W. POHLIT
Max Planck - Institut für Biophysik, Francoforte - Germania

Cybernetic problems in radiation effects in living cells

Radiation effects in tumor cells embedded in normal living tissue of an organism are very complicated processes. Radiation effects in single cell cultures are much simpler, but still complicated enough to show some of the basic and the most important reactions of irradiated tissue. For single cells there exist some very interesting and exact experiments especially those of Elkind and Sutton (1960), and it should be possible to explain the results of these experiments by means of well known radiation effects on both the chemical molecules in solutions, and the fundamental processes in living cells.

Therefore, in the first part a simple model for radiation effects in solutions is developed by the combination of two basic reaction possibilities and this model is used for the interpretation of dose effect relations for single cells. The second part deals with the basic cybernetic processes in cells. These are estimated quantitatively and are used to explain the behaviour of cells in recovery processes or during later irradiation.

1) Radical reactions in solutions and dose effect relations

When an aqueous solution is irradiated, radicals are produced and these radicals may react in three ways, as shown in Fig. 1:

a) recombination with each other to form normal water;

b) reaction with susceptible atoms or molecules and;

c) recombination forming active molecular products like H_2O_2.

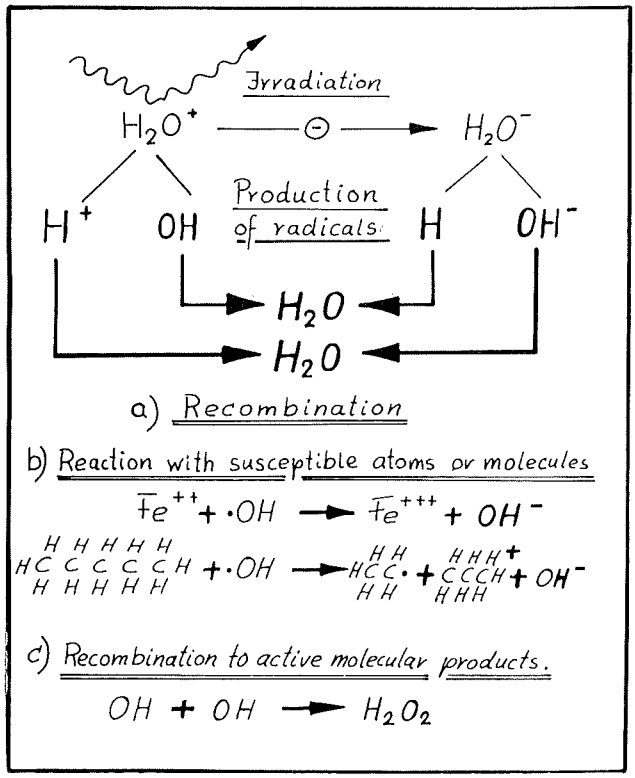

Fig. 1 · *Radiation reactions in solutions*

If the irradiation is carried out with weak ionizing radiation like x- or gamma-rays, as in the experiments of Elkind and Sutton, the third possibility can be neglected, since it is of minor importance. Because of the recombination effect, a radical has a given mean life span during which it can react. In other words, it can diffuse only a certain mean distance, « r », through the solution before it recombines (Fig. 2). Equally, one can surround the susceptible atom with a sphere v of a radius r, with the condition that a radical will react on the average with the atom or molecule, only if it is produced inside the sphere. If it was produced outside the sphere it will recombine with another radical. This recombination takes place only with radicals from the track of the same particle, so no dependence on dose rate is present.

