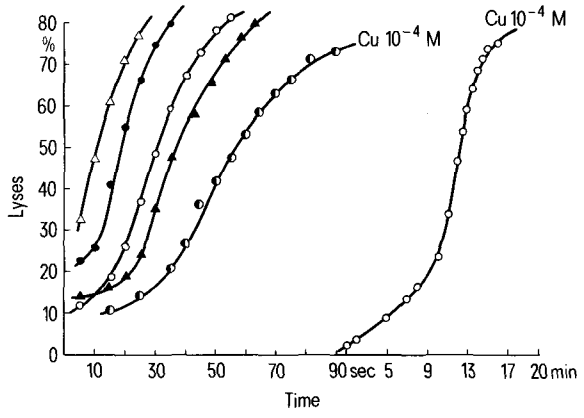
cells of each unit retained their characteristic permeability during the prolonged storage in ACD and CPD. There was the expected slight delay with glycerol monoacetate[9] and very rapid curves of glycerol diacetate and glycerol triacetate. This pattern did not change with routine storage. As intracellular ATP level did decrease with storage, there could not be any correlation between glycerol permeability and ATP levels. Moreover, when intracellular ATP has reduced from 3.0 µmoles ATP/g Hb to 0.2 µmoles ATP, by incubation of fresh cells with sodium fluoride, permeability to glycerol and its derivatives did not change at all. The effect of 10^{-4} M Cu^{++} on the glycerol permeability is plotted on the right, showing marked inhibition, (20-25-fold prolongation, with GLT_{50}-values around 12–14 min).

A much less dramatic effect was obtained by studying the effect of Cu^{++} on permeability to glycerol derivatives. The GLT_{50} of glycerol monoacetate did increase but only by 2-fold. However, the permeability to glycerol diacetate and triacetate was completely unaffected, in fresh and stored cells. Our data demonstrate that substitution of 1 hydroxyl decreases slightly the permeability, and abolishes the inhibitory effect of copper ions. This suggests a different kind of permeation mechanism; the increased size of the molecule may contribute to this decrease in permeability[10]. The additional acylations increase the permeability markedly in spite of the increased molecular size. This again suggests a transport mechanism which is dependent on lipid solubility, but is not energy dependent.

Our data indicate that the major pathway of permeation of the acylated glycerols is via the non-facilitated mechanism, as it is nearly unaffected by copper ions. It seems that, while the main mechanism of permeation of glycerol into the erythrocyte is via facilitated diffusion, this mechanism is negligible in the derivatives.

Another conclusion which may be drawn from these studies is that the intact membrane of the fresh erythrocyte and the changed membrane after storage have similar permeability patterns for glycerol and its derivatives.



Glycerol hemolysis time curves in fresh and outdated blood. Glycerol is represented as O——O, glycerol monoacetate as ▲——▲ and ◐——◐. The presence of copper ions is notted on the curves. The curves of glycerol diacetate (●——●) and glycerol triacetate (△——△) are identical with and without copper ions.

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The boar-pheromone steroid identified in vegetables

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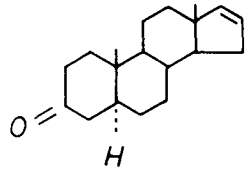
Summary. The steroid 5a-androst-16-en-3-one, known as a boar pheromone, was identified in parsnip *(Pastinaca sativa)* and celery *(Apium graveolens)*. Concentrations are in the range of 8 ng/g plant.

The steroid 5a-androst-16-en-3-one (figure) is synthesized in the testes of boars and released to the bloodstream where it is measurable in the range of ng/ml bloodplasma. It is stored in fat tissue, concentrations being in the range of µg/g fat, and it is delivered to the salivary glands and the saliva. Though it is a C-19-steroid, it has no androgenic activity. However, it has characteristic smell properties, usually described as urine-or perspiration-like, thus causing problems in the utilization of meat of intact male pigs. For female pigs in oestrus, however, it is a very desirable 'male perfume' which is released by the boar's saliva before mating and stimulates the female's 'standing-reflex', thus acting as an aphrodisiac pheromone[1]. Occurrence of this compound in boars has led to the term 'boar-taint-steroid' and so far its presence has not been demonstrated in other species, with the exception of man[2, 3].

As a result of a chance observation, we have now demonstrated the presence of the 'boar-taint-steroid' 5a-androst-16-en-3-one in roots of the parsnip plant (*Pastinaca sativa* L.) and in celery *(Apium graveolens)*. The initial impetus for these investigations was provided by the wife of one of the authors.

