A REPLY TO RECENT CRITICISMS OF THE THEORY OF A RELATIONSHIP BETWEEN VITAMIN E AND THE OESTROGENS

By E. V. SHUTE

From the Department of Obstetrics and Gynaecology, University of Western Ontario, London, Ontario

(*Received 23 May 1940*)

Recently Drummond, Noble & Wright [1939] and Cuthbertson & Drummond [1939] have criticized the method employed by the author in determining relationships between vitamin E and blood oestrogens. It would seem appropriate to discuss the questions raised in these papers and to present such further evidence as has accrued to the subject.

In the article by Cuthbertson & Drummond a number of *a priori* objections to the validity of the test are presented. It is stated that the reaction produced can scarcely be due to proteolysis by trypsin because there is no initial increase in the formol titration. However, this characteristic of tryptic digestion was observed years ago by Cole and confirmed by both Wigglesworth [1928] and Fine [1931]. Fine held that trypsin had two active components: a tryptase which acted primarily to liberate free acid and a polypeptidase, which subsequently yielded amino acids. Northrop & Kunitz [1932] say: 'The increase of formol titration . . . measures principally the later stages of digestion and . . . can hardly be considered as the result of hydrolysis of the protein itself.' As the preliminary phase of the digestion only is pertinent to the technique of the writer's test, it is conceivable that no amino acids may appear within the period of observation.

Further, Cuthbertson & Drummond express doubt that trypsin could be responsible for any 'digestion' produced, because the author claimed that acid production might occur over a temperature range of 20–90° C.—although, needless to say, these were not suggested as optimum temperatures for the digestion test. It is to be noted that the solutions are exposed to the ferment for only 40 minutes, and even temperatures considerably higher than 65° take at least this time to inactivate trypsin, especially if a crude preparation is used and it is in acid solution, both of which conditions decrease the lability of trypsin. The experiments of Mellanby & Woolley [1913] and Eddie [1914] showed that trypsin in dilute acid solution could be heated to 100° C. with very little loss in activity. The upper temperature ranges mentioned above were used in order to inactivate antitrypsin, an alternative explanation of the phenomenon observed.

The digestion of trypsin-serum-buffer mixtures produces a gradual rise of acid. The digests are then titrated against alkali, using phenolphthalein for indicator. Many observers have difficulty in detecting slight changes in pink colours. Possibly some other indicator would prove more generally useful, although it is hard to visualize a more readily detectable end-point than that afforded by phenolphthalein. Fine said of this indicator under similar conditions: 'The end-point was remarkably sharp.' The writer found the indicator mixture evolved by Cuthbertson & Drummond much less satisfactory. When phenolphthalein is used it may be difficult to detect the actual end-point, but it is easy to ascertain when one has gone one drop *beyond* it. It appears to the writer that the curves published in Cuthbertson & Drummond's paper (Figs. 1, 2, 3) merely indicate difficulty in determining an end-point. I have had many such curves sent to me by those first attempting the test, have then had these workers do the test under my own supervision and thereafter they have secured satisfactory results.

How 'absorption of CO_2' could account for such variations in the digests as Cuthbertson & Drummond observed is difficult to understand, since the tubes used in the digestions are small, are more than half-filled with fluid, and are kept corked almost throughout the test. It would be still more difficult to believe that a potent trypsin solution could be in contact with serum protein and produce no evidence of digestion at all after even a 'period of two hours and 20 minutes'.

The author's method is designed to detect small initial variations in digestion rate and is more sensitive than a viscosimetric technique. It does not invalidate the results to say that a viscosimetric method, where a number of additional components were involved, failed to show changes analogous to those seen in the titrations. The titration method was deliberately chosen because of its sensitivity and simplicity.

A qualitative test for blood oestrogens or E-deficiency (assuming for the moment one test can detect both) must be only a temporary phase of the development of this subject. However, one can bring forward such sound objections to any conclusions based upon studies of urinary oestrogens only [Polonsky, 1936; Hain, 1939; Moller-Christensen & Pedersen-Bjergaard, 1936], or upon feasible biological assays [Freed, Hechter & Soskin, 1939; Hechter, Lev & Soskin, 1940; and Fluhmann, 1936], that a simple and rapid chemical test upon blood such as the author has attempted to formulate should not be discarded prematurely. Several writers have reported that they have found the test unsatisfactory; others have used it successfully. The latter have usually been those trained in the technique by its originator.

In the paper by Drummond, Noble & Wright [1939], the authors

discuss the physiological basis of the theory that vitamin E acts in the organism principally as an antagonist of oestrogens [Shute, 1936], or to be more specific, that the evidences of E-deficiency are produced by the liberation of an excess of oestrogens. Mention is made, indeed, of Underhill's [1939] work which indicated that vitamin E actually was oestrogenic. Neither Demole [1939] nor Drummond *et al.* [1939] could, however, confirm Underhill's observations.

