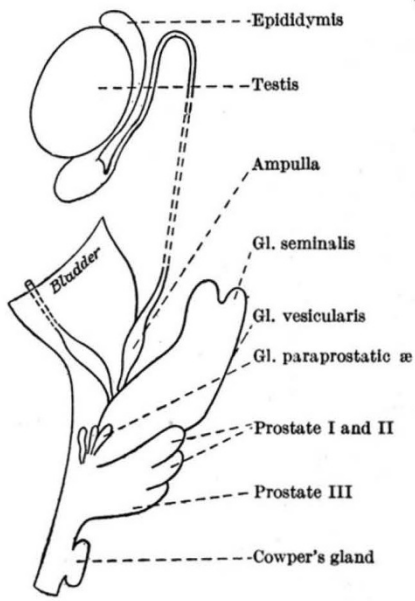
Functional Development of Accessory Glands and Spermatogenesis

WHEN fructose was established as the normal nutrient material for spermatozoa and its origin traced to the seminal vesicles[1], it remained to be explained how certain animals, such as the rabbit, secrete fructose in semen in spite of the absence of seminal vesicles. The rabbit, however, has a large complex organ, sometimes referred to as the 'prostate', which has been described hitherto as composed of three glands, 'gl. seminalis', 'gl. vesicularis' and 'prostate proper', each with an independent urethral outlet[2]. However, our recent investigation of the development of the male reproductive system in the rabbit has established that the gl. seminalis develops in conjunction with the gl. vesicularis from the same diverticulum of the Wolffian duct; further, both glands possess a common urethral outlet, and gl. vesicularis as a whole, rather than the gl. seminalis by itself, should be regarded as homologous to the seminal vesicle in other mammals[3]. This, incidentally, explains the presence of fructose in the gl. vesicularis. It should be pointed out, however, that in the rabbit, apart from this gland, fructose also occurs in the ampulla of the vas deferens as well as in the 'prostate proper'. The distribution of fructose in the various parts of the reproductive system of the male rabbit is shown in the accompanying sketch.



	Weight of organ (mgm.)	Fructose in organ (mgm.)	Fructose (mgm./100 gm. tissue)
Epididymis	600	0·03	5
Testis	2900	0·06	2
Ampulla	130	0·03	23
Gl. seminalis	150	0·04	26
Gl. vesicularis	920	0·79	85
Gl. paraprostatic æ	190	0·092	48
Prostate I and II	335	0·368	110
Prostate III	475	0·272	57
Cowper's gland	1000	0·0	0·0

In the course of investigations on the development of the reproductive system in the rabbit, we noticed that fructose appeared in the accessory glands at an early stage when there was as yet no sign of active spermatogenesis. In a four-months-old animal, both the gl. vesicularis and the prostate showed already a fairly high concentration of fructose (21 and 44 mgm. per cent, respectively) in spite of the complete absence of spermatozoa in the testis or the epididymis. When, in the sixth month of life, the spermatozoa finally made their appearance, the accessory glands were filled with secretory fluid containing the normal high level of fructose. Experiments on bull-calves gave similar results, showing that the appearance of fructose in the secretory fluids of the accessory glands precedes the onset of active spermatogenesis.

Thus it appears that the male reproductive organs first accumulate a store of nutrient material, so that when the motile spermatozoa make their appearance in the generative tract, the fructose reserve is available, ready to be utilized. Together with the recent finding that the formation of seminal fructose requires the presence of the testicular hormone[4], our experiments provide additional evidence that the testicular hormone begins to function in the body some time before actual spermatogenesis.

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Oxidation of Insulin by Performic Acid

FROM the determination of the terminal residues of insulin, it was suggested that the submolecule of molecular weight 12,000 is made up of four peptide chains bound together by —S—S— linkages[1] Thus if one could break the —S—S— linkages without affecting any other part of the molecule, it should be possible to split the insulin into its separate polypeptide chains, two of which have terminal glycyl residues and the other two phenylalanyl residues. Toennies and Homiller[2] showed that the only amino-acids that are appreciably oxidized by performic acid are tryptophan, methionine and cystine, the latter reacting with five atoms of oxygen and presumably forming cysteic acid. Since insulin contains no tryptophan or methionine, this seemed a suitable way of splitting the —S—S— linkages.

Using the procedure of Toennies and Homiller, it was found that the oxidation of cystine to cysteic acid is complete in five minutes. With insulin an oxidation-time of 15 minutes was generally used. The oxygen consumption was the theoretical one for the cystine content, and paper chromatography[3] showed no qualitative difference in the amino-acid composition except the replacement of cystine by cysteic acid. There was no destruction of the free amino-groups[1]. The oxidation product was studied in the electrophoresis apparatus of Tiselius. Unfortunately, it was not possible to dialyse the material, due to its low molecular weight (about 3,000), so that the results were not always entirely reproducible. Fig. 1 illustrates a typical experiment, which shows three components and indicates that the mixture is not unduly complex.

Fig. 1. ELECTROPHORETIC DIAGRAM OF OXIDIZED INSULIN

Fig. 2. ELECTROPHORETIC DIAGRAM OF FRACTION *A*

By neutralization at *p*H 6, two fractions of about equal weight can be obtained. The solution (fraction *A*) contains predominantly (80 per cent) glycyl terminal residues, and the precipitate (fraction *B*) predominantly phenylalanyl terminal residues. By further treatment of fraction *A* a product can be obtained which has no phenylalanyl terminal residues and appears to be homogeneous by electrophoresis (Fig. 2). It contains no lysine or arginine. Estimation of the terminal glycyl residues suggested a molecular weight of about 2,500. Fraction *B* has not yet been further purified.

The work is being continued, and a more detailed report will be published later.

