observed in favorable cases in normal cells.

The infection caused by the virus seems to be nuclear and was found in several individuals of one group of larvae. Indications of different degrees of infection were found in cells of a pair of gastric caeca, which are attached near the crop of the larva, and also in cells of the distal region of the median intestine. The general appearance of the hypertrophied chromosomes of the cells of these two tissues is very similar (Fig. 3). They show very distinct bands, and only a few of them are puffed, which makes these chromosomes show a larger number of condensed bands than the corresponding chromosomes of cells infected by the protozoan, which normally show numerous puffs. This higher activity of the hypertrophied chromosome induced by the protozoan, when compared with the activity induced by the virus, holds only with respect to the production of RNA and not to the duplication of DNA. The multiplication of the chromonemata is very high in both infections (*5*). A multibranched nucleolus can be found in the base of the X chromosomes in many cells infected by the virus. There is one indication that the hypertrophied chromosomes induced by the virus infection have defects in their structure. In several cells, chromosome A showed two or three specific weak points which produced chromosomal breakage even in preparations where small pressure was applied to the cover slip (Fig. 3). These easily breakable regions were never found in chromosomes of normal cells or in cells infected by the protozoan. One of these weak points is located near or in a heterochromatic band of chromosome A. In cells infected by the virus we could not detect any difference between the behavior of the X chromosome and the behavior of the autosome as we had found in the cells infected by the protozoan.

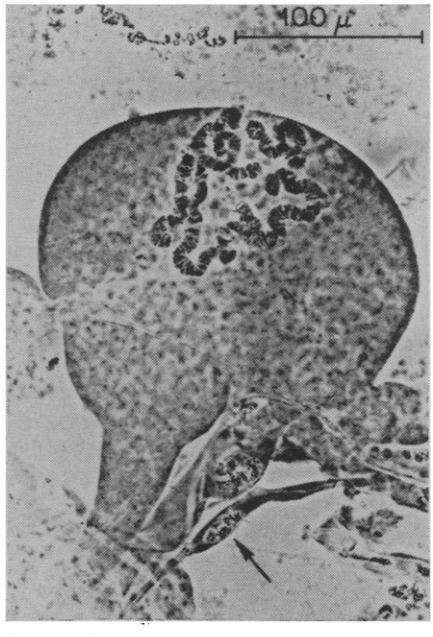

Fig. 1. Three cells of the visceral muscles which connect the gastric caeca and the median intestine of *R. angelae*. The cell at the bottom (indicated by the arrow) is normal, the one whose nucleus is above it is a little infected, and the larger one on the top of the other two is heavily infected with the protozoan. As no pressure on the coverslip was applied in this preparation, the protozoans in the cytoplasm of the infected cells can hardly be distinguished. For hypertrophied chromosomes of cells of the salivary gland, see Diaz and Pavan (*1*, Figs. 1 to 5).

In this virus infection, as in that described by Diaz and Pavan (*1*), after a certain stage of the infection the cell reacts by producing a great number of polyhedral crystals that characterize the infection.

The morphology and behavior of the polytene chromosomes of infected cells of *R. angelae* vary according to the type of infection, to the tissue to which the cell belongs, and also to the developmental stage of the organism. The protozoan develops and multiplies, destroying the cytoplasm of the cell; and the cell reacts with a higher activity in the nucleus in order, perhaps, to reconstruct what is being destroyed. We still do not know what part of the activity represents an enhancement of the normal activity and what part represents the particular reaction of the chromosomes to the change in the internal environment induced by the infective agent. Comparing the chromosomal behavior in infected cells of different tissues, one can see that in many cases similar puffs are induced in chromosomes of different cells, but also the activities of many chromosomal regions are peculiar to the infected cell of a particular tissue. This indicates that the protozoan, at least in the beginning of the infection, in many cases induces an increase in the normal activity of the cell and also leads to specific reactions that are common to cells of different tissues.

In the cells infected by the protozoan, the X chromosomes of both males and females show a higher activity than the autosome. This seems to indicate that, in the infected cells of *R. angelae*, the X chromosomes have different behavior from that shown by the X chromosomes of normal cells of several other organisms including man (*6*). The aberrant morphology of the chromosomes of some infected cells may be caused by differences in cellular activities, or may also

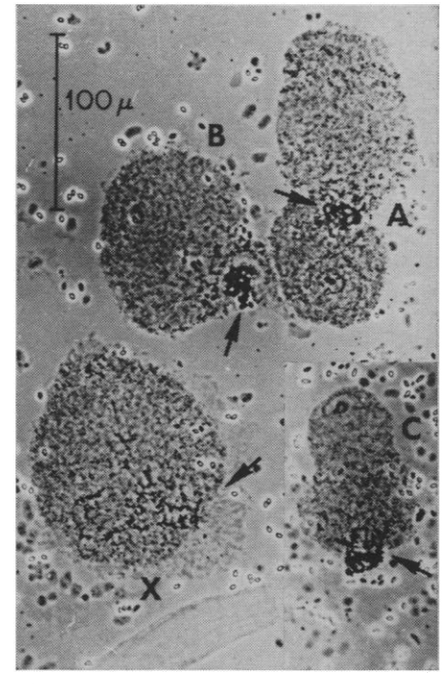

Fig. 2. Photomicrograph of the polytene chromosomes of an isolated cell of a larva of *R. angelae* infected by the protozoan (composite photomicrograph). In heavily infected larvae, several of these isolated cells can be found in different parts of the body, and practically all of these cells, when infected, have these pompon-like chromosomes. The letters indicate the chromosomes, and the arrows indicate the heterochromatic regions of the chromosomes. At the base of the X chromosome, indicated by the arrow, a fan-like nucleolus is present. The spotted bodies in the background represent the protozoa, which can be seen in various forms.

represent cases of chromosomal differentiation (*3*, *7*).

