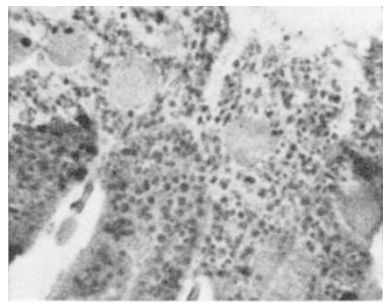

Fig. 1

as an example a section of mouse liver. The droplets of neutral fat (rather few in this section) stain an intense blue, appearing black in layers a few microns thick, the mitochondria grey or blue-grey.

V. B. Wigglesworth

Department of Zoology,
University of Cambridge.
April 1.

[1] Wigglesworth, V. B., *Proc. Roy. Soc.*, B (in the press).
[2] Bolles Lee, A., *La Cellule*, **4**, 107 (1887).
[3] Kolossow, A., *Z. wiss. Mikr.*, **9**, 38 (1892).
[4] Palade, G. E., *J. Exp. Med.*, **95**, 285 (1952).
[5] Sjöstrand, F. S., "Physical Techniques in Biological Research" (edit. by Oster, G., and Pollister, A. W.), **3**, 241 (1956).

## Use of Antipyrene and N-Acetyl 4-Aminoantipyrene in the Measurement of Body Water and the Intraluminal Water of the Gastro-intestinal Tract of Living Cattle

It was demonstrated recently that a knowledge of the water content of the empty body allows the entire proximate composition and the calorific value of the bodies of cattle[1] and pigs[2] to be resolved by means of the well-known inverse relationship between the fat and water contents of the animal body. Indirect methods of measuring body-water have, therefore, a potentially great usefulness in nutritional experiments.

Experiments with eleven cows confirmed that the commonly used reference substance antipyrene is metabolized by the body, less than 2 per cent of doses injected intravenously appearing in the urine in 50 hr. The theoretical distribution in blood at the time of the injection may, however, be calculated by extrapolation from the concentrations in samples of blood taken after distribution in body-water is complete, and the body-water content or space is then readily determined. The same method may also be used with N-acetyl 4-aminoantipyrene as the reference substance. The experiments confirmed that this compound is not metabolized, $99{\cdot}5 \pm 1{\cdot}79$ per cent of the injected dose being excreted in the urine in 50 hr. In consequence, the body-water content at any given time after injection could also be found by an alternative method[3] requiring only single determinations on blood and urine. The two compounds proved compatible in solution, and with certain refinements each could be determined in the presence of the other in blood, urine or digesta by the methods of Brodie[4].

In the course of these experiments, it was observed invariably that values for the water content of animals was greater when using antipyrene than when using N-acetyl 4-aminoantipyrene. The difference was approximately equal to the amount of water to be expected in the gut, suggesting that antipyrene might become distributed in the total body-water and N-acetyl 4-aminoantipyrene in only the empty body-water.

This hypothesis was examined with two steers and two cows each equipped with a rumen cannula and consuming rations of hay, grass or hay and concentrates and with liveweights ranging from 325 kgm. to 680 kgm. The weight of water in the reticulo-rumen was measured by temporary removal of the contents, and from this value the water content of the whole gut was determined assuming that 73 per cent of the gut water is found in this compartment[5]. Examination of rumen contents also showed that the diffusion of antipyrene into the gut was very much more rapid than that of N-acetyl 4-aminoantipyrene. The results (Table 1) showed that when allowance was made for the amounts of the marker entering the gut, the difference between the water spaces for the two reference substances closely approached the value for the amount of water in the gut.

Table 1. Estimates of the Intraluminal Water of the Gastro-intestinal Tract

| Animal | Difference between water space for the two reference substances | | Water in total tract calculated from amount found in reticulo-rumen (kgm.) |
|---|---|---|---|
| | Uncorrected (kgm.) | Corrected for amount of reference substance entering tract (kgm.) | |
| Steer *A* | 46·8 | 55·0 | 53·6 |
| Steer *B* | 75·1 | 92·4 | 90·8 |
| Cow *K* | 51·1 | 64·4 | 65·2 |
| Cow *K* | 47·9 | 65·1 | 66·2 |
| Cow *D* | 69·0 | 80·3 | 82·0 |

These studies encourage the hope that concurrent use of two reference substances will allow the water content to be determined simultaneously in the whole body, the empty body and in the contents of the alimentary canal. They are being continued in an attempt to derive means of making allowance in non-fistulated animals for the amounts of the reference substances entering the gut.

This work was largely carried out while one of us (J. T. R.) was on leave from Cornell University; the award of the John Simon Guggenheim Memorial Foundation that made this possible is gratefully acknowledged. We also wish to thank Mr. D. Millard and Mr. V. W. Johnson for their help with the experiments.

J. T. Reid
C. C. Balch
M. J. Head

National Institute for Research in Dairying
(University of Reading),
Shinfield, Reading.

J. W. Stroud

Cornell University,
Ithaca, N.Y.
Nov. 7.

[1] Reid, J. T., Wellington, G. H., and Dunn, H. O., *J. Dairy Sci.*, **38**, 1344 (1955).
[2] Clawson, A. J., Sheffy, B. E., and Reid, J. T., *J. Anim. Sci.*, **14**, 1122 (1955).
[3] Brodie, B. B., Berger, E. Y., Axelrod, J., Dunning, M. F., Porosowska, Y., and Steele, J. M., *Proc. Soc. Exp. Biol. Med.*, **77**, 794 (1951).
[4] Visscher, M. B., edited by, "Methods in Medical Research", **4**, 31 (Chicago, 1951).
[5] Mäkelä, A., *Acta Agralia Fennica*, **85**, 1 (1956).