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Life Span of Tissue Cells

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The life cycle of tissue cells, for example those of the liver, is determined by measuring the rate of incorporation of ^{32}P, ^{14}C, of ^{3}H-thymidine or other suitable markers into DNA of the cells. An alternative method is the measurement of the rate of loss of the labelled DNA and thus of labelled cells from a uniformly labelled organ. Both methods and the results obtained by them are discussed.

Besides expressing his cordial wishes on this memorable day for the happiness and further success of Hugo Theorell, the author wishes to contribute to this volume by a discussion of the problem of the life span of tissue cells.

At the end of the thirties J. Ottesen and the present writer were interested in the determination of the life span of erythrocytes by labelling these with a stably adhering radioactive marker. The disappearance of the radioactivity should in such a system indicate the end of the life span of the erythrocyte. The time however which ^{32}P, ^{24}Na, ^{42}K and some other radioactive tracers, which were at that time at our disposal, spend in non-nucleated red corpuscles is much shorter than is the life span of the latter, correspondingly we had to abandon our plan to determine the life span of the mammalian red corpuscle by the above mentioned method. Earlier investigations carried out by us suggested that the radioactive label attached to the DNA of nucleated red corpuscles is stable and we therefore concentrated our interest on the determination of the life span of the nucleated erythrocytes of the hen[1]. The plasma orthophosphate of the hen was kept on a constant specific activity level and at intervals the latter was compared with the specific activity of the DNA phosphorus of the erythrocytes. After the lapse of about 1 month the two specific activities were found to be equal indicating that the full renewal of the erythrocyte DNA and thus of the erythrocytes took place in the course of about 1 month. One year later Rittenberg and Shemin[2] found in the stable ^{15}N a very suitable tracer of the hemin of hemoglobin and determined in a classical investigation the life span of the red corpuscle of the man to be about 120 days. When I shortly afterwards mentioned our results with Ottesen at the Solvay Congress in Brussels I met an incredulous audience.

The objection to our results (an entirely unjustified one) was that if the red corpuscle of the man prevails for 120 days the nucleated red corpuscle of the hen should live longer than 120 days, thus very much longer than 30 days. Soon after Rittenberg repeated our experiments using ^{15}N as a tracer and corroborated our results. After the availability of ^{14}C, ^{15}N was in most cases replaced by the first mentioned isotope in life span determinations of erythrocytes. Several other methods of determination of life span became available as well. In a pioneer work Paul Hahn and collaborators[3] observed that ^{59}Fe released from the dying red corpuscle is reutilized in the formation of new erythrocytes, thus ^{59}Fe could not be used as a tracer in the determination of the life span of red corpuscles. When much later ^{59}Fe of very high specific activity became available it was shown that about 2 % of the ^{59}Fe released by the dying erythrocyte is not re-utilized, or not reutilized within a reasonable time, in the formation of new red corpuscles. It became now possible to use even ^{59}Fe as a tracer in the life span determinations of red corpuscles. Nowadays mostly ^{51}Cr and DF ^{32}P are used for that purpose. All these methods found a far-reaching application and we have today quite an extended knowledge of the life span of the red corpuscle both under physiological and pathological conditions. We have very much less knowledge on the life span of tissue cells. In determining the life span of erythrocytes we introduce in several of the methods applied a labelled component into the heme during its formation which is renewed only when the erythrocyte is destroyed. In the determination of the life span of nucleated erythrocytes an alternative method is the introduction of a labelled component into the DNA molecule of the nucleus in the course of its formation. The latter procedure is the only one which can be applied in the determination of the life span of tissue cells. The DNA molecules of the latter, apart from a few very special cases, are not renewed during the life of the cell. The disappearance of the label of the DNA molecule indicates thus the termination of the life span of the tissue cell. The human red corpuscles have the same life span apart from a small fraction which is decaying at random. In the liver, for example, we find cells of very different life time. The parenchymal cells live longer than the reticulo-endothelial cells and indications are not lacking that even within these two groups we find cells of different life time. It therefore encounters difficulties to state the life time of liver cells. We can however state after the lapse of a day what fraction of those originally present are still present or of those present after 100 days how many still prevail after the lapse of 200 days and so on.

