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NUTRITION AND THE LIVER

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The Chemical Architecture of the Liver Cell

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Recent extensive analyses by Harrison (1953*a*) show the following amounts of material in 100 g fresh male rat liver: water 69·3 g, protein 18·0 g, glycogen 4·3 g, neutral lipids 1·5 g, phospholipids 2·8 g, ribonucleic acid (RNA) 0·96 g, deoxyribonucleic acid (DNA) 0·21 g, sodium 3·30 m-equiv., potassium 8·10 m-equiv., chloride 2·55 m-equiv., iron (total) 17 mg, copper 0·42 mg, and zinc 4·2 mg. Part of this material is present in the extracellular space which occupies some 23–27% of the liver substance, but most is contained in the liver cells themselves which, in the male rat, are of average diameter 20μ, mass $45 \times 10^{-4}\mu$g and volume $4{\cdot}2 \times 10^{-9}$ cu.cm with nuclei of diameter 7·7μ, and a nucleocytoplasmic ratio of 8% (Harrison 1953*a*). Female cells are slightly smaller. The polygonal liver cells or hepatocytes are most abundant (60%); the remainder are derived from such structures as bile ducts, blood vessels, Kupffer cells.

The cytoplasm of the liver cell contains in addition to the secretory granules (0·5–1μ diam.), lipid droplets (2–3μ diam.) and the Golgi apparatus, the mitochondria (dimensions $1{-}4\mu \times 0{\cdot}3{-}0{\cdot}7\mu$) which have recently been intensively studied by electron microscopists using very thin tissue sections (0·05μ) (Palade, 1953). They are embedded in the basophilic 'submicroscopic' ground substance of the cell (the ergastoplasm) which shows up in the electron microscope as a fine network of strands forming the endoplasmic reticulum (Porter, 1953). Many methods of fixation cause clumping of the cytoplasmic material into easily stainable, ultraviolet-absorbing basophilic masses of nucleoprotein (Davidson & Waymouth, 1946; Lagerstedt, 1949).

When liver tissue is disrupted or 'homogenized' in a suitable medium (e.g. 0·25 M-sucrose) the cell contents are released and may be separated by the process of differential centrifugation (Claude, 1943; Schneider & Hogeboom, 1951). Low centrifugal speeds (600 *g*) bring down cell nuclei, higher speeds (8500 *g*) the mitochondria, and still higher speeds (25,000 *g*) the 'submicroscopic' particles (60–150 mμ diam.) known as microsomes which probably represent the remains of the endoplasmic reticulum. The supernatant material which is not sedimented is derived

from the cell sap. Of the total nitrogen in the cell 17% is in the mitochondria, 26% in the microsomes, 42% in the cell sap and 15% in the nucleus, whereas of the total RNA 4% is in the mitochondria, about 50% in the microsomes, 34% in the cell sap and 12% in the nucleus. These proportions are significantly altered during starvation or protein depletion (Munro, 1954). Though the mitochondria and microsomes form two quite distinct populations of particles of different dimensions, chemical constitution and enzyme content, the mitochondria can nevertheless be subdivided into at least four chemically and enzymologically distinct fractions (Kuff & Schneider, 1954; Paigen, 1954).

The cell nucleus contains DNA, a characteristic component of the chromosomes. The mean amount of DNA per nucleus, as determined by chemical analysis of nuclei in bulk, is approximately constant for the diploid nuclei of all the tissues of a given species although it varies widely between one species and another (Vendrely & Vendrely, 1948; Davidson, 1953*a*,*b*). Haploid nuclei in sperm cells contain half this amount. Most rat tissues, e.g. kidney, contain about 0·65 pg (picogram, 1 pg $=10^{-12}$g) DNA phosphorus per nucleus but liver tissue shows the much higher value of about 0·913 pg (Thomson, Heagy, Hutchison & Davidson, 1953), owing to the presence of many tetraploid nuclei with twice the normal diploid DNA complement as determined by estimations of DNA by spectrophotometric methods on individual nuclei (Swift, 1950; Frazer & Davidson, 1953). From cytological evidence it is known that in the adult rat liver 70% of hepatocytes are tetraploid (McKellar, 1949) so that the mean DNA phosphorus content of the hepatocyte nucleus is $(0{\cdot}65 \times 0{\cdot}30) + (2 \times 0{\cdot}65 \times 0{\cdot}70)$ pg, i.e. 1·105 pg. But hepatocytes account for only 60% of total liver cells (Abercrombie & Harkness, 1951) so that the mean DNA phosphorus content of the rat-liver nucleus should be $(1{\cdot}105 \times 0{\cdot}60) + (0{\cdot}65 \times 0{\cdot}40)$ pg or 0·923 pg which agrees with the figure of 0·913 pg found by Thomson *et al.* (1953).

Thomson *et al.* (1953) have shown that for rats of the same age, sex and initial body-weight drastic alterations in the diet (e.g. fasting, protein-free diet) cause no statistically significant changes either in the total amount of DNA per liver or in the mean amount of DNA per liver nucleus. Consequently it is possible to use DNA as a reference substance in terms of which the composition of a tissue may be expressed as amounts of a constituent per unit of DNA or per cell.

