The radiation doses were computed from the known activity of the tritium, the mean energy of its beta-radiation (5.7 keV[6]) and the duration of the experiment. It was 29.2 rad per day for 1 mc/10 ml. The range of the tritium beta-rays is so small that the loss of energy in the walls of the flasks can be neglected. On the other hand, it is large enough to provide a practically uniform radiation dose over the whole of the system of cells + medium, whatever the distribution of the tritium within the cells[6]. The contribution of the radiocarbon to the dose is negligible. In Table I the cell counts, and in Table II the radiocarbon contents of the protein are given as functions of amount of tritium and irradiation time.

It is seen that the cell count is significantly affected by irradiation at the level of 5 mc/10 ml. However, the incorporation of radioleucine, i.e. the synthesis of protein, is noticeably depressed only at the 50 mc/10 ml level. Because of the higher sensitivity to radiation of cell count compared with protein production, the average amount of protein synthesized per cell shows an increase with increasing dose (Table III). The cell numbers used for calculating Table III are not the numbers at the end of the relevant time intervals, but calculated average numbers during these intervals.

It has been shown before, both by colony counting[7] and by cell counting[8], that cells in culture are remarkably sensitive to radiation in respect of their capacity for division. The metabolism of the cells is affected much less[7,9]. Our result, that protein synthesis goes on with little change while cell division is largely inhibited, is in agreement with these earlier findings. The results suggest that the suppression of antibody formation by radiation occurs by inhibition of formation of active cells rather than by inhibition of protein synthesis by the competent cells[10].

Table III. Mean protein activity per cell (relative values)

Activity of tritium (mc/10 ml)	After 24 h		After 48 h	
	soluble	insoluble	soluble	insoluble
0	100.0 ± 8.0	100.0 ± 9.0	100.0 ± 10.6	100.0 ± 11.6
0.5	98.8 ± 14.1	98.6 ± 10.0	92.7 ± 11.2	105.0 ± 15.1
50	107.6 ± 10.0	111.5 ± 11.2	107.0 ± 11.1	117.0 ± 10.9
5	105.5 ± 11.3	121.2 ± 12.6	122.0 ± 19.0	146.0 ± 25.8

Zusammenfassung. Bei der Züchtung von HeLa-Krebszellen in tritiumhaltiger Nährlösung während 48 h wurde festgestellt, dass die Mitoserate durch viel kleinere Strahlendosen nachweisbar herabgesetzt wird als die radiochemisch gemessene Rate der Proteinsynthese.

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A Demonstrable Local and Geometric Increase in the Chromosomal DNA of *Chironomus*

Investigations on the incorporation of H^3-thymidine in the salivary gland chromosomes of *Chironomus thummi* have shown that local DNA synthesis begins approximately simultaneously in all bands, and that thick, DNA-rich bands require somewhat more time to complete replication than do thin ones with less DNA content[1]. These autoradiographic findings were confirmed by microspectrophotometric measurements of DNA content in salivary gland chromosomes of different size classes[2]. These facts are understandable if it is assumed that the bands enclose sections of the chromatids in which DNA synthesis proceeds independently of one another. From similar observations of H^3-thymidine autoradiography, Taylor[3] concluded that long mitotic chromosomes are composed of many such subunits.

Keyl and Pelling[1] took advantage of the special situation in the salivary gland chromosomes of hybrids between the two subspecies, *Ch. th. thummi* and *Ch. th. piger*, viz. the occurrence of structurally different, unpaired regions, to study the relationship between the amount of DNA and the duration of DNA synthesis. Within the unpaired segments, homologous bands occur in precisely the same sequence as in paired segments. Nonetheless, the homologous chromosomes can be distinguished from one another because of distinct differences in their dimensions[4,5]. The chromosomes of *Ch. th. thummi* possess numerous bands whose appearance suggests that they contain a larger

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amount of DNA than those of *Ch. th. piger* (Figure 1). While the duration of DNA synthesis, as measured by the thymidine incorporation, was longer in bands of higher DNA content, a quantitative measure of this difference was not possible.

However, by employing the methods indicated below, precise measurements of DNA content were obtained. These results permit an insight into the neglected type of localized DNA differentiation which occurs in chromosome evolution.

Microspectrophotometric measurements of the DNA amounts of homologous bands of salivary gland chromosomes in hybrids of *Ch. th. thummi* and *Ch. th. piger* were made on Feulgen stained preparation employing a Zeiss microspectrophotometer (UMSP). By this technique a comparison of extinctions can be made only within individual preparation. Separate preparations can be compared using extinction ratios.

Until now 30 bands of thummi-piger hybrids manifesting subspecific differences in DNA amount have been measured[6]. The differences follow a simple relationship. The ratio of extinction values (E) of two homologous bands (E *th*/E *pi*) always fall into a doubling series, i.e. a specific band of *Ch. th. thummi* contains 2, 4, 8, or 16 times more DNA than does the homologous band of *Ch. th. piger*. It should be emphasized that the homology between bands in the unpaired sections is made on a morphological basis[5]. Uncertainties concerning band homology arise only in those chromosome sections with numerous and large local structural differences. The duplication hypothesis was supported by the additional finding that within and between populations of the subspecies *th. thummi* differences in the DNA amount of the same band also fall into a doubling series. In this case no problem of homology comparable to that in thummi-piger hybrids occurs and the bands of *Ch. th. piger* whose DNA amount is constant serve as reference values.