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Malonate and Plant Respiration

SINCE Quastel and Whetham's discovery in 1925 [1], biochemists have come to regard the malonate ion as one of the very few specific enzyme inhibitors—as a competitive inhibitor of succinic dehydrogenase[2] Malonate has therefore been widely used with animal tissue, in work designed to elucidate the Krebs cycle. Recently it has also been applied to plant tissues, but with discordant results. Machlis[3] showed that malonate inhibited part of the respiration of barley roots; but several other workers have independently stated that malonate does not inhibit and may even stimulate plant respiration[4–7].

In 1938, Turner[8] pointed out that the apparent differential effect of iodoacetate on respiration and fermentation was probably explicable in terms of the different rates of penetration of the drug into the cells, under aerobic and anaerobic conditions. In spite of a general increase in our knowledge of cell permeability, this aspect of work with inhibitors and living tissue is still neglected. It is highly probable that the reported lack of inhibition of plant respiration by malonate is due simply to the use of the malonate ion instead of the malonic acid molecule as a cell penetrant.

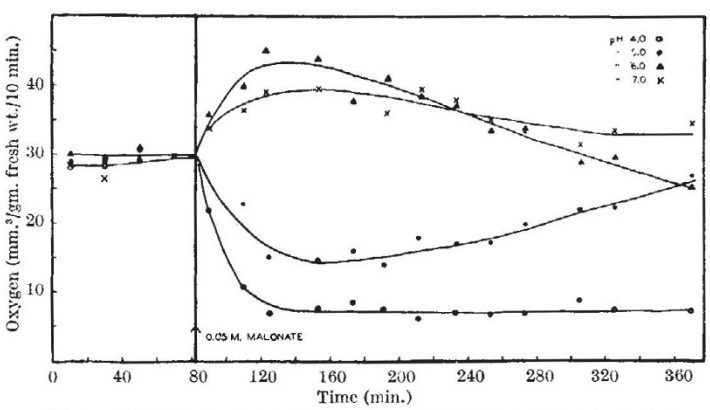

Fig. 1. REACTIONS OF TISSUE RESPIRATION TOWARDS POTASSIUM MALONATE SOLUTIONS OF INCREASING ALKALINITY. UNDISSOCIATED ACID IN SOLUTION: *p*H 4, 98%; *p*H 5, 84%; *p*H 6, 33%; *p*H 7, 5%. CARROT TISSUE, 308 HOURS FROM CUTTING

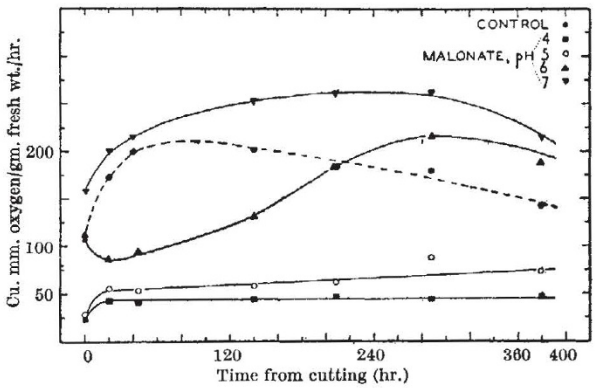

Fig. 2. DRIFT OF RESPIRATORY RESPONSES OF CARROT TISSUE TOWARDS POTASSIUM MALONATE SOLUTIONS, WITH TIME FROM CUTTING. THE VALUES PLOTTED ARE THE MINIMUM (OR MAXIMUM) RATES OF OXYGEN UPTAKE ACHIEVED IN THE SEVERAL EXPERIMENTS

In work with tissue slices of carrot root we have found (Figs. 1 and 2) that potassium malonate (0·05 *M* in distilled water) at *p*H 4·0 causes inhibition of that fraction of the respiration which is inhibited by cyanide[9] The malonate effect at *p*H 4·0 therefore supports the view of Marsh and Goddard[10] that the cyanide-sensitive respiration in carrot is mediated by the cytochrome-oxidase system, for this is coupled with succinic dehydrogenase.

At *p*H 4, about 98 per cent of undissociated malonic acid is present and might be expected to penetrate cell membranes rapidly. As shown in Fig. 1, very different results are obtained if malonate is applied at *p*H 5, 6 or 7, rather than at *p*H 4. At *p*H 7·0, when only 3 per cent of the undissociated acid is present, the potassium malonate causes a clear stimulation of the oxygen uptake. Because of the associated *R.Q.* effects, we provisionally assign this stimulation to the effect of the potassium ion in bringing about an 'Ulrich' effect, that is, a shift in the balance of the organic acids which leads to an increased respiration-rate[11]. Malonate effects at *p*H between 5·0 and 7·0 (but not at 4·0) vary markedly with the period of time that elapses between the cutting of the slices and the experiment (Fig. 2). For example, at *p*H 6·0 (33 per cent molecule) malonate applied to freshly cut tissue produces a clear-cut but partial inhibition of the cyanide-sensitive respiration. If the tissue is first washed in distilled water for 300 hours, the malonate causes stimulation of the respiration; if the tissue is washed for only about 200 hours, the malonate has no effect whatever upon the rate of oxygen uptake. These curious effects are difficult to explain at present; but at least they emphasize once again the fact that freshly cut tissue slices differ remarkably from long-washed, 'aged' slices in their physiological behaviour[12].

Where the authors who have noted no inhibition of plant respiration by malonate have stated fully their experimental technique, it is usually possible to explain their results in terms of the facts recorded above. Malonic acid appears to enter the cell membrane at an appreciable rate only as the undissociated molecule. As the actual enzyme inhibitor is probably the malonate ion, it is to be presumed that the