Iodination of angiotensin II

SIR,—Recent reports concerning radioimmuneassay methods for the measurement of angiotensin II, using angiotensin iodinated with radioactive iodine, have shown that both synthetic angiotensin II, and the naturally occurring hormone may be estimated at very low concentrations (Catt & Coghlan, 1967; Boyd, Landon & Peart, 1967; Catt, Cain & Coghlan, 1967). It has also been suggested that immunologically active but biologically inactive fragments of angiotensin II in the circulation may interfere with the estimation since the antigenic portion of the molecule is close to the C-terminal end and this could result in differing values for angiotensin II obtained by bioassay and radioimmuneassay (Catt & Coghlan, 1967). A further possible source of error arises from the iodination procedure used, as a result of which there is contamination of the iodinated angiotensin with either unchanged angiotensin or with angiotensin which has been modified in ways other than the introduction of iodine atoms. Of the contaminating reactions the most likely is oxidation, since a potent oxidizing agent is used in all published methods of iodination.

I have investigated the efficiency of the iodination method of Hunter & Greenwood (1962) and found that using 5 μg of angiotensin II and 1 mc of $Na^{131}I$ in the reaction mixture, only about 1% of the angiotensin molecules were iodinated. Neither paper nor column chromatography using several systems proved particularly effective in separating the iodinated hormone from unchanged angiotensin and from other products of the reaction. However, after a prior precipitation of most of the inorganic salts present with methanol, paper electrophoresis using acetic acid: sodium acetate buffer, pH 3·2 and an applied voltage of 7 V/cm resulted in clear separation of iodinated angiotensin from angiotensin and the other products of the reaction. The iodinated angiotensin migrated much more slowly towards the cathode (0·2 cm/hr compared with 1·5 cm/hr) than angiotensin. The iodinated angiotensin could be readily eluted from the paper with methanol. The use of iodinated angiotensin prepared in this way should improve both the specificity and sensitivity of radioimmuneassay procedures using this substance.

Because of previous reports concerning the loss of the biological activity of iodinated angiotensin II (Cruz-Coke, 1946; Wolf, Mendlowitz & others, 1961, 1962a, b, c; Barbour & Bartter, 1963) the effect of modifying the method of Hunter & Greenwood for iodinating synthetic angiotensin II has also been investigated. Excess iodine has been used in place of excess chloramine T, sufficient iodine being liberated from the NaI to ensure complete iodination. The iodinated derivative was eluted after electrophoresis. Amino-acid analysis by Dr. T. Bellair of the Russell Grimwade School of Biochemistry, University of Melbourne, according to Spackman, Stein & Moore (1958) showed that the iodinated material obtained contained the amino-acids present in angiotensin II in approximately the expected proportions but it had no biological activity when tested by two methods (Regoli & Vane, 1964; Osborn, Louis & Doyle, 1966). Subsequent experiments also showed that it differed from angiotensin II in the ability of chymotrypsin to destroy it, as judged by a comparison of the rate of formation of iodo-asparaginyl-arginyl-valyltyrosine and the loss of biological activity of angiotensin II under comparable conditions.

The present results and of those of Catt & Coghlan (1967) suggest that structural modifications of angiotensin involving loss of biological activity do not necessarily affect radioimmuneassay procedures.

Acknowledgments. This work was supported by a grant (G-64) from the National Heart Foundation of Australia. I am indebted to Dr. R. Melick for advice and criticism.

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References

Barbour, B. H. & Bartter, F. C. (1963). *J. clin. Endocr. Metab.*, **23**, 313–314.
Boyd, G. W., Landon, J. & Peart, W. S. (1967). *Lancet*, **2**, 1002–1005.
Catt, K. J., Cain, M. C. & Coghlan, J. P. (1967). *Ibid.*, **2**, 1005–1007.
Catt, K. J. & Coghlan, J. P. (1967). *Aust. J. exp. Biol. med. Sci.*, **45**, 269–282.
Cruz-Coke, E. (1946). *Experimental Hypertension*. III, pp. 32–50. New York: New York Academy of Sciences.
Hunter, W. M. & Greenwood, F. C. (1962). *Nature, Lond.*, **194**, 495–496.
Osborn, E. C., Louis, W. J. & Doyle, A. E. (1966). *Aust. J. exp. Biol. med. Sci.*, **44**, 475–483.
Regoli, D. & Vane, J. R. (1964). *Br. J. Pharmac. Chemother.*, **23**, 351–359.
Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.*, **30**, 1190–1206.
Wolf, R. L., Mendlowitz, M., Gitlow, S. E. & Naftchi, N. (1961). *Circulation*, **23**, 754–758.
Wolf, R. L., Mendlowitz, M., Gitlow, S. E. & Naftchi, N. (1962a). *Circulation Res.*, **11**, 195–205.
Wolf, R. L., Mendlowitz, M., Pick, J., Gitlow, S. E. & Naftchi, N. (1962b). *J. Lab. clin. Med.*, **60**, 150–159.
Wolf, R. L., Mendlowitz, M., Pick, J., Gitlow, S. E. & Naftchi, N. (1962c). *Proc. Soc. exp. Biol. Med.*, **109**, 308–313.

Evaluation and control of variability in hormone-stimulated lipolysis in rat adipose tissue

SIR,—We use adrenaline-stimulated lipolysis in isolated rat epididymal fat pads to assay the antilipolytic property of various prostaglandins (Pike, Kupiecki & Weeks, 1967). For accurate assays and screening tests, small pieces of tissue have proved to be impractical because of the variation in the lipolytic response. This variation has been minimized (Schusterová, Krčíková & others, 1964; Carlson, 1965; Finger, Page & Feller, 1966) by distributing tissue from several rats among replicate vessels or by mincing the fat with scissors and using samples from the minced pool. We have extended and evaluated a method using finely chopped tissue pools to control variability between replicate samples whereby up to 30 replicates with 200 mg of tissue each may be used.

The distal portion of fat pads from 12 to 16 rats (male, 260 to 300 g) are removed under ether anaesthesia and incubated for 2 hr in about 125 ml of Krebs-Ringer bicarbonate solution (without glucose, with 3·2% albumin, always aerated with air 95% and carbon dioxide 5%. This pre-incubation allows basal lipolysis to become minimal (Vaughan, 1967). Each fat pad is cut into "cubes" with a McIlwain chopper (H. Mickle, Gomshall, Surrey) (McIlwain & Buddle, 1953), set for 0·73 mm thickness and 80 to 85 strokes/min. The chopped tissue is pooled in 75 ml of Krebs solution and mixed continuously using a magnetic stirrer. Any tissue not completely chopped is removed and the mixture is then centrifuged for 3 min at 1000 rev/min to separate shreds of filter paper from the chopper pad. The floating cake of chopped tissue is then divided among two or three 50 ml beakers and resuspended with stirring in 25 ml of Krebs solution at room temperature. Tissue may be kept for at least 2 hr without loss of lipolytic activity while stirred and aerated at room temperature, but no more than 30 min in a cake. Just before tissue samples are weighed, they are centrifuged again, and samples of 200 to 205 mg are