Table I

Enzyme activity	Date of analysis	*n*-plants of "17/51 strain"	*an*-plants of "17/51 strain"	plants of line No. 5
Catalase in cm^3 O_2 developed in 3′ at 25°C and pH = 8	25–7–′52	13·75	10·35	
	4–8–′52	14·50	14·00	
	12–8–′52	12·60	9·50	
Carbonic anhydrase in mm^3 CO_2 developed in 2′ at 15°C and pH = 6·8	17–7–′52	550	380	
	12–8–′52	660	600	
Polyphenol oxidase in mm^3 O_2 absorbed in 10′ at 25°C and pH = 5 .	4–8–′52	1800	2400	
	11–8–′52	880	1850	
	12–8–′52	1070	2030	1030
	3–8–′53		1550	450

Table II

Enzyme activity	*in vitro* treatment		*in vivo* treatment							
	essay	blank	after 5 days	test	after 8 days	test	after 20 days	test	after 30 days	test
Catalase in cm^3 O_2 developed in 2′ at 25°C and pH = 8	15·5	22·5	23·2	25	14·2	15·2				
Carbonic anhydrase in mm^3 CO_2 developed in 2′ at 15°C and pH = 6·8	470	300	610	410	510	470				
Polyphenol oxidase in mm^3 O_2 absorbed in 10′ at 25°C and pH = 5	360	460	900	1000	1290	1150	1250	400	1000	550

polyphenol oxidase and to successive accumulation of hydrogen peroxide due to an insufficient catalase activity[1], or to some disorder occurring in the system that produces the energy employed in the processes of synthesis themselves.

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Zusammenfassung

Blätter von Tabakpflanzen wurden *in vivo* und *in vitro* mit 20‰ Acridinorange-Lösung behandelt und danach die Aktivitäten von Katalase, Polyphenoloxydase und Kohlensäureanhydrase bestimmt. Nach zwanzigtägiger *In-vivo*-Behandlung machte sich eine Störung der Oxydationsprozesse bemerkbar. Ähnliche Befunde ergaben sich bei Pflanzen, welche das Bild einer Viruskrankheit zeigten und von einer Mutterpflanze stammten, deren Blütenknospen mit 2‰ Acridinorange-Lösung behandelt worden waren.

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Preliminary Studies on the Metabolism of Vacuolated Cells Following Hypoxia

Researches on the metabolism of vacuolated cells are still in progress in this laboratory. The present paper is an account of exploratory experiments.

Some difficulties have been encountered in producing experimentally a satisfactory and well-reproducible cell vacuolation in rat tissues. The use of substances which directly interfere with some enzymatic systems has been intentionally avoided, owing to the difficulty of discerning the changes due to the direct action of the drug from those connected with cell vacuolation. The ureter ligation, succesfully used by Vernoni[1] on rabbits, induced in the rat kidney a mild vacuolation and a marked necrosis. On the other hand, a slight steatosis took place in the liver together with vacuolation after partial hepatectomy according to the technique of Price and Lard[2]. At least for our purposes, the most satisfactory liver cell vacuolation was obtained by exposing the rats to hypoxia. Soda lime (Trowell[3]) or decompression (Pichotka[4]) did not permit a well-adjustable degree of hypoxia, as shown by the necrotic areas that complicated the cytological picture. In our study, the liver cell vacuolation was induced by keeping the rats in a continuously renewed atmosphere of nitrogen (97 %) and oxygen (3 %) for 2 h, according to Pichotka's technique[4]. It should be noted that even with this method the cell vacuolation did not appear uniform nor very typical. Apart from a number of plain vacuolated cells, many other elements were present showing a particular appearance. These cells presented well-defined limits; their cytoplasm consisted of hyperchromic areas alternated by small and pale zones irregularly bordered. All the cells did not differ in size from the normal ones. The meaning of this cellular appearance is at present under study, but presumably the change represents an early stage of cell damage preceding the formation of true vacuoles.

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Table I.–Oxydative metabolism and total water content of rat liver slices*.

Atm.: air
Temp.: 37° C.

