Water Content in Vacuolated Liver

In a previous paper[1] on some aspects of the metabolism of vacuolated cells, the hypothesis was formulated that the vacuole formation, at least in the rat liver, is not necessarily connected with an increased water content of the cells. In fact, the vacuolated cells did not appear to differ in size from the normal ones, and, on the other hand, no modification of the total water content was observed in vacuolated livers. However, there was the possibility that an irregular water distribution between the extra- and intracellular phases existed, with a probably increased intracellular water content and a correspondingly diminished size of the extracellular phase.

Further findings[2] showed that the treatment adopted to obtain cellular vacuolation *in vivo* (keeping the rats in an atmosphere of 97% nitrogen and 3% oxygen) when applied *in vitro* on rat liver sections caused a lowered Q_{O_2} and an increased water content, without any appreciable vacuolation.

It seemed therefore justified to extend our researches to the water distribution in the extra- and intracellular phases of normal and vacuolated rat livers.

Adult albino rats, previously starved for 16–20 h, were used throughout our experiments. The vacuolation in the liver cells was obtained by the usual technique, that is by keeping the rats in a continuously renewed atmosphere of N_2 + 3% O_2 for 2 h (PICHOTKA[3]).

Blood serum samples were obtained by heart puncture under slight ether anesthesia, just before killing the animals.

The rats were killed by decapitation. The livers were quickly removed, gently squeezed on blotting paper and immediately weighed.

The H_2O and Cl^- content of the livers and sera were determined and calculated on the basis of fat-free fresh tissue, in order to eliminate variations due to different fat content of the tissues from animal to animal.

For the determination of the water content, weighed samples of tissue and serum were heated in an oven at 103°C to reach constant weight. The total water content was calculated as the difference between wet and dry weight.

In order to remove neutral fat, the dried tissues were extracted with ether for 5–6 h, and then left overnight in petroleum ether (b.p. 40–60°C). The organic solvent remaining in the tissues was evaporated off by heating in an oven at 103°C. The weight after extraction was taken as the weight of the fat-free solids in the samples. For the Cl^- determinations, the fat-free dried tissues were powdered, to ensure uniform sampling.

Chlorine was determined by the WILSON and BALL method[4].

According to the formulae proposed by HASTINGS and EICHELBERGER[5] and successfully used by POPJÁK[6] in studying the mechanism of parenchymatous degeneration (cloudy swelling), the values of chlorine and water content in liver and serum were used to calculate the size of extracellular phase (g/kg fat-free fresh tissue) and the water content of the intracellular phase (g/kg intracellular phase) of the liver.

Table.–Extracellular phase and water content of intracellular phase in normal and vacuolated rat livers.

		Serum Cl^- (mEq/kg)	Serum H_2O (g/kg)	Liver Cl^- (mEq/kg fat-free tissue)	Liver H_2O (g/kg)	Extracellular phase (g/kg)	H_2O in intracellular phase (g/kg intracellular phase)
Normal animals	1	103	925	38·8	676	343	585
	2	110	928	35·8	717	298	634
	3	99	902	41·8	716	350	602
	4	99	922	32·1	702	290	620
	5	101	915	37·0	700	324	609
	6	102	920	38·7	707	336	595
	7	112	936	33·0	716	379	560
	8	105	918	40·0	703	337	570
	9	106	921	32·0	692	266	594
	10	99	931	34·0	705	309	695
	Mean	—	—	—	703·4	323·2	606·4
	s.e.m.				±4·0	±10·4	±12·0
Hypoxic animals	1	101	922	37·8	710	333	614
	2	109	924	36·8	703	300	604
	3	100	927	33·0	703	294	604
	4	104	924	37·6	705	320	585
	5	106	918	39·4	718	329	621
	6	98	933	34·6	706	316	607
	7	100	929	42·9	707	382	594
	8	97	932	38·1	685	353	593
	9	103	933	33·0	707	347	585
	10	97	936	26·5	708	245	634
	Mean	—	—	—	705·2	321·9	604·1
	s.e.m.				±2·6	±12·0	±5·0
	t	—	—	—	0·38	0·26	0·56
	P	—	—	—	0·8>P>0·7	0·8>P>0·7	0·6>P>0·5

The results are shown in the Table.

The total water content, the chlorine space (or extracellular phase) and the water content of the intracellular phase are not significantly different in normal and treated animals. Therefore, as no increased hydration can be noted in the experimentally vacuolated liver cells, we cannot consider vacuolar degeneration as closely associated with hydropic degeneration.

As already noted, surviving rat liver sections, in the Warburg apparatus, take up water when kept in hypoxia (gas phase: N_2 + 3% O_2): but in living animals conditions seem to be altogether different. This difference is to be emphasized, as some information on vacuolar degeneration is derived from studies on isolated cells.

This work has been supported by research grants from the *Lega Italiana per la Lotta contro i Tumori*.

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Institute of General Pathology, University of Milan, February 11, 1955.

Riassunto

È stata studiata, nel ratto, la distribuzione dell'acqua nelle fasi extra- ed intracellulare in fegati normali e vacuolizzati.

Nessuna variazione del contenuto in acqua è stata notata nei fegati in degenerazione vacuolare.

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