

my method<sup>1</sup>, especially devised for this purpose. The hormone yield is seen in Table 1.

TABLE 1.

Estrogenic substance	In the body	In the excreta
Æstrone .. .. .	1-2 per cent	0 per cent
Æstrone benzoate ..	80 " "	Not examined
Æstradiolbenzoate ..	40 " "	1 per cent
Æstrone methyl ether	70 " "	5 " "
Stilbæstrol .. .. .	25 " "	25 " "

These experiments show that roughly 50 per cent of stilbæstrol can be recovered and that, in this respect, it resembles the hormone esters. It differs from those substances, however, in that half the amount is eliminated in the excreta.

We further investigated the question whether the slow decomposition in the organism is due to storage at the site of injection, or whether the substances are subject to slow decomposition during their circulation in the body. We injected stilbæstrol and other œstrogenic substances (1,000 I.U. in 0.1 c.c. of oil) into the hind-leg of infantile rats, killing the animals after forty-eight hours, and extracted separately the anterior and posterior half (without viscera). The result was that stilbæstrol was exclusively found in that part which contained the site of injection (Table 2).

TABLE 2.

Estrogenic substance	Anterior half	Posterior half
Æstradiolbenzoate ..	0	40 per cent
Æstrone methyl ether	0	70 " "
Stilbæstrol .. .. .	0	25 " "

The experiments lead to the following conclusions:

(1) In contrast to œstrone, stilbæstrol is only rendered inactive in the organism to a small extent, resembling, in this respect, the hormone esters.

(2) Similarly to the hormone esters, stilbæstrol remains deposited at the site of injection for a considerable time (depot formation) and from there it is slowly absorbed.

(3) Stilbæstrol is distinguished from the hormone esters by the fact that large amounts are eliminated in the excreta, particularly in the urine.

While after absorption the hormone esters are rapidly rendered inactive in the organism, this is not the case with stilbæstrol. In the excreta large amounts of the active substance are found. The fact that the organism is unable to inactivate considerable amounts of stilbæstrol probably helps to explain its eventual toxic activity (compared with œstrone) particularly if large doses are used.

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<sup>1</sup> Zondek, R., *Lancet*, 227, 356 (1938); *Scand. Arch. Physiol.*, 70, 133 (1934); *NATURE*, 143, 282 (1939).

<sup>2</sup> Dingemans, E., and Laqueur, E., *Amer. J. Obst. and Gyn.*, 33, 1000 (1937); Frank Anniversary Volume.

<sup>3</sup> Dodds, E. C., Golberg, L., Lawson, W., and Robinson, R., *NATURE*, 141, 247 (1938).

<sup>4</sup> Zondek, B., and Bergmann, E., *Biochem. J.*, 32, 641 (1938).

#### Estimation and Occurrence of Keto-Acids in Green Plants

ACCORDING to the present conception the keto-acids occupy a central position in the synthesis of amino-acids. Attention has been paid especially to the 'fundamental keto-acids', namely, oxaloacetic acid and keto-glutaric acid, since the amino group of aspartic and glutamic acids formed from them is transferred to other keto acids. In green plants,

however, keto-acids other than oxaloacetic acid—the presence of which in the growing legumes has recently been demonstrated in this laboratory<sup>1</sup>—have hitherto not been found, evidently due to the absence of suitable methods.

Lately we have developed the following technique for the determination of keto-acids in plants.

Caustic soda solution and fine quartz sand are added to plant material<sup>2</sup>. The mass is crushed in a mortar and 2-4-dinitrophenylhydrazine solution, strongly acidified with hydrochloric acid, is added to it. The mixture, the pH of which is less than 1, is allowed to stand for one hour at room temperature, whereupon anhydrous sodium sulphate is added until the mass is dry. The mass is extracted with ether in the Soxhlet apparatus. The ether solution, which contains the dinitrophenylhydrazones formed, is shaken with sodium carbonate solution. The solution is then made acid with hydrochloric acid and the hydrazones are again precipitated from it by allowing the solution to stand for three days in the ice-box. The precipitate is separated, dissolved in alcohol and hydrated according to Tafel<sup>2</sup> but at near to 0° C. with sodium amalgam. Amalgam must be added in small quantities to the reaction solution. Thereby the hydrazones are decomposed so that the keto-acids form the corresponding amino-acids as a reduction product. Alcohol is distilled away *in vacuo* and the rest is dissolved in water. The amino-acids formed are determined in the solution. By means of the method in its present form, 80-90 per cent of the keto-acids are found.

In the above manner we have succeeded in finding and determining oxaloacetic acid, keto-glutaric acid and pyruvic acid in growing peas and red clover. For instance, in an experiment with red clover there were found 42  $\gamma$  keto-glutaric acid, 18  $\gamma$  oxaloacetic acid and 12  $\gamma$  pyruvic acid per one gram of fresh material. Investigations are being continued also with non-legumes. The method is applicable also to the determination of keto-acids in animal material and micro-organisms.

A detailed account of the experiments will be given in the *Biochemical Journal*.

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<sup>1</sup> Virtanen and Arhimo, *NATURE*, 144, 36 (1939).

<sup>2</sup> Tafel, *Ber. deutsch. chem. Ges.*, 19, 1924 (1886).

#### Effect of Ethylene on *Mangifera indica*

THE variations in the chemical changes of the respirable substrate accompanying the process of fruit development and ripening of the different varieties of the tropical fruit *Mangifera indica* were investigated with the view of finding out the nature and the response of the substrate to the different methods of storage. Such investigations would provide helpful information for devising methods of storage of this delicate and easily perishable fruit. Accordingly, investigations as to the changes in the respirable substrate under the different conditions of storage were planned and carried out, first at a lower temperature of 8-12° C., secondly in ordinary room conditions of 28-32° C., and thirdly the effect was examined of different concentrations of ethylene gas as a means of artificial ripening.

The presence of ethylene gas is detected in developing *Mangifera indica* by stunted growth of pea