If the susceptible molecules « A » are so highly concen-

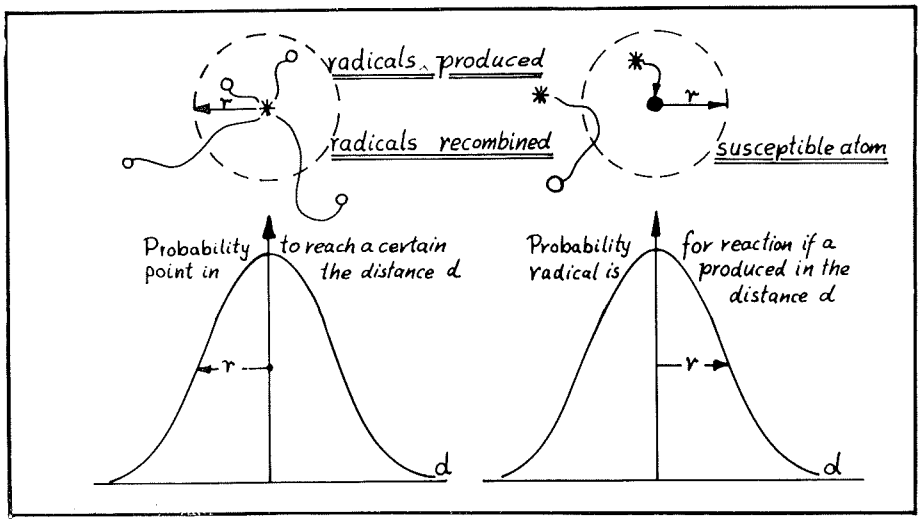

Fig. 2 - *Diffusion of radicals and reaction probability*

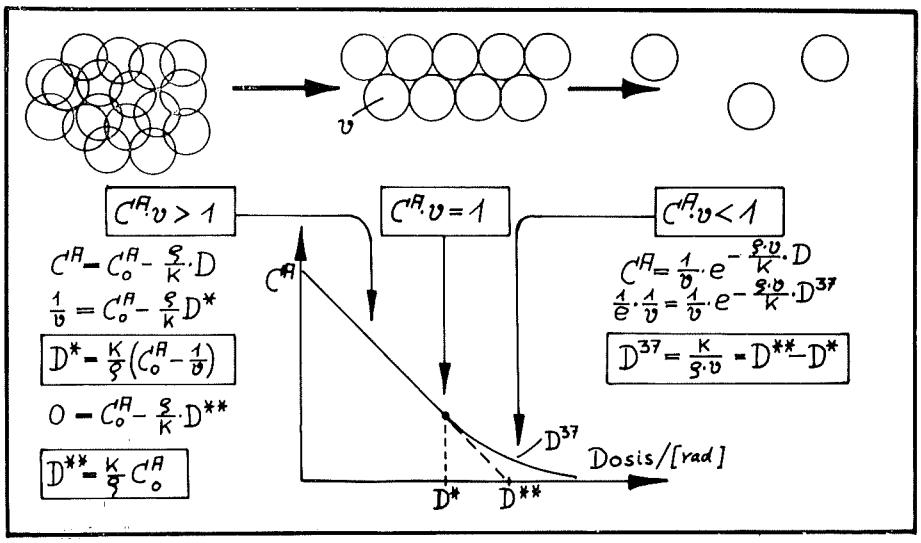

Fig. 3 - *Radiation effects at different concentrations*

trated in the solution (see Fig. 3) that the single spheres v around each molecule « A » overlap, then each radical produced by the radiation will reach a molecule « A » and lead to a chemical reaction of molecule « A » into another form

« B ». This means that ΔC^A, the decrease of the concentration of « A », is proportional to the absorbed dose ΔD:

$$\Delta C^A = - \frac{\rho}{k} \cdot \Delta D$$

ρ is the density of the irradiated medium composed of the solvent and the susceptible molecules, and k is the energy necessary to convert a molecule « A » into the form « B » by irradiations. A linear dose effect relation is the result:

$$C^A = C^A_o - \frac{\rho}{k} \cdot D,$$

where C^A_o is the initial concentration of « A » before irradiation. During irradiation the concentration of « A » decreases and after a certain absorbed dose, D^x, the volumes v just touch each other. In this case, the concentration C^A is 1/v or $C^A \cdot v = 1$. By further irradiation more and more of the produced radicals are lost by recombination between the spheres v. The resulting decrease of the concentration of « A » is proportional not only to the absorbed dose D, but also to the concentration C^A:

$$\Delta C^A = - C^A \frac{\rho \cdot v}{k} \cdot \Delta D$$

An exponential dose effect relation is the result in this region ($C^A \cdot v < 1$):

$$C^A = \frac{1}{v} e^{- \frac{\rho \cdot v}{k} \cdot D}$$

The total dose effect curve is composed of two parts: a linear portion for $C^A \cdot v > 1$, with the characteristic slope given by ρ/k; and an exponential portion, characterized by a D^{37} of $k/\rho \cdot v$. From both parts, it is possible to determine the two quantities of interest: the energy k, necessary for the reaction, and the volume v due to the radicals involved. Beyond this there exists a check of such a dose effect curve by the determination of $D^{37} = k/\rho \cdot v$ from the absorbed dose D^x and the absorbed dose D^{xx} where the linear portion would strike the axis:

From $C^A = \frac{1}{v} = C^A_o - \frac{\rho}{k} D^x$ it follows:

$$D^x = \frac{k}{\rho} (C^A_o - 1/v)$$

$$\text{and from } O = C^A_o - \frac{\rho}{k} D^{xx} \quad : \quad D^{xx} = \frac{k}{\rho} \cdot C^A_o$$

$$\text{Therefore } D^{xx} - D^x = \frac{k}{\rho \cdot v} = D^{37}$$

This additional test can be used to see if the inspected reaction is in agreement with the used model. This test will be used later in this paper.

In Fig. 3 such dose effect curves are drawn for different initial concentrations. The scale for the concentrations is normalized to v, and the scale for the absorbed dose is normalized to D^{37}. In the upper part, one can see that if the concentration $C^A = 1/v$ is reached, the linear relation always proceeds exponentially.

If one draws these curves on a semilogarithmic scale, the exponential parts yield straight lines and the linear parts yield curves. Together both parts produce the so called « shoulder curve », well known from many radiobiological experiments.

Very often such curves are normalized to $C^A/C^A_o = 1$, since only this relative concentration is known, as shown in the lower part of Fig. 4. One can see from the comparison of these curves that there is no reason to speak of a lower « radiation sensitivity » in this case. An example can be found in the comparison of curves 4 and one in Fig. 4. The difference is due only to different concentrations of the susceptible molecules « A » before irradiation.

