Table 1.

200° C, 8 ml N_2/min	Steel gauze		NaCl
Outlet pressure, mm Hg	50	20	20
Pressure drop » »	4	4.5	52
Inlet pressure » »	54	24.5	72
HETP* for C_{12} cm	2.2	4.2	3.6

* HETP has been calculated according to a formula given by van Deemter *et al.* in "Symposium on Vapour Phase Chromatography, London, 30th May—1st June 1956", Butterworth's Scientific Publications, p. A 21.

further enables the use of lower column temperatures or higher outlet pressures, and in both cases the degree of separation obtained from a column is increased considerably. It is also possible to use long columns, the pressure drop in the case of a 4 m column being for instance 8 mm mercury.

It remains to be ascertained whether the type of packing described is also applicable for vapour-phase chromatography at normal pressures, but considering the great similarity between vapour-phase partition chromatography and fractional distillation there is every probability that this is the case.

1. Cropper, F. R. and Heywood, A. *Nature* **174** (1954) 1063.

Received November 15, 1956.

Distribution of Enzymes Involved in the Breakdown of Uracil to β-Alanine in Rat Liver Cells

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Previous studies of the catabolism of ^{14}C-labeled uracil, dihydrouracil (DHU) and β-ureidopropionic acid (BUP) in the rat [1,2] demonstrated the following reactions in rat liver slices *in vitro* and in the intact rat:

Uracil → DHU ⇌ BUP → β-alanine

The results further indicated that the liver is the main site of these reactions. The localization of the enzymes responsible for the different reactions in this process in the liver cells, has now been investigated.

White rats were decapitated, and the livers were homogenized in 0.25 M sucrose as described by Ernster and Löw [3]. Uracil-6-^{14}C *, DHU-6-^{14}C and BUP-3-^{14}C were incubated with the homogenate and with three fractions of the homogenate obtained by successive removal of nuclei, mitochondria and microsomes by fractional centrifugation at 1 600 × g for 10 min, 4 100 × g for 15 min, and 105 000 × g for 45 min, respectively. The reaction mixtures were analyzed for radioactive compounds by paper strip chromatography in *n*-butanol-acetic acid-water (4:1:5) and in collidine saturated with water, and the distribution of radioactivity along the strips was determined, as described previously [1,2]. The total radioactivity of the incubation mixtures before and after incubation was determined by plating aliquots on planchets and subsequent counting, as described by Canellakis [5]. The results showed, within the limits of experimental error, that no volatile, radioactive compounds were formed during incubation, indicating that all the radioactive products can be detected in the chromatograms. The results are recorded in Table 1. The incubations with BUP as substrate show that the yield of β-alanine is the same in the presence of the homogenate and the particle-free supernatant fluid. The removal of nuclei and mitochondria has no effect on the enzymatic activity. These facts clearly demonstrate that the enzyme catalyzing the conversion of BUP to β-alanine is present entirely in the supernatant fraction. The data from the incubations of DHU with homogenate and supernatant fluid are consistent with the finding that the enzyme catalyzing the interconversion of DHU and BUP was present entirely in the supernatant fraction of isotonic KCl homogenates of rat liver [6]. The incubation of uracil with the supernatant fluid demonstrates that β-alanine is the only radioactive product which can be clearly detected in the chromatograms.

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* The numbering system is that in which uracil is 2,4-dihydroxypyrimidine. The preparation and tests of purity of the labeled compounds are described elsewhere [4,2].

Table 1. DHU-6-^{14}C and BUP-3-^{14}C incubated for 1 h at 30° C with aliquots of liver preparations representing 0.17—0.19 g of fresh tissue in a final volume of 3 ml of 0.25 M sucrose. Gas phase, air. Uracil-6-^{14}C was incubated at 37° C.

Substrate	Liver preparation	Amounts of radioactive compounds detected after incubation *				Radioactivity recovered after incubation ** (in per cent)
		Uracil	DHU	BUP	β-alanine	
8 μmoles BUP-3-^{14}C	Homogenate	0	0	77	23	
	Mitochondria + microsomes + supernatant	0	0	75	25	
	Microsomes + supernatant	0	0	75	25	
	Supernatant	0	0	77	23	96
8 μmoles DHU-6-^{14}C	Homogenate	0	72	6	22	
	Supernatant	0	73	10	17	102
1 μmole uracil-6-^{14}C	Supernatant	78	0	0	17	96

* Expressed in per cent of the total radioactivity on the paper strip after chromatography.
** Determined by plating TCA-treated aliquots of the incubation mixture.

Additional data from the work of Rutman *et al.*[7] indicate that the ureido carbon of uracil is released as carbon dioxide in the supernatant fluid from rat liver homogenates. The particulate fraction was found to inhibit the enzymatic activity. These results were confirmed by the recent work of Canellakis [5], and it was demonstrated that the enzymatic activity of rat liver slices approximated that of the supernatant fluid. On the basis of these findings it can be concluded that the enzyme catalyzing the reduction of uracil to DHU is present mainly or entirely in the part of the cytoplasm represented by the nonparticulate system.

The author wishes to express his deep appreciation to Dr. Hans Löw, Wenner-Gren Institute, for his valuable advice and assistance in connection with tissue preparations.

1. Fritzson, P. *J. Biol. Chem.* (1957) *In press.*
2. Fritzson, P. and Pihl, A. *J. Biol. Chem.* (1957) *In press.*
3. Ernster, L. and Löw, H. *Exptl. Cell Research*, Suppl. **3** (1955) 133.
4. Fritzson, P. *Acta Chem. Scand.* **9** (1955) 1239.
5. Canellakis, E. S. *J. Biol. Chem.* **221** (1956) 315.
6. Grisolia, S. and Wallach, D. P. *Biochim. et Biophys. Acta* **18** (1955) 449.
7. Rutman, R. J., Cantarow, A. and Paschkis, K. E. *J. Biol. Chem.* **210** (1954) 321.

Received November 24, 1956.

On the Molecular Weights of Human and Ox Pituitary Growth Hormones

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A growth-hormone-like principle in human pituitaries has recently been demonstrated by Gemzell and Heijkenskjöld[1]. The human pituitary growth hormone has now been isolated in an electrophoretically pure form [2], and investigations are being carried out concerning its chemical and physical nature. Some preliminary experiments on the sedimentation behaviour and molecular weight of this hormone are re-