She was familiar, from her husbands work, with the characteristic smell of boar taint, and noticed this smell when cooking parsnips grown in her garden. Therefore analytical screening in parsnip was carried out by introducing extracts from the root into the routine radioimmunoassay (RIA) for 5a-androst-16-en-3-one[4]. The 1st extracts assayed gave positive results. Consequently further analytical investigations in various vegetables were carried out: 100 g of vegetable were homogenized with 250 ml of water. Aliquot portions (0.5–2.0 ml) were removed, extracted with methylene chloride and the evaporated extract was directly measured radioimmunologically. The main part of the homogenate was mixed with 200,000 dpm ^{3}H-5a-androst-16-en-3-one (sp. act. 15 Ci/mM), extracted 2 times with methylene chloride/ethyl acetate = 1/1 (v/v) and the solvent was evaporated.

Extracts were purified on silica gel columns (270 × 10 mm) using cyclohexane/ethyl acetate = 98/2 (v/v), further on thin-layer plates (silica gel; Merck, Darmstadt) by the system benzene/acetone = 90/10. Aliquot parts of the eluate were measured radioimmunologically. Thus parsnip samples were measured from the 1977- and 1978-harvest and from the market. For comparison, carrots (*Daucus carota* L.), potatoes (*Solanum tuberosum*), fennel (*Foeniculum vulgare*), radish (*Raphanus sativus*), scorsonera (*Scorzonera hispanica*), parsley (*Petroselinum hortense*) and – due to some similarities to the taste of parsnip – celery were treated and measured identically. Results of the radioimmunological measurements in crude and purified extracts of parsnip roots are shown in the table.



5a-androst-16-en-3-one ('boar taint')

5a-androst-16-en-3-one ('boar taint steroid') in roots of the parsnip plant (concentrations in ng/g root; measurements after purification corrected for procedural losses)

Grown 1977		Grown 1978		From the market	
Crude extract	Purified extract	Crude extract	Purified extract	Crude extract	Purified extract
6.3	5.5	11.4	9.6	9.5	8.0

In all 3 pools of samples, the presence of 5a-androst-16-en-3-one could be clearly demonstrated by RIA. Concentrations in crude extracts are only slightly higher compared to extracts where the steroid was isolated by chromatography. In crude extract from celery, a concentration of 9 ng/g plant was detectable, in purified extracts 5.6 ng/g plant were measured.

Surprisingly, the concentrations are remarkably high. For comparison, concentrations in peripheral blood-plasma of mature boars (expressed in ng/ml plasma) are in the same range. In the other vegetables, measurements revealed negative results.

The identity in parsnip as well as in celery was confirmed by combined gas chromatography – mass spectrometry (GLC-MS; LKB 2091 instrument, 1.5 m GLC glass column with 1% OV 3 on chromosorb WHP operated at 195 °C; 20 ml He/min). GLC retention time (5.8 min) as well as molecular weight (m/e 272) and the fragmentation pattern of the substance from parsnip and celery extracts were identical with authentic 5a-androst-16-en-3-one[2].

The occurrence of steroidal compounds in plants is not unique. Oestrogens, for example, were isolated from plants as early as 1933[4]. It is also known that some plants may mimic pheromonal substances[5]. At the moment, biological function – if any – of boar taint substance in parsnip is not known. Neither is it known if the boar taint substance in celery contributes to the 'libido-supporting' property for which this plant has some popularity.

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Control of the hypothalamus-pituitary-adrenal axis in young and older rats injected with thyroxine[1-3]

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Summary. Administration of T_4 on alternate weeks for 30 weeks at a dosage which does not alter body weight depresses basal serum corticosterone levels in older rats (575 days), but not in young animals (260 days). Similar serum corticosterone response to HPA axis stimulation occurs regardless of age or T_4 injection.

The depression of activity of the hypothalamus-pituitary-adrenal (HPA) axis with age has been reported in numerous species from mice[4] to men[5], including rats[6]. On the other hand, some investigators have reported similar HPA response 15 min after stress in 2.5-, 7.5-, 12-, 18-and 26-month-old rats[7]. Studies of other aging neuroendocrine axes have included demonstration of altered control of hypothalamus-pituitary-thyroid system, resulting in depressed circulating thyroid hormone levels[8-10]. Since hypothyroidism alters the response of the HPA axis to stress in the rat[11] and mouse[12], the present study was conducted to determine the influence of low doses of thyroxine on HPA function in young and older rats.

Materials and methods. Male Holtzman rats were obtained from the supplier and started on experiment at either 50 days or approximately 1 year (retired breeders) of age. L-thyroxine (T_4, sodium salt) was injected s.c. 6 days per week, every other week for 30 weeks, according to the schedule of Everitt[13] based on the data of Grad and Hoffman[14] for a low hormone dosage. Young rats received a total of 0.825 mg T_4 in 90 injections and older rats received 1.65 mg on the same schedule. Control animals were administered saline injection vehicle. At the end of the 30th week, 44 animals per age and thyroid status group were randomly divided into 4 HPA test groups of 11 animals each. HPA response was measured by comparing the adrenal and serum corticosterone (B) concentrations in unstimulated rats (basal) to those in animals 15 min after either: exposure to ether fumes for 1 min (ether stress); s.c. injection of 8 IU depot adrenocorticotropin[15] (exogenous