This is an important fact for the explanation of the recovery process in cells later in this paper. As an example, in Fig. 5 and 6, two of the several experimental results of Elkind and Sutton (1960) are drawn in linear coordinates to test, if the « shoulder » in the semilogarithmic plot is a linear dose effect relation. This is the case within the small experimental error of these investigations. The additional test to see if $D^{xx} - D^x$ from the linear portion is equal to the D^{37} of the exponential portion was also made, and showed a good agreement in these two (and all other) examples of these experiments.

To get some quantitative data on the susceptible molecules involved in the cell, a comparison with the radical reaction in aqueous solutions was made between the experiments of DAINTON, 1958, involving the oxydation of ferrosulfate solution, and the more precise investigations designed especially for this purpose by KRETSCHKO and POHLIT, 1964. Here the same dose effect relationships were observed with a linear

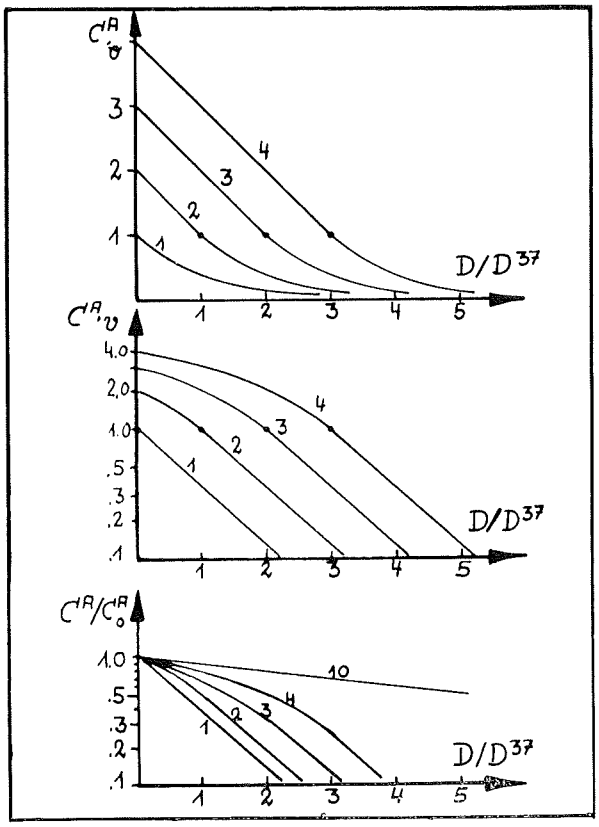

Fig. 4 - *Dose effect curves, linear, semilogarithmic*

and an exponential part (in linear coordinates) when only radical reactions were involved.

Also, the D^{37} estimated from the exponential part is equal to the D^{37} from the linear part:

$$D^{37} = D^{XX} - D^{X} = 1{,}2 \text{ krad or}$$

$$\rho \cdot D^{37} = 7{,}5 \cdot 10^{16} \text{ eV/cm}^3.$$

From the slope of the straight line k was determined to be 12,5 eV per atom and from the exponential part, v was determined to be

$$V_R = \frac{k}{\rho \cdot D^{37}} = \frac{12{,}5 \text{ eV}}{7{,}5 \cdot 10^{16} \text{eV/cm}^3} = 1{,}6 \cdot 10^{-16} \text{ cm}^3$$

or $r_R = 3{,}3 \cdot 10^{-6}$ cm.

