

partial loss of this terminal $-\text{CH}_2$ group. (5) Finally, in the region above 8.5μ , the bands at 11.15 , 12.50 and 13.85μ (Fig. 2) appear to be characteristic for both crude and purified cryptococcal polysaccharides.

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Carnitine in Lipid Metabolism

THE finding by Fraenkel and Friedman¹ that carnitine (4-trimethyl amino 3 hydroxy butyric acid) is an essential growth factor for certain organisms (vitamin B_T) and its availability by synthesis have renewed interest in the problem of its biochemical function.

Recent reports suggest that carnitine is implicated in lipid metabolism. Schaepdryver and Vleeschhouwer² showed that it increased the fat content of lymph; Fritz and McEwan³ postulated a role in fat transport; and it has been reported present in vagotonin⁴ and lipocain⁵. Binon and Deltour⁶ reported that, contrary to the belief that the nitrogenous base of dog serum phospholipid was all choline, one-third was actually carnitine.

The chemical similarity of carnitine and choline suggests that carnitine might possess anti-lipotropic activity. This possibility was examined by the 6-day mouse test of Welch and Welch⁷. Seventy young male mice were fed on (a) high fat, negative control diet; (b) positive controls containing 0.5 per cent choline; (c) test diet containing 0.5 per cent carnitine; and (d) test diet containing 1.0 per cent carnitine. The results (Table 1) show that carnitine had no effect on the fat content of the liver. This result substantiates the finding of Fritz⁸ on rats.

As a further examination of the role of carnitine in lipid metabolism the finding of Binon and Deltour⁶ was investigated. These authors analysed the total lipid fraction from dog serum by differential reineckate precipitation⁹. The precipitate at pH 10 was assumed to be choline, and that at pH 2, carnitine. The carnitine was not further identified.

Table 1. TOTAL LIPID CONTENT OF MOUSE LIVERS (FOUR ANIMALS GROUPED IN EACH DETERMINATION)

Group	High diet fat. Negative controls (choline + carnitine absent) (gm./100 gm.)	Plus 0.5 per cent choline. Positive controls (gm./100 gm.)	Plus 0.5 per cent carnitine (gm./100 gm.)	Plus 1.0 per cent carnitine (gm./100 gm.)
Total animals used	20	20	20	10 (2 per group)
1	54.2	10.9	58.6	58.5
2	55.4	12.4	55.0	57.2
3	52.2	19.9	55.4	47.5
4	59.0	11.2	51.3	47.1
5	60.7	22.5	53.0	62.1

The lipids from 300 ml. of foxhound serum were extracted with ethanol/ether and hydrolysed by boiling with methanolic hydrochloric acid (following the procedure of Binon and Deltour). The two fractions obtained by differential reineckate precipitation at pH 10 (voluminous) and pH 2 (trace) were examined by paper chromatography. They were run in the presence of silver nitrate¹⁰ in ethanol/ammonium hydroxide/water (95:5:5) and in a second experiment in isopropanol/ammonium hydroxide/water (20:1:2). The spots were located with iodine and with potassium bismuth iodide. Choline was the only quaternary ammonium compound detected in both reineckate fractions. Direct chromatography of the phospholipid hydrolysate, without reineckate treatment, also failed to reveal the presence of carnitine.

In addition to the serum investigation the phospholipid fraction of beef muscle was examined. The fraction was obtained by petroleum ether/ethanol extraction followed by acetone precipitation¹¹. The hydrolysed material showed no reineckate precipitate under acid conditions, and chromatography revealed choline as the only quaternary ammonium compound.

It thus appears that carnitine is not present in phospholipids of blood or muscle, and that it does not play a part in transmethylations reactions that lead to the formation of choline or betaine.

A detailed account of this work and further investigations into the role of carnitine will be published elsewhere.

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17-Oxosteroids of Pregnant Ewes' Urine

IN view of the increasing use of steroid hormones and stilbene derivatives in agriculture and agricultural research, it was felt that it was necessary to investigate steroid excretion by the ewe.

The isolation of 5β -pregnane- $3\alpha:20\alpha$ -diol from the urine of the pregnant ewe was recently reported by Robertson and Coulson¹. As a continuation of this work the neutral ketonic fraction of pregnant ewes' urine was investigated.

22.5 l. of urine representing twenty 24-hr. collections from two ewes in the fourth month of pregnancy, were acid hydrolysed and extracted in the usual way. The neutral extract was separated into ketonic and non-ketonic fractions by the method of Dorfman².