Zusammenfassung

Erste Versuche zur Ultrazentrifugierung isolierter Zytoplasmapartikelfraktionen aus Krallenfroschleber (*Xenopus laevis* Daud.) in «Diodon»-Saccharose-Gradienten mit verschiedenen Dichtebereichen zeigen, dass mitochondrien- und mikrosomenartige Zellpartikel in verschiedenen Zonen der Gradienten ins Dichtegleichgewicht gebracht werden können. Die Lage der Partikelzonen in den Gradienten ist abhängig von den gewählten Dichteextremen, woraus abzuleiten ist, dass die Trennung der Fraktionen im wesentlichen durch die Unterschiede in der Partikeldichte bedingt ist. Für die isolierten Mitochondrien wurde so eine Dichte von 1,10 bis 1,20, für die Mikrosomen hingegen eine solche von 1,25 bis 1,30 gefunden.

Hydrogen Peroxide in Tumours

Its Possible Significance in Carcinogenesis

During the last twenty years, we have studied the action of hydrogen peroxide on proteins and high split-products of proteins consisting in an aggregation of molecules with an increase of N precipitable by trichloroacetic acid[1] and of turbidity[2]. This phenomenon was further investigated and called[3] "aggregation effect" of H_2O_2. While formerly we considered such effect as chiefly due to an interaction with an enzymatic system, and therefore corresponding to a true polymerisation, we were lately inclined to admit an oxidation of hydrophilic groups of proteins and peptides molecules and subsequent process of physical aggregation. The formation of S-S-bridges between molecules through oxidation of SH-groups was also taken into consideration.

A relation of this effect of H_2O_2 to similar processes and their importance for the protein synthesis were largely discussed[4].

YAMAFUJI and coworkers[5] paid great attention to the action of H_2O_2 on proteins, considering it to be a true polymerisation[5]. They think that poisoning catalase in the living organism (e.g. by hydroxylamine), permitting the H_2O_2 formed in the oxidative metabolism of cells to escape destruction by this enzyme, may give rise to polymerisation of some protein constituents and bring about the formation of virus particles. These authors therefore admit an endogenous origin of some virus by means of abnormal H_2O_2 concentration in tissues.

One of the most important features of enzymatic pattern in tumours is represented by a strong decrease of catalase activity. This was first shown by GREENSTEIN and coworkers[6], who studied the activity of some enzymes in rats' hepatomas, in normal and regenerating livers. The relationship between catalase activity in hepatoma and in normal adult liver was 1:1000; whereas the differences concerning other enzymes were much smaller. Regenerating liver has nearly the catalase activity of normal liver. A decrease of catalase activity in livers of tumour-bearing rats was also demonstrated by GREENSTEIN and coworkers, and later throughly investigated by other authors[1]: thus CUDKOWICZ[2] found a strong decrease of catalase in livers of benzpyrene-treated rats as soon as 60 days after the injection of the hydrocarbon, i.e. before the appearance of a sarcoma at the injection site.

A working hypothesis was advanced by RONDONI[3], who said: "The strong diminution of catalase activity in a tissue may induce an accumulation of H_2O_2 having an aggregation or polymerisation effect on some proteins and high split-products of proteins. Such aggregation effect may be the starting point for that proteins rearrangement, perhaps ultrastructural in nature, which brings about the malignant change in cell" (p. 272).

According to such hypothesis the carcinogenic agents might be considered as catalase poisons: really CUDKOWICZ found that liver catalase can be partially inhibited *in vitro* by two powerful carcinogenic hydrocarbons (3,4-benzpyrene and methylcholanthrene) dissolved with caffein, and not by some non-carcinogenic hydrocarbons. However, water suspensions of hydrocarbons were in every case devoid of inhibiting activity.

It may be mentioned that according to recent investigations the mode of action of ionising and exciting radiations on cells can be at least partially explained by the formation of hydroxylic radicals from water, i.e. a chemical mechanism involving oxidation reactions and very likely some kind of protein denaturation. Besides this, a formation of H_2O_2 by irradiation of water or aqueous solutions has been found in some experiments[4].

A direct proof of the role of H_2O_2 in carcinogenesis has neither been given nor proposed, so far as we know. Such a demonstration may be very difficult, because the peroxide after formation in sufficient amount to induce the cellular change can be very quickly destroyed in the metabolic pool. However, we have attempted to estimate the contents of H_2O_2 in normal and tumour tissues (rats and mice) by the following method, permitting an approximative and comparative evaluation.