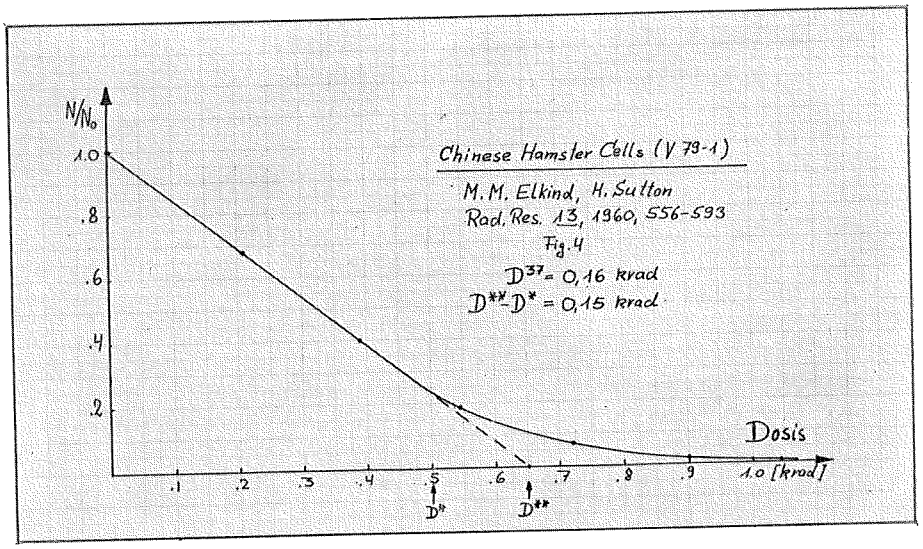

Fig. 5 - *Radiation effect on living cells. Linear plot.*

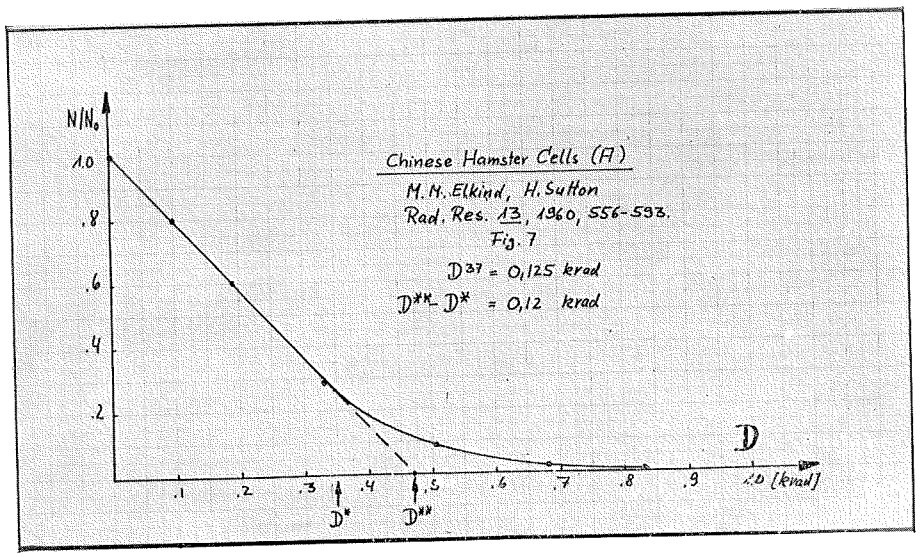

Fig. 6 - *Radiation effect on living cells. Linear plot.*

In the biological experiment of ELKIND and SUTTON the D^{37} was determined to be approximately 0,16 krad or

$$\rho \cdot D^{37} = 1 \cdot 10^{16} \text{ eV/cm}^3.$$

An estimation of k is impossible because of the absence of absolute values for the initial concentration of the susceptible molecules; so as a first approximation the same value from the reaction in acqueous solution of ferrosulfate was used. Then for the susceptible biological molecule:

$$v = \frac{k}{\rho \cdot D^{37}} = \frac{12{,}5 \text{ eV}}{10^{16} \text{ eV/cm}^3} = 1{,}2 \cdot 10^{-15} \text{ cm}^3.$$

This volume is of a factor nearly eight times larger than the volume v_R in the reaction of radicals with the iron atoms. This is due to the fact that the essential biological molecules are long chains of several hundred atoms. Therefore the volume must be considered to be roughly a cylinder with the radius r_R and the length 1. Then

$$v = 1{,}2 \cdot 10^{-15} \text{ cm}^3 = \pi r^2 \cdot 1$$

$$\text{and } 1 = \frac{1{,}2 \cdot 10^{-15} \text{ cm}^3}{\pi \cdot (3{,}3 \cdot 10^{-6})^2 \text{ cm}^2} = 34 \cdot 10^{-6} \text{ cm}.$$

This length is adequate for a molecule of RNA of 280 triplets of nucleotides, or for the same number of corresponding amino acids in a polypeptide; both are well known to be essential in the cell. Later it will be shown that the messenger RNA is most probably involved in this radiation effect.

As a conclusion, one can estimate that the dose effect relationship for the death of single cells is in astonishing accordance with the relation for radical reactions in aqueous solutions, and that the quantitative analysis gives reasonable values for the molecules involved in the reaction.

2) Cybernetic behavior of the cell processes

Due to the indication that large molecules like RNA or enzymes are involved in the radiation reaction in cells, the cycles of protein synthesis should be quantitatively investigated in more detail.

In the cell nucleus the information is stored in the desoxyribonucleic acid in a text using four different letters: A (adenine), C (cytosine), G (guanine), and T (thymine) (see Fig. 8). The pairs adenine - thymine and guanine - cytosine