The Table includes the results of microspectrophotometric measurements (at 546 mμ) of the Feulgen stained band b 3/11 in the third chromosome of thummi-piger hybrids. Hybrids were obtained by crossing *Ch. th. piger* from mass culture S with *Ch. th. thummi* from mass culture A/62 and from two natural populations of Northern Germany, Altenau and Vienenburg. The extinction ratios (E *th*/E *pi*) in the Table demonstrate that the DNA content of the particular band b 3/11 of *Ch. th. thummi* falls into four discrete classes, 2, 4, 8, or 16 times that of the homologous band in *Ch. th. piger* or alternatively 2, 4, or 8 times greater than the lowest value found for *Ch. th. thummi*. Within the *Ch. th. thummi* strain A/62 and Vienenburg two types of bands were found whose DNA content was in the ratio 1:2. Correspondingly, in the Altenau strain two types also occurred whose DNA was in the ratio 1:4. Among individuals of all three strains it was possible to demonstrate that these types of bands occur as natural structural heterozygotes. Within *Ch. th. thummi* these heterozygotes are identifiable only by the difference in size of the halves of a band. DNA measurements could not be effectively made, due to the too intimate pairing of the homologous chromosomes.

Fig. 1. A structurally different section of salivary gland chromosome II in the hybrid *Chironomus th. thummi* × *Ch. th. piger*.

Two facts should be borne in mind when discussing the results. (1) The differences in total DNA content of salivary gland nuclei between the two subspecies has also been demonstrated for the spermatocytes[7]. This means that the difference in DNA content is not a consequence of polyteny. (2) From a cytological investigation of 20 Chironomus species closely related to *Ch. thummi*, it was concluded that the banding pattern of *Ch. th. piger* is phylogenetically older than that of *Ch. th. thummi*[8,9]. This means that the difference in DNA content of the homologous bands is caused by an increase in the DNA of *Ch. th. thummi*.

The fact that the DNA content of specific bands falls into a geometric series (1:2:4:8:16) suggests that the bands of higher DNA content arose by a doubling of the DNA of bands of lower content.

The mechanism of the DNA increase in specific bands is not yet clear. It might be imagined that the initial doubling of DNA in a band arose by unequal crossing-over, resulting in a direct duplication and therefore a doubling of the DNA content. However, subsequent unequal crossing-over in the homozygous duplication should

Integrated extinctions of the band 3/11 of salivary gland chromosome III of *Chironomus th. thummi–th. piger* hybrids

Crosses	Sources *th. thummi*	Integrated extinctions (means of 5 measurements of the same band) *th. thummi*	*th. piger*	Ratio E *th*/E *pi*
♀ *th.* × ♂ *pi.*	A/62	35.6	18.4	1.93
♂ *th.* × ♀ *pi.*	A/62	25.4	12.4	2.04
♀ *th.* × ♂ *pi.*	A/62	24.4	11.8	2.06
♀ *th.* × ♂ *pi.*	A/62	24.8	12.8	1.93
♂ *th.* × ♀ *pi.*	A/62	21.2	10.2	2.07
♂ *th.* × ♀ *pi.*	Altenau	37.4	17.6	2.12
♂ *th.* × ♀ *pi.*	Altenau	57.6	27.6	2.08
♂ *th.* × ♀ *pi.*	A/62	41.0	10.0	4.10
♂ *th.* × ♀ *pi.*	A/62	40.4	10.0	4.04
♀ *th.* × ♂ *pi.*	A/62	44.0	11.6	3.79
♀ *th.* × ♂ *pi.*	Altenau	146.2	18.0	8.12
♂ *th.* × ♀ *pi.*	Altenau	60.4	7.6	7.94
♂ *th.* × ♀ *pi.*	Altenau	143.8	17.6	8.17
♂ *th.* × ♀ *pi.*	Altenau	155.0	18.8	8.24
♂ *th.* × ♀ *pi.*	Vienenburg	119.0	15.0	7.93
♂ *th.* × ♀ *pi.*	Vienenburg	506.6	31.4	16.13
♂ *th.* × ♀ *pi.*	Vienenburg	394.2	24.6	16.02

[6] Manuscript in preparation.
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[8] H.-G. KEYL, Verh. Dtsch. zool. Ges. (1960), p. 280.
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also produce a triplication, or a DNA content of three times that of the original band. The fact that the DNA content follows the ratio 1:2:4:8:16 strictly, and that intermediate values expected on the basis of unequal crossing-over were never found, suggests that this mechanism is inadequate to explain the higher DNA amounts. A more reasonable explanation might be based on disturbances in DNA replication. In this connection it is important that in several Chironomus species instances have been observed where an increase in DNA amount of an individual band occurs in association with specific inversions[10–12]. In each of these cases the increase is found in those bands located at one breakage-point of the inversion. Such an increase was also found in association with an X-ray induced transposition in *Ch. th. thummi* in a band adjacent to one breakage-point[5]. It is therefore conceivable that spontaneous and induced chromosome breaks can cause a localized increase in the DNA amount. As mechanism for such a DNA increase, the following possibilities are imaginable: (1) local failure in the separation of the replicated DNA strand, (2) localized stimulation of DNA synthesis limited to a replication unit (= band).