That E-deficient rats continue to exhibit fertility and oestrous cycles, as Drummond *et al.* point out, is no argument against the theory that oestrogens are liberated by prolonged E-deficiency. Wade & Doisy [1935] gave large daily doses of theelol to forty-one female rats over periods of as long as 316 days, and consistently observed the early re-establishment and continuation of oestrous cycles and that there was unimpaired fertility during such treatment. They found, too, that uterine and ovarian weights revealed an initial decrease during the course of their experiment, but soon returned to normal.

In 1937 the writer published a note [Shute, 1937*a*] on one of the interesting early pregnancies which give a negative Friedman test. In this case the administration of vitamin E was accompanied by a rapid change, over a period of only 48 hours, to a strongly positive Friedman. If this change can be fairly ascribed to the vitamin E that had been given, it certainly suggests that it neutralized an oestrogen excess, allowing prolan to be excreted as in most normal early pregnancies.

However, it would not be surprising if E-deficiency displayed certain specific characters, and from Mason's [1933] work it appears that the effect on the testis is dissimilar to that produced by oestrogen administration. The studies of Einarson & Ringsted [1938], Bicknell [1940] and others suggest, too, that it has a specific action on certain portions of the spinal cord and its nerve roots, as well as on the skeletal musculature. Bicknell points out that this is not due to α-tocopherol but to some other factor in the wheat germ. However, Wechsler [1940] has secured therapeutic results in amyotrophic lateral sclerosis with synthetic *dl*-α-tocopherol. Moreover, the very close analogy between the gynaecological and obstetrical properties of vitamin E and those evinced by progesterone calls for an attempt at a single explanation, the most obvious being that both are effective anti-oestrogens.

The writer has used his proteolysis test upon blood samples taken from nearly 2,000 women showing many different obstetrical and gynaecological conditions, and has consistently found it feasible to consider vitamin E as an anti-oestrogen, in this field at least. It has been possible to time the oestrogen therapy of certain secondary amenorrhoeas and predict the time when menstruation should recur. Weeks, or even months,

before spontaneous abortion or miscarriage or a late toxaemia of the haemorrhagic type [Shute, 1939*a*] has developed clinically it has been possible to predict that such a complication lay in the offing. Late pregnancy toxaemias have been distinguished on this basis when no other differential evidence could be discerned and appropriate therapy, widely different for the two main types, has dispelled the incipient toxaemia in many instances. Cases of senile vulvovaginitis have been analogously differentiated and treated. Many of the blood specimens studied by us have come from outlying points and from patients we have never seen. We know of no instance in which the advice based upon our test has led to improper therapy, although, for example, senile vulvovaginitis associated with a high blood oestrogen, is, to say the least, not helped by the administration of oestrogens. The same remark is even truer of the late pregnancy toxaemias. The clinical field in which a blood oestrogen test has value is enlarging steadily. It has an essential place in the management of hyperemesis, dysmenorrhoea, menorrhagia and many post-menopausal complaints, and in differentiating acute appendicitis and placenta praevia from premature placental detachment. It may be added, in passing, that the test appears to have no significance in cases of habitual abortion. In ten such personal cases, vitamin E in any dose has been valueless. Yet three of these have since carried pregnancies far beyond the usual time of abortion without the assistance of E! These pregnancies have not yet reached term. We define habitual abortion, of course, as the condition in which at least the last three abortions have occurred consecutively at or before the third month. Because this definition has not been adhered to, much of the literature on this topic is fallacious.

For some years reports have appeared which indicate that post-menopausal women often or occasionally excrete oestrogens in the urine [Shute, 1937*b*]. The writer has indicated recently [Shute, 1939*b*], entirely on the evidence derived from his proteolysis test, that at least 67% of 82 post-menopausal women (quite inadequately tested for the presence or absence of cycles) and 75% of 12 of these tested rather more completely, revealed blood oestrogens cyclically or occasionally even long after the cessation of the menses. If more trials had been feasible, it is fair to assume that this percentage would have been appreciably raised. Fluhmann & Murphy [1939] still more recently gave a figure of 89%, derived from a study of 76 such women, using Fluhmann's mucification test (a histological assay technique) and checking the women adequately.

Smith & Smith [1935] were the first to indicate that the blood of eclamptic women revealed low oestrogen values. This observation was confirmed by the writer by means of the proteolysis test, and later by

Mühlbock [1939]. The writer described the beneficial effects of oestrogens in the treatment of these cases [Shute, 1937*a*] and now has observations on ten true pre-eclamptics and six convulsive eclamptics to confirm his first impression. Siegler [1939] has also used this form of therapy with satisfactory results.

It is an easy inference from the theory of E-oestrogen interbalance that if a true pre-eclamptic woman with low values for blood oestrogen be given vitamin E, her blood oestrogen values should fall still lower; if this oestrogen level has any relation to her pathological state perhaps convulsions should then appear.