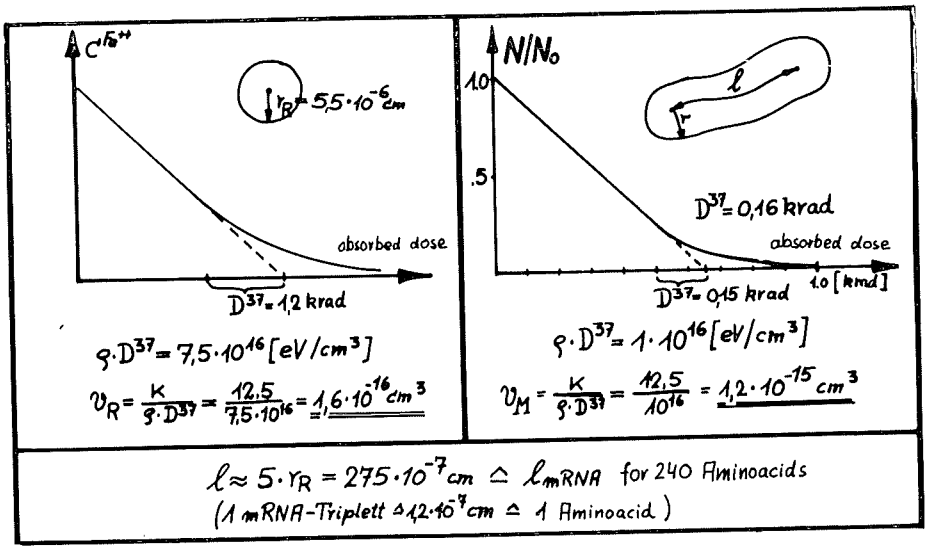

Fig. 7 - *Comparison of the effect in solution and cells*

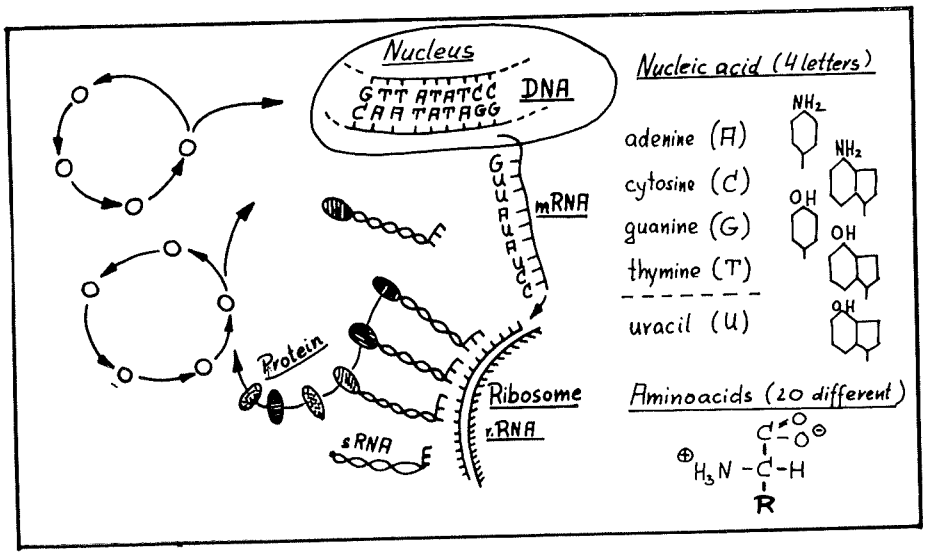

Fig. 8 - *Proteinsynthesis in the cell*

geometrically suit each other very well, as a positive and a negative or a script and its template. This explains the double helix of DNA propagated by Watson and Crick. It is also the expression for a good « shielding » of the information in the cell against all disturbing influences, in addition it provides for the possibility of repairing defects in the « positive » by means of the intact « negative » and vice versa. If both lines are injured the damage is irreparable.

From the information in the DNA, parts are copied in a very similar script. The ribonucleic acid (RNA) uses the same letters as the DNA but instead of thymine the letter U (uracil) (see Fig. 8). Because of its transport of messages from the cell nucleus into the cytoplasm, this type of RNA is called messenger RNA (m-RNA). These messages are trans-

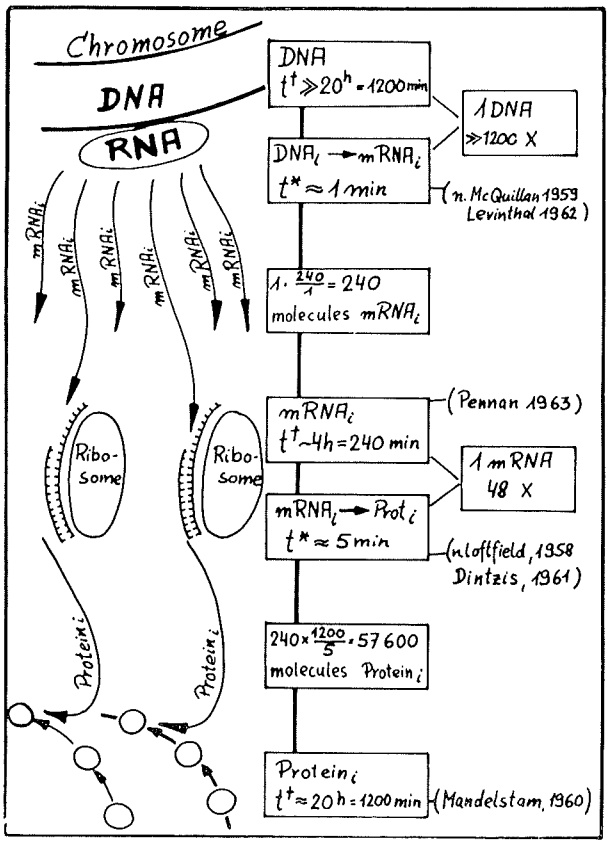

Fig. 9 - *Quantitative values for protein syinthesis*

lated on the surface of ribosomes into another script, the polypeptides or enzymes, which use twenty different amino acids as letters. The enzymes now control the metabolic processes in the cell in a catalytic way; for example those cycles where new constituents of RNA are produced.

Such storage and transportation of information in a script with few symbols is relatively insensitive to disturbances. The morse code for the translation of morse signals into letters is similar to the code for the translation of RNA-signals into amino acid letters. This code is approximately known, since there are chemical methods to read the text of polypeptides, and to carry out such translations in invitro experiments. From information theory it is clear that at least three letters of RNA are necessary for one letter of the amino acid script.