The virus-infected larvae frequently show clear symptoms of disease, and, although surviving for several weeks, they are unable to grow at normal rates. The virus seems to induce a higher metabolic activity, resulting mainly in an increase in the size of the chromosomes. As revealed by autoradiography, synthesis of DNA in the chromosomes is greatly enhanced after the infection. The presence of easily breakable points in some of the chromosomes is an indication that the multiplication of virus inside the cell interferes with synthesis of some chromosomal constituent. This is a point of interest, since in *R. angelae* the size and structure of chromosomes permit us to do some detailed work on the relation of the virus to the chromosome. This perhaps will help us to understand the deleterious effects of many viruses on mitotic chromosomes described in several papers dealing mainly with mammalian chromosomes (*8*). Paton *et al.*, and others as well (*9*), have described interesting cases of changes in morphology and behavior of chromosomes induced by mycoplasma in tissue cultures of human cells. Some of these results may be related to the virus-induced changes observed in the chromosomes of *R. angelae*.

Rhynchiosciara angelae was previously one of the most favorable organisms for study of the activity of chromosomes and specific chromosomal regions in differentiation (*2*, *3*). The present findings demonstrate that *R. angelae* is also uniquely suitable for studies on the response of chromosomes and genes to infective agents. It is the only known organism in which gene activities can be studied morphologically in at least eight different tissues. Probably many of the reactions of the chromosomes and genes of *R. angelae* to those two microorganisms are good indications of what occurs in many infections in other organisms.

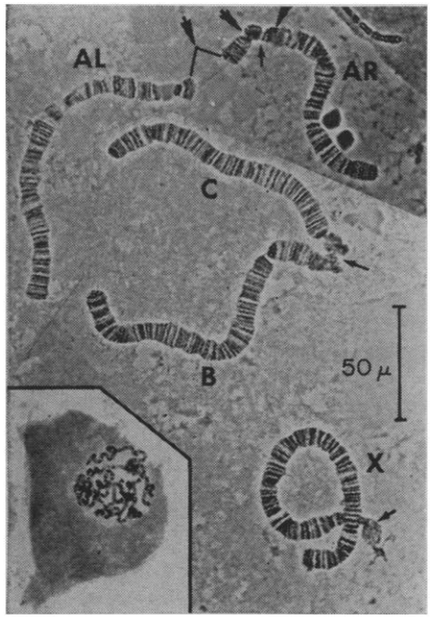

Fig. 3. The polytene chromosomes (A, B, C, and X) of a virus-infected cell from the gastric caecum (composite photomicrograph). *AR* and *AL* represent respectively the right and the left arms of chromosome A. The short arrows indicate the heterochromatic portions at the centromeric region of the chromosomes. At the base of the X chromosome, the nucleolus-organizing region is dispersed, a branched structure inside a dispersed nucleolus not visible in this picture being formed. The long arrows near the centromeric region of chromosome A indicate three easily breakable points frequently seen in chromosomes from cells infected by the virus. Inset: a normal cell of the same gastric caecum, at the same scale to show the increase in size of the chromosomes of infected cells.

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Dreaming Sleep in Man: Changes in Urine Volume and Osmolality

Abstract. *Epochs of dreaming sleep, as measured by rapid eye movements, consistently correlated with biphasic change in urine volume and osmolality in catheterized human subjects. Marked decrease in volume and increase in osmolality were followed by a hypotonic diuresis.*

Recent studies (*1*) demonstrating autonomic and respiratory changes associated with the rapid eye movement (REM) state during sleep prompted us to attempt to elucidate neuroendocrine correlates associated with this state, because subcortical systems influencing peripheral physiological events have been shown to act through the pituitary-adrenal axis (*2*); we studied urinine volume, osmolality, creatinine concentration, and steroid and catechol amine metabolites serially collected during all-night sleep in man. We now report a consistent urinary-volume correlate of the REM state and some findings that suggest the mechanism involved.

Seven male urology patients aged 45 to 74, whose renal function was normal and who were habituated to urinary catheters for several days, were studied for a total of 11 nights by means of conventional electroencephalographic, electromyographic, and electrooculographic recordings in a sound-attenuated, darkened room (*3*). The urinary catheters were led from the room into a volume-regulated fraction collector which permitted continuous collection of urine samples of uniform volume, the volumes being measured accurately after collection. The electrical recordings were scored for REM state without knowledge of the data from the urine studies, by use of Dement's criteria (*4*). The time of onset and duration of the REM periods were carefully determined. The filling of each tube in the fraction collector was time-locked to the electrophysiologic data by an event recorder fed into an amplifier of one channel of the electroencephalograph. Urinary creatinine was determined by the method of Jaffe (*5*). The osmolality of the urine samples was measured by freezing-point depression, by use of an Advanced osmometer.

All 41 REM-state epochs recorded during the 11 nights were associated with urine-volume responses of varying magnitude (Figs. 1 and 2). With some