One of the writer's patients developed a fulminating toxaemia ending in convulsions when large doses of wheat germ oil had been administered without the precaution of a preliminary test of blood oestrogen level. Unfortunately there is a large gap in the chain of evidence and inference here, but a colleague has had two similar experiences and Barrie [personal communication, 1940] has found that some of her rats have displayed clonic convulsions after the administration of large doses of α-tocopherol; these animals at autopsy showed the histological changes she had earlier reported [Barrie, 1939] as bearing a resemblance to those of toxaemic human pregnancy.

If vitamin E and the body's oestrogens are in modifiable equilibrium throughout the year, summer and winter diets should alter the results of oestrogen assays. Such a seasonal change in the bloods of normal male medical students had been observed by the author [Shute, 1938]. A more or less corresponding observation on castrated mice has been reported by Duszynska [1938], who found that more oestrogen was required to produce changes in these animals during the summer months than in the late winter.

In a series of 63 dysmenorrhoeic women, taken at random, the writer found high blood oestrogen values, by the use of his test, in 49% [Shute, 1940]. Kotz & Parker [1937] tabulated their results in 100 such women and reported a figure of 68%. They used the Frank-Goldberger technique.

All of the above evidence indicates that sufficient confirmation of the writer's deductions from both theory and practice has already appeared to stretch the long arm of coincidence very far indeed. It is improbable that these conclusions have been derived from the use of methods involving an excessive margin of error.

REFERENCES

Barrie, M. M. O. [1939]. *J. Obstet. Gynaec.* **46**, 49.
Bicknell, F. [1940]. *Lancet*, I, 10.
Cole, S. W. Quoted by Wigglesworth [1928].
Cuthbertson, W. F. J., & Drummond, J. C. [1939]. *Biochem. J.* **33**, 1621.

Demole, V. [1939]. *Z. Vitaminforsch.* **8**, 338.
Drummond, J. C., Noble, R. L., & Wright, M. D. [1939]. *Journal of Endocrinology*, **1**, 275.
Duszynska, J. [1938]. *Nature*, **142**, 673.
Eddie, E. S. [1914]. *Biochem. J.* **8**, 84.
Einarson, L., & Ringsted, A. [1938]. *Effect of Chronic Vitamin E Deficiency on the Nervous System and Skeletal Musculature in Adult Rats.* Copenhagen.
Fine, J. [1931]. *Biochem. J.* **25**, 647.
Fluhmann, C. F. [1936]. *Amer. J. Obstet. Gynec.* **32**, 612.
Fluhmann, C. F., & Murphy, K. M. [1939]. *Amer. J. Obstet. Gynec.* **38**, 778.
Freed, S. C., Hechter, O., & Soskin, S. [1939]. *Journal of Endocrinology*, **1**, 268.
Hain, A. M. [1939]. *Quart. J. exp. Physiol.* **29**, 139.
Hechter, O., Lev, M., & Soskin, S. [1940]. *Endocrinology*, **26**, 73.
Kotz, J., & Parker, E. [1937]. *Amer. J. Obstet. Gynec.* **34**, 38.
Mason, K. E. [1933]. *Amer. J. Anat.* **52**, 153.
Mellanby, J., & Woolley, V. J. [1913]. *J. Physiol.* **47**, 339.
Moller-Christensen, E., & Pedersen-Bjergaard, K. [1936]. *Acta obstet. gynec. scand.* **16**, 142. (Abstracted: [1937]. *Zbl. Gynäk.* **61**, 830.)
Mühlbock, O. [1939]. *Lancet*, I, 634.
Northrop, J. H., & Kunitz, M. [1932]. *J. gen. Physiol.* **16**, 313.
Polonsky, J. [1936]. *Lpool med.-chir. J.* **44**, 458. (Abstracted: [1936]. *J. Obstet. Gynaec.* **33**, 615.)
Shute, E. V. [1936]. *J. Obstet. Gynaec.* **43**, 74.
Shute, E. V. [1937*a*]. *Endocrinology*, **21**, 594.
Shute, E. V. [1937*b*]. *Canad. med. Assoc. J.* **37**, 350.
Shute, E. V. [1938]. *Amer. J. Obstet. Gynec.* **35**, 609.
Shute, E. V. [1939*a*]. *Vitamin E*, p. 67. London: Soc. Chem. Ind.
Shute, E. V. [1939*b*]. *Endocrinology*, **24**, 744.
Shute, E. V. [1940]. *Canad. med. Assoc. J.* **42**, 145.
Siegler, S. L. [1939]. *J. Lab. clin. Med.* **24**, 1277.
Smith, G. V. S., & Smith, O. W. [1935]. *Surg. Gynec. Obstet.* **61**, 27.
Underhill, S. W. F. [1939]. *Vitamin E*, p. 38. London: Soc. Chem. Ind.
Wade, N. J., & Doisy, E. A. [1935]. *Endocrinology*, **19**, 77.
Wechsler, L. S. [1940]. *J. Amer. med. Assoc.* **114**, 948.
Wigglesworth, V. B. [1928]. *Biochem. J.* **22**, 150.