Some quantitative values for this information transport estimated from different experiments, are given in Fig. 9. To print a m-RNA molecule from the DNA takes approximately one minute. The m-RNA then goes into the cytoplasm and is involved in the translation process, but after a mean life span of approximately four hours it is decomposed. Since each minute a new m-RNA molecule of the type « i » is produced and each molecule lives 240 minutes, the average 240 molecules of type « i » are always present in the cell. The translation process into the amino acid script at the ribosomes takes somewhat more time. One can calculate that each five minutes an enzyme molecule of type « i » is produced. The lifespan of these enzymes is about twenty hours, so on the average approximately 60000 enzyme molecules of the type « i » are present in the cell. From this one can see that at the ribosome not only a translation is carried out, but also an amplification of the number of messages. If by irradiation, a certain number of molecules are destroyed, there will be a large effect in the small number of messenger RNA, but only a small effect in the large number of enzymes of the same type. Therefore, it is obvious that the m-RNA is the most radiation sensitive component in this cell cycle, compared with the few but safely arranged DNA molecules and the large number of enzymes and metabolic products.

The temporal relations of this cycle can be treated by the diagram given in Fig. 10. Here precursors A_i for the m-RNA of the type i are delivered to the cell nucleus in a concentration (A_i). By the printing process in the cell, m-RNA is then produced with a concentration (X_i) and translated in an enzyme Y_i. This enzyme induces the production of a metabolite M_i, which is stored in a reservoir or pool. From there, a certain concentration (R_i) reaches the nucleus and

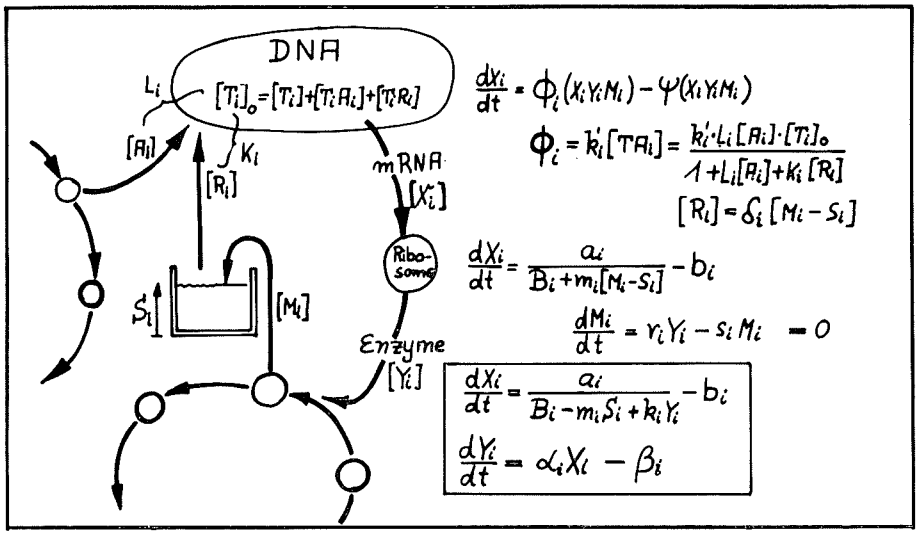

Fig. 10 - *Cybernetic model of protein synthesis*

acts at the DNA template like a molecule A_i with the only difference being that it doesn't produce a « legible » m-RNA. In this way the production of m-RNA is repressed and the metabolic product R_i is called a « repressor ».

If a large number of m-RNA$_i$ molecules is produced, then a large number of repressor molecules is the result causing a damping of the m-RNA production, and vice versa. This represents a regulating cycle.

The differential equation for the time dependent concentration of m-RNA (X_i) and enzyme (Y_i) derived by Goodwin (1963), gives fluctuations of the concentration of X_i and Y_i around the mean values as shown in Fig. 11. The fluctuations are not sinoidal but more like a saw tooth and the fluctuation time is approximately four hours.

An irradiation of the cell should be an abrupt reduction in the number of m-RNA molecules in the regulating cycle and should lead to an overproduction of m-RNA approximately two hours later and also should lead to a synchronization of the cycles in all cells.

Irradiation of this system a second time approximately two hours after the first irradiation should produce small irradiation effects since the concentration of the m-RNA is high; if the cells are irradiated approximately five hours later, then the effect should be large due to the small concentration

