of riboflavin, lumiflavin and second UV-maximum of lumichrome reflect transitions between these orbitals. The fact that the calculated value for the position near UV-maximum of lumiflavin is of lower frequency than measured experimentally is most probably caused by using riboflavin molecular orbital energies (these two compounds have not exactly the same spectra). An opposite irregularity, however in higher degree, is observed for lumichrome second UV-maximum. In both cases, the effect of specific interaction with dioxane cannot be excluded. In addition, positions of considered maxima of both compounds are slightly over the limits permitted for such calculations[6]. In the solvent used, measured positions of longest wavelength maxima of two of the compounds considered are shifted a little towards shorter wavelengths. Positions of this maximum calculated from energies of highest filled and lowest empty molecular orbitals are not exactly the same (Table).

Zusammenfassung. Der Einfluss organischer Lösungsmittel auf die Erhöhung der Lichtempfindlichkeit und auf die Formen der Absorptionskurven von Riboflavin, Lumiflavin und Lumichrom wurde untersucht; dabei wird eine deutliche Übereinstimmung der zwei längstwelligen Maxima-Positionen in Dioxanlösung und der theoretisch berechneten Positionen festgestellt.

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Influence of Ionizing Radiation on Protein Production by HeLa Cells in Culture

The production of proteins by HeLa human carcinoma cells in culture has been studied by radiochemical methods. The cells were incubated in a medium containing ^{14}C-leucine, the soluble proteins formed were separated into fractions by physico-chemical and immunological methods, these fractions were burned, and their radioactivity estimated with the gas counter[1-3].

We have now examined, by the radiochemical method, the influence of ionizing radiation on the synthesis of (soluble and insoluble) proteins by HeLa. The question of this influence is of interest, among other reasons, in view of the well-known inhibition of antibody formation by irradiation of whole animals. Additional information on the mechanisms responsible on the level of the cell is desirable.

The cells were grown in flat-bottomed flasks as monolayers in 10 ml of a medium consisting of Gey solution, human cord serum and hydrolyzed lactalbumin. 0.5 μc D,L-radioleucine were added as a precursor of protein. For irradiation, the medium in the flasks contained various quantities of tritiated water. Radioincubation lasted either 24 or 48 h. Thereafter, the cells were taken off the wall of the flask with complexon, and an aliquot part of the cells was counted in the hemocytometer. The cell suspension was then used to determine the newly synthesized protein, i.e. the protein containing radiocarbon. In a number of parallel flasks, cell counts were made for the time of the start of the irradiation.

To isolate the soluble proteins, cells and medium were centrifuged at low speed (giving supernatant I), the cells broken by freezing, and the fragments separated by centrifugation (giving supernatant II) and washed with NaCl. The supernatants I and II and the wash solutions were combined, inactive leucine was introduced as a hold-back carrier, trichloroacetic acid (TCA) was added, the precipitate freed from the solution by centrifugation and thorough washing with TCA, burned, and the radioactivity of the CO_2 measured with the gas counter[4,5]. The cell debris, containing the insoluble proteins, were dissolved in warm *n* NaOH, aliquots of these solutions were neutralized with HCl, burned, and measured independently. All values in the Tables are mean values from 2 or 3 flasks; the errors are standard errors.

Table I. Cell counts per flask ($\cdot 10^{-6}$)

Activity of tritium (mc/10 ml)	Start	After 24 h	After 48 h
0	3.25 ± 0.15	4.61 ± 0.40	5.94 ± 0.39
0.5	3.25 ± 0.15	4.28 ± 0.47	6.06 ± 0.54
5	3.25 ± 0.15	3.88 ± 0.23	5.01 ± 0.53
50	3.25 ± 0.15	3.17 ± 0.31	2.61 ± 0.37

Table II. Protein production per flask

Activity of tritium (mc/10 ml)	Activity of protein (^{14}C) (dpm)			
	After 24 h		After 48 h	
	soluble	insoluble	soluble	insoluble
0	9680 ± 570	11620 ± 840	17140 ± 1280	20300 ± 570
0.5	9130 ± 1160	10980 ± 850	15390 ± 1020	20680 ± 2020
5	9430 ± 790	11720 ± 1080	15900 ± 1110	20670 ± 1050
50	8340 ± 780	11510 ± 1000	13890 ± 1860	19680 ± 2950

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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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