Method.—The animals were killed by bleeding. The tissues were immediately homogenised in an aq. 6·5%-solution of KCN in order to destroy every enzymatic activity. Estimation of dry weight. A 20%-solution of trichloroacetic acid is added (1:1) to the homogenate. After a few minutes filtration through paper, a clear solution is obtained, to a known amount of which some milliliters of a solution of titanium sulphate are added. Such a solution is made by dissolving the titanium salt in warm n H_2SO_4. A yellow colour is developed, which is photometrically (FISCHER electrophotometer) checked against a standard (homogenate + trichloroacetic acid + n H_2SO_4 without titanium salt). As the colour changes to some extent, an empirical curve ought to be established for every homogenate (using a part of homogenate where H_2O_2 has been destroyed by silver powder and another with known amount of H_2O_2), in order to evaluate the H_2O_2 contents from the photometric figures.

We must remark that photometric values do not always give a regular straight line, and the colour derived from the reaction $H_2O_2 + Ti_2(SO_4)_3$, which is quite stable in simple water solution, fades away at a different speed in the homogenates.

We may summarize our relative results in the following Tables.

[1] P. RONDONI and L. POZZI, H. S. Z. phys. Chem. *219*, 22 (1933); *235*, 81 (1935).

[2] P. RONDONI, H. S. Z. phys. Chem. 254, 207 (1938).

[3] P. RONDONI and M. BASSI, Enzymologia *15*, 70 (1951).

[4] P. RONDONI, Ergebn. Enzymforsch. *10*, 65 (1949).

[5] Many papers in Enzymologia *13*, *14*, *15* (1949–1951); see. chiefly *15*, 223 (1952). – K. YAMAFUJI and K. ROSA, Biochem. Z. *317*, 81 (1944). – K. YAMAFUJI and F. YOSHIHARA, Biochem. Z. *317*, 87 (1944). – K. YAMAFUJI and Y. SHIROZU, Biochem. Z. *317*, 94 (1944).

[6] J. P. GREENSTEIN, V. J. WENDELL, and J. WHITE, J. nat. Cancer Inst. *2*, 17 (1941). See also: J. P. GREENSTEIN, *Biochemistry of cancer* (Academic Press Inc., New York, 1947.

[1] H. v. EULER and L. HELLER, Z. Krebsforsch. *56*, 395 (1949).

[2] G. CUDKOWICZ, Tumori, *38*, 176 (1952).

[3] P. RONDONI, Boll. Oncologia (Lega ital. per la lotta contro i tumori) *26*, 245 (1952).

[4] C. BIAGINI, Nuntius radiologicus *18*, 97 (1952) (a review).

Table I

H_2O_2-contents (γ pro 17 mg dry weight) in organs of normal rats and in organs and tumour of rats with transplants of Walker-carcinoma

Rat	Liver	Kidney	Tumour
1 (normal)	2·1	25·0	
2 (normal)	4·8	50·0	
3 (normal)	2·5	45·0	
4 (normal)	3·0	34·0	
5 (Ca 10 days after graft)	—	—	16·0
6 (Ca 10 days)	1·8	23·0	32·6
7 (Ca 12 days)	1·7	28·5	12·8
8 (Ca 14 days)	3·0	33·0	13·5
9 (Ca 16 days)	4·2	45·0	4·1
10 (Ca 18 days)	4·7	51·3	4·4

Table II

H_2O_2-contents (γ pro 12 mg dry weight) in organs of normal mice and in organs and tumours of mice with transplants of Ehrlich-cancer

Mouse	Liver	Kidney	Tumour
1 (normal)	12	40	
2 (normal)	11·3	53	
3 (normal)	12·7	37	
4 (normal)	10	35	
5 (normal)	6	30	
6 (normal)	9	60	
7 (Ca 10 days after graft)	12	50	12
8 (Ca 10 days after graft)	14	47	13
9 (Ca 10 days after graft)	7	60	13
10 (Ca 10 days after graft)	6	50	11
11 (Ca 13 days)	11	55	5
12 (Ca 13 days)	8	43	6

In all animals the kidney shows the highest contents of H_2O_2, a finding which may be in accord with some old observations of BLASCHKO[1] that an appropriate concentration of hydroxylamine brings about a strong decrease of respiration of kidney sections, whereas it does not disturb the respiration of testis. A catalase poison exerts a stronger action where H_2O_2 is more largely formed, i.e. in kidney. This organ is a site of the most intense oxidation processes and H_2O_2 may be considered as a result of a very active oxidative metabolism.