Medium: NaCl . . . . . 135 × $10^{-3}M$
KCl . . . . . 27 × $10^{-4}M$
$CaCl_2$ . . . . . 19 × $10^{-4}M$
Phosphate buffer (pH 7·4) . . . . . 96 × $10^{-3}M$
Octanoic or succinic acid (Na-salts) . . . 5 × $10^{-3}M$

	Controls	Hypoxic animals	*t*	P
Q_{O_2}	5·70 ± 0·20	6·00 ± 0·35	0·60	0·6 > P > 0·5
Q_{O_2} octanoic	7·90 ± 0·33	7·97 ± 0·55	0·11	P > 0·9
Q_{O_2} succinic	19·90 ± 0·98	21·20 ± 0·57	1·19	0·3 > P > 0·2
RQ	1·01 ± 0·05	0·65 ± 0·04	5·39	P < 0·01
Total water content (mg/g wet tissue)	711 ± 3·7	711 ± 3·8	–	–

* Each value represents the mean of 10 animals ± s.e.

Adult albino rats were used throughout our experiments. Both normal and hypoxic animals were fasted for 16–20 h, after which they were killed by decapitation. Since an appreciable vacuolation after hypoxia was present only in the liver, our metabolic researches have been limited exclusively to this organ. Oxygen-uptake, octanoic-oxidase and succinic-oxidase activities were measured on liver slices according to the direct WARBURG method[1]. Respiratory quotient was determined according to the DIXON modification[2] of DICKENS' and ŠÍMER's first method[3]. The total water content was calculated by the difference between the wet weight and the dry weight of the tissue. The results obtained are summarized in Table I. The data on the water content appear of some interest. Since no modification has been observed in vacuolated cells, it is reasonable to assume that vacuolar degeneration is not necessarily always a hydropic degeneration. From the examination of the data in Table I, it appears also evident that the RQ is significantly lowered in vacuolated cells as compared with the controls. On the contrary, oxygen-uptake, octanoic-oxidase and succinic-oxidase activities remain unchanged. At present a satisfactory explanation of the lowering of the RQ is impossible, since the change may be the consequence of several metabolic deviations, all of which are possible.

In addition to the above experiments, acid-soluble phosphorus fractionation has been carried out in normal and vacuolated tissues. Livers were removed from both normal and hypoxic rats under $MgSO_4$ anaesthesia according to DUBOIS *et al.*[1]. Phosphorus fractionation was performed after PINCHOT and BLOOM[2], using the method of FISKE and SUBBAROW[3] for the phosphorus determination in the samples. The data given in Table II show clearly that labile P(ATP, ADP) and total high energy phosphate bonds (~P) are decreased in vacuolated tissues. Since this diminution was also found in hypoxic rats brought to the normal O_2 tension 15 min before decapitation, the ~P reduction cannot be due to the lowered O_2 tension. Therefore it seems logical to conclude that the biochemical change is in relation to the cytological alterations.

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Riassunto

È stato studiato il metabolismo ossidativo di fette di fegato di ratto in degenerazione vacuolare da ipossia. Si è rilevata una diminuzione del quoziente respiratorio, mentre il consumo globale di ossigeno, senza substrati o in presenza di ottanoato o di succinato, rimane invariato. Nei fegati degli animali tenuti in ipossia si ha diminuzione del P labile (ATP, ADP) e dei ~P totali.

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Table II.–Acid-soluble phosphorus fractions of livers from controls and hypoxic rats (mg P/100 g wet tissue)[1]

	Controls	Hypoxic animals					
		treatment *a*	*t*	P	treatment *b*	*t*	P
180 min P	54·5 ± 3·42	47·9 ± 2·30	1·68	0·2 > P > 0·1	51·6 ± 2·64	0·63	0·6 > P > 0·5
Inorg. P	26·9 ± 0·90	28·7 ± 1·60	0·48	0·7 > P > 0·6	28·8 ± 1·55	0·82	0·5 > P > 0·4
Ester P	14·2 ± 1·79	11·5 ± 0·96	1·33	0·3 > P > 0·2	14·6 ± 2·09	0·16	0·9 > P > 0·8
Labile P (ATP, ADP)	10·5 ± 0·40	5·0 ± 1·69	3·14	0·02 > P > 0·01	4·4 ± 1·20	4·77	P < 0·01
Phosphocreatine P	2·9 ± 1·10	2·7 ± 0·76	0·18	0·9 > P > 0·8	3·8 ± 0·39	0·79	0·5 > P > 0·4
~P	13·4 ± 1·39	7·7 ± 1·91	2·41	0·05 > P > 0·02	8·2 ± 1·17	2·85	0·05 > P > 0·02

[1] Each value represents the mean of 5 animals ± s.e. Treatment *a*: animals killed immediately after 2 h hypoxia. Treatment *b*: animals brought to normal O_2 tension 15 min before sacrifice.