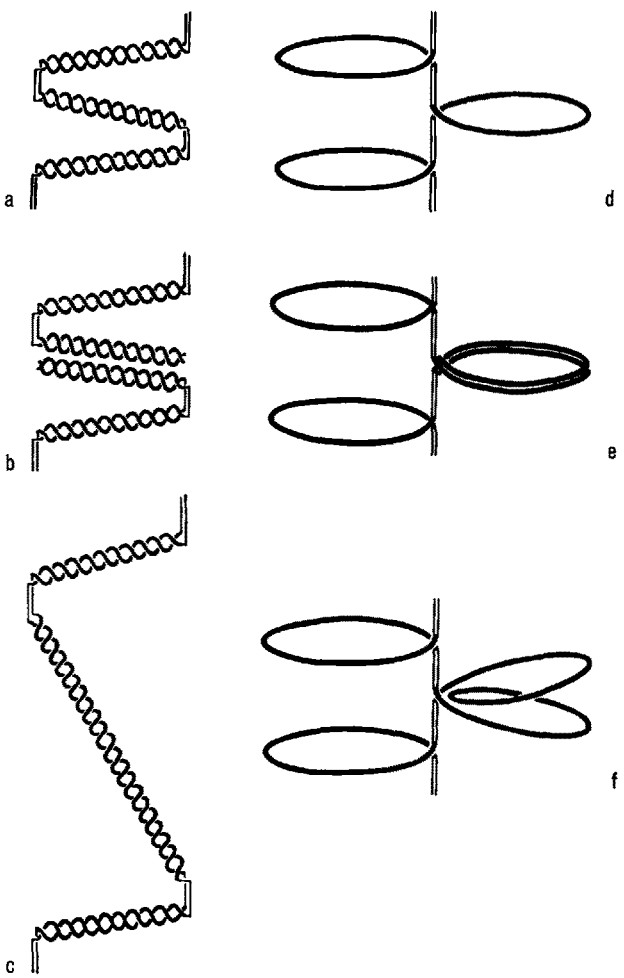

Fig. 2. Schematic representation of the doubling in the DNA amount of a replication unit. a–c According to Taylor's model (somewhat simplified), d–f according to model of ring-like replication units (see text for details). Thick lines in d–f represent double helices as shown in a–c.

Decisive for the concept of the structural and genetic consequences of localized DNA increase is the interrelationship between this increase and the duration of DNA synthesis in the bands. It has been shown that in *Ch. th. thummi* not only do the bands rich in DNA require more time than their homologues in *Ch. th. piger*, but also within *Ch. th. thummi*, whose third chromosome bands b 3/11 of higher DNA amount require a significantly longer time to replicate than do those of lesser DNA amount[1].

The prolongation of replication time can be explained by the direct correlation between increase in DNA amount and increase in length of the replication unit. This assumption appears more plausible than one which assumes that the duration of DNA synthesis depends upon a limited pool of DNA precursors.

The frequency and precision of structural differences in association with a regular, step-wise doubling of the DNA amount permits the assumption that this is not a chance process. Unfortunately the arrangement of the DNA molecules in the chromosomes is not yet fully understood, so that only a tentative model of the doubling process in the bands is possible. TAYLOR[3] has developed a chromosome model which takes into account certain cytological facts. In this model chromatids consist of a series of replication units (replicons[13]) attached together by linkers of unknown nature (Figure 2a). On the basis of this model an increase in the length of the replication unit can occur if, after a DNA replication limited to this unit, the two parts become attached end-to-end as shown in Figure 2 b, c. The processes which bring about the progressive lengthening in a 1:2 ratio of the replication unit could perhaps be more easily understood by assuming an alternative mechanism which more readily explains the progressive nature of the duplication process than does TAYLOR's model. This alternative mechanism assumes that the replication units are organized into rings or ring-like DNA structures (Figure 2d). From the diagram (Figure 2 e, f) it can be easily seen how the end-to-end attachment of two identical rings can be accomplished without essential changes in their basic relationship. These relationships apply to all subsequent steps in the doubling of the DNA amount.

Zusammenfassung. Mikrospektrophotometrische Messungen an den Speicheldrüsenchromosomen des Bastards zweier *Chironomus thummi*-Unterarten ergeben Differenzen des DNS-Gehalts von homologen Querscheiben. Diese Differenzen folgen der Beziehung $1:2^n$ (n = 1, 2, 3 oder 4). Für bestimmte Querscheiben wurden Verdopplungsstufen des DNS-Gehalts von unterschiedlicher Höhe gefunden. Mechanismen, die geeignet sind, das Duplikationsphänomen zu erklären, werden diskutiert.

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