The results in Table 1 for the livers of fed and fasted rats, expressed in the usual way in terms of units of protein nitrogen (PN), lipid phosphorus (LP), ribonucleic-acid phosphorus (RNAP) or deoxyribonucleic-acid phosphorus (DNAP) per 100 g tissue show that a fast of 48 h is followed by a sharp rise in DNA concentration owing to loss of lipid and stored protein resulting in an increased number of cells per unit weight of liver tissue and by a rise in the concentration of LP, PN and RNAP. These figures may easily be misleading since the absolute amounts of DNA per liver do not alter on fasting while those of the other constituents fall. This is clearly shown when the mean amounts per cell are calculated (Table 1). Liver is in many ways a peculiarly complicated tissue since in addition to polyploidy it shows the presence of many binucleate cells. Harrison (1953*a*,*b*) has devised a method of

Table 1. *Effect of a 48h fast on the composition of rat-liver tissue in terms of deoxyribonucleic-acid phosphorus (DNAP), lipid phosphorus (LP), protein nitrogen (PN) and ribonucleic-acid phosphorus (RNAP)*

Condition of rat	DNAP	LP	PN	RNAP	Tissue mass
			mg/100 g liver		
Fed	21	111	2380	92	—
Fasted	33	140	2603	108	—
			mg/liver		
Fed	1·7	9·0	193	7·5	—
Fasted	1·8	7·7	146	5·9	—
			pg*/pg* DNAP		
Fed	1	5·3	116	4·4	4810
Fasted	1	4·2	79	3·3	3080

*Picogram, 1 pg = 10^{-12} g.

calculation to allow not only for polyploidy but also for binucleate cells and for extracellular tissue in the calculation of the composition of the mean liver cell; some of her results are shown in Table 2. The most striking features are the loss in water, protein and glycogen on fasting and the sex differences in the content of iron and neutral lipids.

Table 2. *Composition of the diploid liver cell of the fed and fasted adult rat (Harrison, 1953b)*

(Values in $\mu g \times 10^{-4}$)

	Male rats		Female rats	
Constituent	Fed	Fasted 6 days	Fed	Fasted 6 days
Water	12·5	7·7	10·3	7·1
Protein	4·35	3·39	3·56	2·68
Glycogen	1·17	0·15	0·87	0·06
Neutral lipids	0·41	0·40	0·13	0·85
Phospholipids	0·76	0·57	0·59	0·50
RNA	0·26	0·20	0·24	0·17
DNA	0·06	0·06	0·06	0·06
Iron	0·0035	0·0041	0·0096	0·0101
Copper	0·00011	0·00011	0·00012	0·00010

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The Influence of the Protein and Energy Content of the Diet on the Liver

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Pflüger (1903) in the course of a long paper on glycogen claimed that the liver is an organ for the storage of protein. This deduction, for which Pflüger offered only indirect evidence, led his pupil Seitz (1906) to study the nitrogen content of the livers and carcasses of hens and ducks receiving a diet rich in protein. In comparison with fasting birds, the feeding of protein caused the retention of large amounts of nitrogen in the liver, but only slight changes in the total nitrogen content of the carcass. These observations have since been confirmed on other species by a number of investigators (see review by Kosterlitz & Campbell, 1945–6), the most notable studies being those of Addis, Poo & Lew (1936*a–c*). They showed that, when rats undergo a 7-day fast, the liver loses 40% of its initial total protein content, the prostate and seminal vesicles 29% each, the alimentary tract 28%, the kidneys and the drawn blood 20% each, the heart 18%, the carcass 8% and the brain 5%; no protein was lost from the eyes, testicles or adrenals. A difference between the proportion of protein lost from the liver and from other tissues was also evident during the first 2 days of feeding a protein-free diet. On re-introducing a large amount of protein into the diet, the liver rapidly increased its protein content but the carcass protein underwent only a small change. A considerable rise in kidney protein on the high-protein diet is attributed by Addis *et al.* (1936*c*) to work hypertrophy.

The protein content of the liver is influenced by energy intake as well as by protein intake. Using diets containing substantial amounts of protein, Campbell & Kosterlitz (1948*a*) showed that a reduction in energy intake *per se* reduced the amount of liver protein in proportion to caloric deficit. Munro & Naismith (1953) confirmed that the total amount of protein in the liver varied linearly with energy intake when the diet provided adequate amounts of protein; however, addition of energy to a protein-free diet tended to reduce the amount of liver protein. On both types of diet, no distinction could be drawn between the effect of energy in the form of carbohydrate or of fat. Comparison of the changes in the liver with those occurring in other tissues showed that energy intake, like protein intake, had a greater effect on the protein content of the liver than on the protein of other tissues generally. The addition of 1000 Cal./sq.m body surface area caused a 23% increment in liver nitrogen, whereas the other viscera underwent a change of only 3%, and so did the carcass.