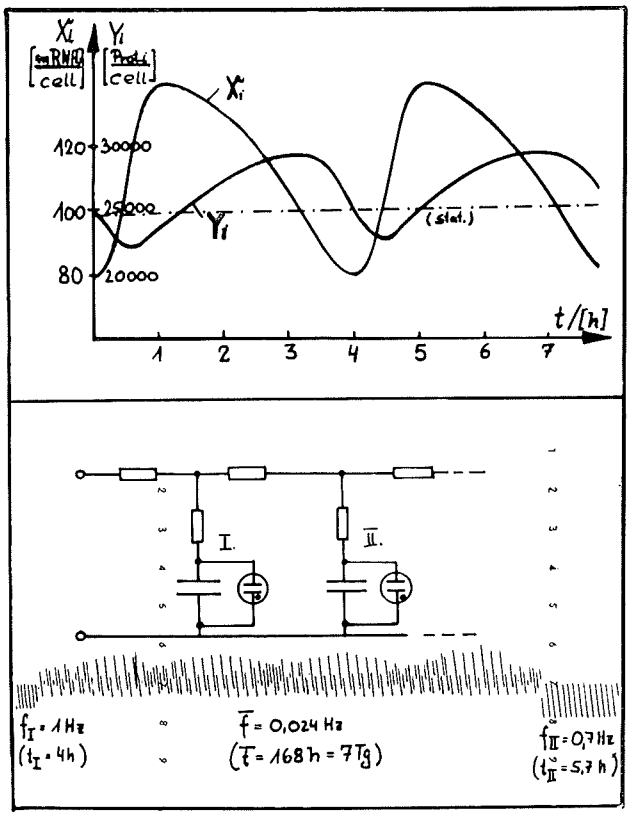

Fig. 11 - *Periods of protein synthesis and coupling of oscillators*

of the m-RNA. If the irradiation is delivered later, the effect will decrease again, corresponding to the fluctuation of molecule concentration. This is shown by the experiments of Elkind and Sutton (1960 given in Fig. 12 and Fig. 13). Here the dose effect relations are drawn for a single dose irradiation and also for consecutive irradiations, where a first dose of 505 rad is followed a certain time later by a second dose of 578 rad. If the second dose is delivered 5,5 hours after the first irradiation, the radiation effect is maximal and a small fraction of the cells survive. In a shorter or a longer time interval between these doses the effect is less, due to the assumed high concentration of m-RNA molecules.

If this is true, the shape of the dose effect curves should also change. When the concentration of m-RNA is high, (after

2,5 or 20 hours) a shoulder should be present in the dose effect curve in the semilogarithmic plot (straight portion of the linear plot) due to the high concentration. On the other hand, it is expected that at 5,5 hours, due to the small concentration of m-RNA molecules, a straight line will be present in the semilogarithmic plot. This is exactly the result in the experiments of Elkind and Sutton, as shown in Fig. 12 and 13.

A more precise examination shows that there are differences between the irradiation of a synchronized population and an unsynchronized population as shown for the single dose experiment.

Here also the correlation between the concentration of the susceptible molecules (m-RNA) and the radiation effect is obvious. One can check the results in the following way: If the concentration of the susceptible molecule is reduced

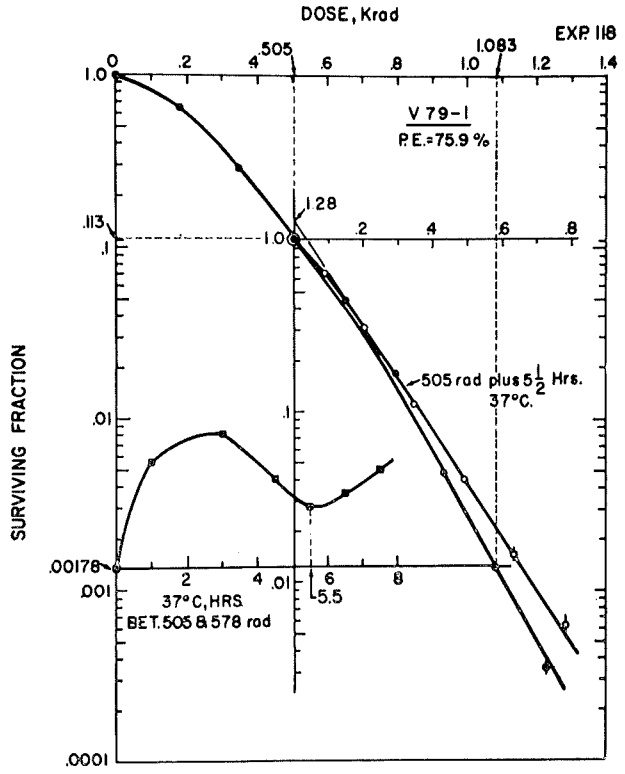

Fig. 12 - *Recovery of irradiated cells, Elkind a. Sutton* (1960)

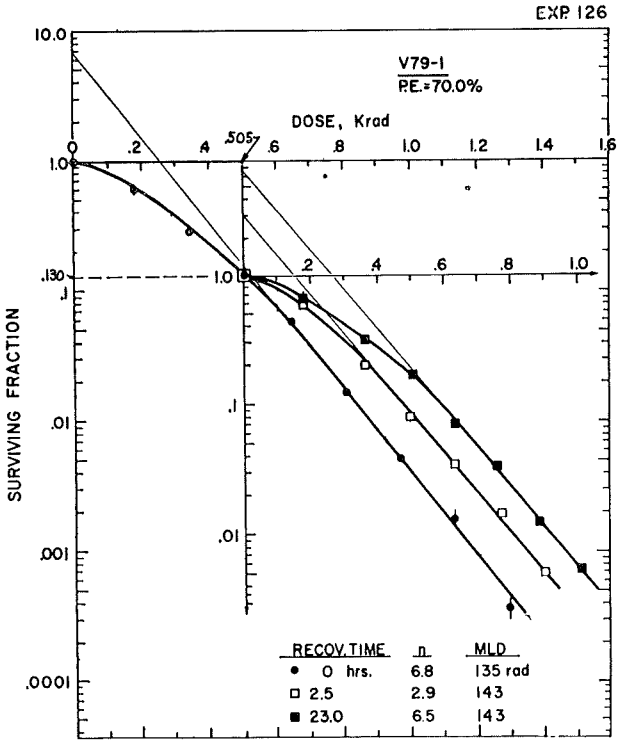

Fig. 13 - *Dose effect curves for second irradiation of cells, Elkind and Sutton* (1960)