The first investigation in which the incorporation of ^{32}P into DNA was studied by the present writer and J. Ottesen[4] brought out the great difference in the rate of incorporation of ^{32}P into DNA and into the acid-soluble components of the liver of the fully grown rat. The latter takes place at a fairly rapid or even very rapid rate, the former at a very slow rate only. In the above mentioned experiments in the course of 4 days about 4 % only of the liver cells of the fully grown rat were found newly formed. These results were interpreted as indicating that while ^{32}P incorporation into acid-soluble components of the liver is due to a molecular renewal that into DNA is to be ascribed to a new formation of tissue cells. The rate of cell formation can thus be easily measured. Possible errors of the method will be discussed later.

Shortly afterwards Lucie Ahlström, Hans von Euler and the present writer[5] carried out experiments taking 2 hours only and found a 0.13 % renewal. Hammarsten and the present writer[6] found a renewal rate of 0.105 % of the DNA molecule of the liver in experiments taking 2 hours. These values are somewhat larger than those found in experiments taking 4 days, where the corresponding (2-hour) value was 0.08 only. The incorporation of ^{32}P into DNA takes place 5–7 hours during the late interphase. In the experiments taking 2 hours we measure not only labelled finally formed cells but also such in the late interphase, while in the experiments taking 4 days happenings during 5–7 hours can almost be disregarded. Differences as mentioned above are to be expected as we have different types of liver cells and these are renewed at different rates. As the most labile ones are already replaced by labelled cells in experiments of short duration, a prolonged experiment cannot be expected to lead to as high per hour renewal figures as found in experiments of short duration. The renewal rate was in all the above investigations assumed to be equal to the ratio of specific activity of the DNA phosphorus at the end of the experiment and the mean value of cellular orthophosphate phosphorus during the experiment. It was assumed that the extracellular labelled orthophosphate first mixes with the intracellular ortophosphate and participates then in the synthesis of organic phosphorus compounds. This assumption can however not be maintained. Ahlström and associates[5] when determining the rate of formation of liver cells in newly born (4 day old) rapidly growing rats found a very high formation rate, 2 % in the course of 2 hours. In the strongly growing rat we can determine the weight increase of the liver in the course of the experiment and calculate, under the assumption that the DNA content increases proportionally with the weight of the organ, the DNA increase in the course of 2 hours. This was found to make out 0.7 times only the figure calculated from radioactive data. As a possible explanation of this difference the view was put forward that beside a formation of labelled, thus new, cells some turnover of DNA molecule takes place as well, which is partly responsible for the high ^{32}P incorporation into DNA. Soon, however, it was found that this assumption cannot be maintained as a renewal of the DNA molecule only takes place in some very special cases. The difference between the measured increase in the DNA content and that calculated from radioactive data is due to another reason than to a turnover of the DNA molecule. It is due to an erroneous assumption when carrying out the calculation from radioactive data. The extracellular ortophosphate does not first mix with the cellular orthophosphate and is then incorporated into organic phosphorus compounds as assumed in the above consideration, but the synthesis of these starts at once after intrusion of the labelled orthophosphate into the cell. The mean specific activity of the reacting orthophosphate is thus larger than assumed when carrying out the calculation leading to too high formation figures and the ratio calculated correspondingly smaller. Thus the percentage of DNA formed in the liver of 4 days old rats in the course of 2 hours is not 2 but $0.7 \times 2 = 1.4$. Possible errors involved in the method described when applied to the measurement of the rate of formation of the liver cells are: a) That even in the fully grown animal some growth takes place, thus the formation of some of the labelled DNA molecules is not due to replacement but to additional formation of cells. b) Polyploid nuclei

may be formed in the course of the experiment. In this case the formation of some of the labelled DNA molecules is not due to the synthesis of new cells but to an increase in the DNA content of cells already present. When determining the rate of renewal of tissue cells it is therefore preferable not to measure the rate of labelling of DNA but the rate at which the DNA label and thus the labelled cells are lost.

Measurement of the rate of loss of labelled tissue cells

Tissue cells are renewed and thus labelled at a very different rate, which for many liver cells is so slow that the life time of the mouse does not suffice to achieve a labelling of a part of the cells. We can, however, label pregnant mice with the result of a uniform distribution of the label in the organs of the offspring taking place. We take, for example, a group of mice the DNA of which is labelled with ^{14}C in utero when 50 days old, another when it reached an age of 100 days and determine the ^{14}C content of the DNA of the liver in both groups. The difference in the ^{14}C content of the DNA of the liver of the two groups indicates the DNA loss and thus the cell loss in the course of 50 days. This method was applied by Forssberg, Dreyfus and the present writer[7]. 20 pregnant rats were labelled by injecting 10 μC of adenine-2-^{14}C. The results of experiments in which the first group is taken 30 days, the second 116 days post partum is seen in Table 1.

