with other sheep both in metabolism cages and under field conditions.

Clearly, where urea is to be used as a supplement for grazing animals, it is desirable to feed it as a spray rather than as part of a concentrated ration, because toxicity is then no longer a problem. However, whether it is beneficial and economic to feed very high urea levels is doubtful.

It is interesting to note that when high levels of urea are fed, rumen pH rises to nearly 8, and both rumination and rumen movement are inhibited. Whether this rumen paralysis is caused by the ammonia *per se*, or is a consequence of rumen alkalosis, or is an osmotic effect, is not yet clear.

This work is continuing, and will be published in full elsewhere.

J. B. COOMBE
D. E. TRIBE

School of Agriculture,
University of Melbourne.

¹ Clark, Oyaert and Quin, *Onderstepoort J. Vet. Res.*, **25**, 73 (1951).

² Peirce, Moule and Jackson, *Queensland J. Agric. Sci.*, **12**, 107 (1955).

Heparin Content of Adipose Tissue

THERE is now a well-established association between heparin and clearing factor^{1,2} and between heparin and the mast cell³. It has long been known by histologists that mast cells "are numerous in parts where fat is being laid down"⁴. This, and the extraction of active clearing factor preparations from adipose tissue⁵, suggested to us that heparin might be an essential agent in fat mobilization and deposition, and in consequence be present in all animal fat. As a preliminary step we have determined the amount of heparin in the adipose tissue of cattle and the study forms the subject of this communication.

The tissues, already carefully trimmed, were kindly supplied by Mr. A. J. Ross of the Glasgow Abattoir and his description of them appears in Table 1.

Table 1. THE HEPARIN CONTENT OF BOVINE ADIPOSE TISSUE BEFORE AND AFTER DEFATTING

Type of fat	I.U./gm. whole adipose tissue	I.U./gm. of defatted adipose tissue residue
Intestine	0.92	21.6
Rectum and anus	1.5	60.0
Liver	1.3	45.3
Cardiac	1.0	40.3
Uterus	2.0	56.9
Lung	3.89	130.5
Kidney	0.44	28.7
Tongue	0.88	28.3
Mesentery	1.5	45.01
Stomach	0.9	59.0

Each sample of approximately two kilos was defatted with hot alcohol and the fibrous residue dried *in vacuo*. The heparin was then extracted as follows.

The defatted tissue was homogenized in 10 per cent aqueous ammonium carbonate, heated to 70° C. and filtered. The residue was twice re-extracted using further ammonium carbonate solution and the extracts were combined. The crude heparin was precipitated with two volumes of alcohol, collected by centrifuging and dried. The precipitate was taken up in ammoniacal water to give a gelatinous solution which was treated with trypsin and then assayed for heparin.

We are indebted to our colleague Mr. J. Pritchard for the anticoagulant assays⁶, and on a random

selection of samples he also demonstrated that the ant clotting activity was inhibited by protamine, indicating that the activity was due to heparin or similar substances.

Table 1 summarizes the results and shows that all samples contain heparin in appreciable amounts.

We are indebted to the Directors of Evans Medical Supplies for providing the facilities to carry out this work.

H. G. HIND
S. M. MCGINN

The Evans Biological Institute,
Runcorn,
Cheshire.
May 5.

¹ Anderson, N. G., and Fawcett, B., *Proc. Soc. Exp. Biol. N.Y.*, **74**, 768 (1950).

² Korn, E. D., *J. Biol. Chem.*, **215**, 1 (1955).

³ Jorpes, J. E., "Heparin in the Treatment of Thrombosis", 59 (Oxford Univ. Press, 1946).

⁴ Sharpey-Schafer, Sir E., "Essentials of Histology", 86 (Longmans, Green and Co., London, 1920).

⁵ Korn, E. D., and Quigley, T. W., *J. Biol. Chem.*, **226**, 833 (1957).

⁶ Pritchard, J., *J. Pharm. Pharmacol.*, **8**, 523 (1956).

Difference between Lipoprotein Lipase and Post-Heparin Esterase in Human Serum

THE basic component of the clearing factor¹ is a lipolytic enzyme which differs from hitherto known lipases². Its lipolytic action can be demonstrated by the hydrolysis of the fatty components of lipoproteins³. When using β -naphthyl laurate⁴, acetylcholine and butyrylcholine⁵ as substrate the results are negative. With tributyrin the findings are conflicting⁶. If ethyl butyrate is used as substrate, raised esterolytic activity of the sera can be observed in man after the intravenous injection of heparin⁷.

We consider that the acceleration of hydrolysis of ethyl butyrate is the result of the action of an enzyme other than the lipase of the clearing factor, which is definitely inhibited by protamine and heparin *in vitro*⁸. As against this, the post-heparin esterase splitting of ethyl butyrate is not inhibited by these substances (Table 1).

Table 1. THE RESISTANCE OF POST-HEPARIN ESTERASE TO INHIBITION BY PROTAMINE AND HEPARIN *in vitro*

Inhibitor of lipoprotein lipase	Amounts of inhibitor added per ml. serum	Esterase activity per ml. (μ mol. of acid liberated from ethyl butyrate in 24 hr.)	
		Pre-heparin serum	Post-heparin serum
Protamine	1,000 γ	130	167
	100 γ	131	166
	Control	130	167
Heparin	1,000 units	133	195
	100 units	135	198
	Control	135	195

In vitro, protamine inhibits post-heparin esterase only when it is added in excess⁹. Ordinary amounts, which inhibit the lipase of the clearing factor, have no effect, however, on post-heparin esterase. In accord with the findings of Levy and Swank¹⁰, we found that protamine has an inhibitory effect on the activity of post-heparin esterase *in vivo*¹¹. It may therefore be concluded that heparin produces an increase of esterase in the blood-stream, but that it does not form a component part of the molecule of this enzyme. As