In regard to tumours, we find in rats with Walker-Ca some figures which are (rats 5–8) in the same order as those of kidney, always exceeding the amounts found in livers, i.e. in an organ with high metabolic rate. Tumours of rats 9–10, examined after a longer delay after graft and showing more necrotic changes, gave lower values. Ehrlich-cancer of mice seems to possess lower amounts of H_2O_2, tumours show figures of same order as livers (mice 7–10), in one case lower (mouse 11). This finding may be related to the large necrotic changes occuring in such a tumour. The decrease of H_2O_2-contents with extending necrosis of the tumour is a general feature.

A series of H_2O_2-estimations has been started on livers of rats fed dimethylaminoazobenzene and an appropriate deficient diet in order to obtain hepatomas and to compare them with normal livers. A report of such experiments will be given later. We may now anticipate that the livers of 4 rats fed the deficient diet (with carcinogen or without) showed very high contents in the range 18–26 γ after only a month of treatment, i.e. in a quite precancerous condition.

[1] H. BLASCHKO, J. physiol. *84*, 52 (Proceed.) (1935).

Arguing from the kidney, which evidently has a particular position owing to its high metabolic rate, we may suppose a content of H_2O_2 in tumours which exceeds (in rats), or equals (in mice), that of the liver. This finding may show a rather active oxidative metabolism in tumours, in contrast to the usual assertion of a prevailing anaerobic metabolism. It is difficult to assess the importance of H_2O_2 for carcinogenesis according to the above working hypothesis. The action of this metabolyte may be different in different tissues, according to the equilibrium of catalase-peroxidase, presence of H-donors, structure of cell proteins and protein split product. A more extensive study concerning chemically induced tumours at different stages may give a clearer answer.

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Zusammenfassung

Die auf Grund älterer Arbeiten festgestellte «aggregierende Wirkung» des Wasserstoffsuperoxyds auf gewisse Proteinsysteme gestattet die Arbeitshypothese aufzustellen, wonach die bekannte Abnahme der Kataläseaktivität im Geschwulstgewebe eine Zunahme des H_2O_2-Gehaltes zur Folge haben dürfte und dadurch zum Umbau gewisser Proteine führen würde, der die Grundlage der krebsigen Umwandlung der Zelle darstellen könnte. Die Bestimmung des H_2O_2-Gehaltes (Titanium-Sulfat-Methode) wurde bei transplantablen Tumoren (Walker-Rattekarzinom und Ehrlich-Mäusekrebs) unternommen. Werte in der Grössenordnung der metabolisch sehr aktiven Organe (Niere, Leber) wurden meistens gefunden. Auch die Lebern einiger mit Dimethylazobenzol und einer mangelhaften Diät gefütterten Ratten wiesen sehr frühzeitig hohe H_2O_2-Werte auf. Die Arbeitshypothese erheischt weitere experimentelle Belege.

Über das Verhalten von Grünalgen bei Einwirkung mehrwertiger Alkohole

Den zahlreichen Untersuchungen über die Wirkung mehrwertiger Alkohole auf die Struktur und die Funktion tierischer Gewebe[1] stehen nur wenige gegenüber, die sich mit dem Einfluss solcher Substanzen auf pflanzliche Zellen beschäftigen.

Nach den Beobachtungen von LOESER und Mitarbeitern[2] wird die Atmung von Hefezellen durch mehrwertige Alkohole – 1,2-Propandiol, 1,3-Butandiol, 1,2,3-Propantriol, 1,2,4-Butantriol – unterschiedlich verändert. Während die untersuchten Substanzen den Sauerstoffverbrauch der Hefe bei *Abwesenheit von Glukose* steigern, schränken dieselben Alkohole bei *Zusatz von Glukose* zur Hefe die Atmung ein. Diese Doppelwirkung ist wahrscheinlich darauf zurückzuführen, dass die Hefezelle bei Fehlen von Glukose die Alkohole als Brennmaterial mit heranzieht und sie in Abhängigkeit von der jeweiligen Konstitution – Zahl, Qualität, Stellung der Hydroxylgruppen – verschieden rasch und vollkommen abbaut. Steht den Hefezellen hingegen Glukose – das natürliche Brennmaterial – zur Verfügung, so werden die zugefügten Alkohole nicht angegriffen, sondern ent-

[1] Zusammenfassung der Literatur siehe A. LOESER: Referat: *Lösungsmittel und Lösungsvermittler*, 19. Tagung der Deutschen Pharmakologischen Gesellschaft, Göttingen 1952, Arch. exper. Path. Pharmakol. *218*, 36 (1953).

[2] H. KLEINSORG, C. G. SCHMIDT und A. LOESER, Arch. exper. Path. Pharmakol. *211*, 235 (1950). – E. STÖRMER, unveröffentlichte Versuche.