Table 1. Daily percentage loss of DNA purine ^{14}C by the liver of mice between 30 days post partum and 116 days post partum.

Liver	Spleen	Brain
0.44	0.42	0.21

A possible error of experiment which would result in a reduction of the measured daily DNA purine ^{14}C loss is the reutilization of the released purine ^{14}C. As the labelled purines will have to compete with the non-labelled ones present, furthermore RNA is a much more powerful competitor for adenine than is DNA, the formation rate of labelled RNA molecules making out more than 30 times that of labelled DNA molecules[6], the probability of reutilization of released purine ^{14}C is a restricted one. Cronkite *et al.*[8] found released labelled thymidine not to be reincorporated into DNA.

The half life of the liver cells of the mouse works out from Table 1 to be 114 days. However this is a resultant of different life times. What we can conclude is that out of 100 cells present in the liver at 30 days post partum 86 days later only 62 are present. In the course of the first 30 days of our experiments with growing mice the percentage daily liver cells increase made out 3 %. Later the daily increase more and more slowed down. Hand in hand with this increase due to growth a daily loss of about 0.5 % of the labelled liver cells took place. The latter get replaced by new-formation. We know from much evidence obtained from studies of partially hepatectomized mice and other animals that loss of liver cells is followed by a replacement. In the rat 32 hours after partial hepa-

tectomy an intense new formation of cells can be observed, the liver regaining ultimately its original size. It is probable that the liver cells removed in the course of phagocytosis or by physiological death are replaced by a similar mechanism as the cells removed by surgical interference. As to the mechanism of the replacement of the through hepatectomy removed cells different views were put forward. The following explanation is that of Paul Weiss[9].

Compensation of the removed liver cell

Weiss assumes that the growth rate is proportional to the concentration of an intracellular specific catalyst, a "template". Under normal conditions the compounds remain confined within the cell. Each cell also produces "antitemplates" to this compound which can inhibit the former species by combining with them into inactive complexes. The "antitemplates" in contrast to the templates are released from the cell and get into the extracellular space and into circulation. They carry the specific tag of their producer cell type which endows them with selective affinity for any cell of the same type. They are in constant production so as to make up for their extracellular decomposition and final excretion. If a part of the liver is removed, less "antitemplate" will be formed and reach the circulation. Correspondingly the liver cells are now exposed to less circulating "antitemplates" than was the case prior to hepatectomy and they shall start to divide. This will result in an accumulation of additional amounts of "antitemplate" which in turn shall more and more inactivate "templates". Growth rate will decline in all cells belonging to that particular strain bathed by the common humoral pool. When stationary equilibrium between intracellular and extracellular concentration is reached, growth will cease. While the hepatectomized liver grows until the original size is reached but not further, an increase in the size of the undamaged liver of one partner of a pair of parabiosed rats following removal of the liver of the other partner takes place. Furthermore an increase in the mitotic figure of the livers of normal rats injected intraperitoneally with serum from partially hepatectomized animals is observed. The "growth-stimulating" effect of embryo extract on the proliferation of tissue cultures and also of organs demonstrated by an increased incorporation of ^{32}P into DNA of various organs[10], is accounted for by the fact that, since embryo extract contains cell debris of all organs the growth of any tissue explanted in it would be favoured.

Formation and loss of liver cells

A possible explanation of the observation that in growing liver a not negligible daily cell loss takes place, is indicated by results obtained in the investigation of the ^{32}P incorporation into the DNA of the liver cells under toxic conditions. Kelly *et al.*[11] studied the effect of endotoxins on the rate of formation of labelled DNA, so did Meissner[12] that of tubercle bacteria. The rate of formation of labelled cells was found appreciably accelerated in these cases, the increased cell formation being partly due to the compensation of cell loss.

It is quite possible that even in the normal mice investigated toxins coming from the intestine or from other sites get partly phagocytised by liver cells which

get in the course of the phagocytic process destroyed and then rebuilt. A cell loss observed would thus be a result of elimination of toxins. The possibility can however not be excluded that the loss and following renewal of liver cells is a physiological process showing some resemblance to molecular renewal. The results of experiments with bacteria free mice may decide between these two alternatives.

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