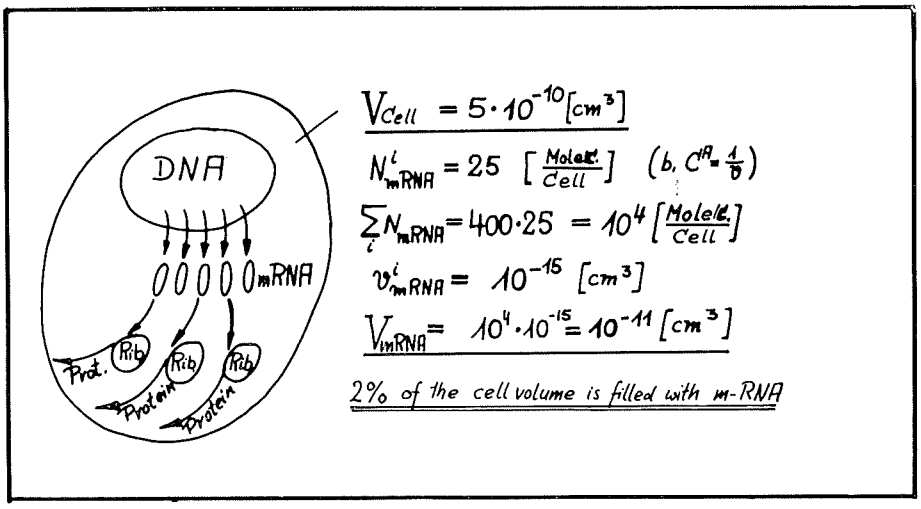

Fig. 14 - *Data on m-RNA in the cell (from irradiation experiments)*

by irradiation with a factor of four or five, down to approximately 25 molecules of type « i » in the cell, the volumes v will lie close together. Since there are probably 400 different types of proteins or of corresponding m-RNA molecules in the cell, the total number of m-RNA-molecules in the cell is:

$$N_{mRNA} = 400 \cdot 25 = 10^4 \text{ molecules per cell}$$

and the total volume filled with all these molecules is:

$$N_{mRNA} \cdot v = 10^{-11} \text{ cm}^3.$$

If the cell volume is assumed to be

$$v_{cell} = 5 \cdot 10^{-10} \text{ cm}^3,$$

2% of the cell volume is occupied by m-RNA. This is a reasonable value.

All these investigations show that there is a striking connection between the radiation damage in cells and the concentration of susceptible molecules (most probably m-RNA) in the cell. The dose effect curve and the temporal behavior after irradiation can be explained quantitatively in this way. From this model, a large number of new questions arise and must be answered to improve radiation therapy. But for successful research in this field, a strong collaboration of physicists, biologists and physicians is absolutely necessary.

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INTERVENTI SULLA RELAZIONE

F. W. SPIERS

From Prof. Pohlit's interesting analysis it would appear that the particular cell survival curves he has considered could be explained in terms of the action on messenger RNA only. Would Prof. Pohlit like to say what part he considers direct action on the nucleus plays and what its relative magnitude might be?

RISPOSTA DEL RELATORE

W. POHLIT

Both effects, direct actions on the cell nucleus and actions on messenger RNA or enzyme molecules must be considered to be generally present in the cell. The quantitative explanation of the dose-effect-curve in my Figure 7 was only a rough estimation from the D^{37} for the lenght of the molecule involved in this reaction. Therefore it is not possible to give a certain value for the relative magnitude of both effects. This relation might be 50 to 50% or 20 to 80% or vice versa. Other and more precise experiments are necessary — and would be done in the next future in my laboratory — to give some information on this ratio. Nevertheless the shoulder of the dose-effect-curve and the recovery after irradiation is explained here to be related to RNA or enzyme molecules only.

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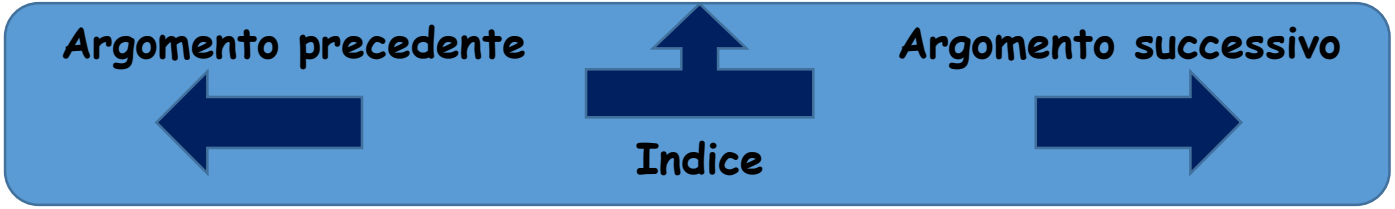
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