hosts. These tumors therefore were phenotypically of host type.

The immunologic mechanisms of HVGD are complex and beyond the scope of this paper. I suggested originally that bidirectional immune reactions, $P \rightarrow F_1$ and $F_1 \rightarrow P$, were probably involved[3]. Further experiments have however provided more evidence that the $F_1 \rightarrow P$ reaction is the crucial reaction[4]. The immunogenetic basis of this reaction has not been clarified but certain features resemble hybrid resistance and its in vitro counterpart, F_1 antiparent cell mediated lympholysis[5].

Tumor incidence in low and high dose $(SB)F_1 \rightarrow B$ HVGD and in $B \rightarrow B$ and B controls

Group	Cell donor	No. mice with tumors (%)[a] / No. mice autopsied	p[b]
Low dose HVGD[c]			
1	$(SB)F_1$	12/23 (52.2)	
2	B	4/20 (20.0)	0.05
High dose HVGD[d]			
1	$(SB)F_1$	22/34 (64.7)	
2	B	1/18 (5.6)	0.001
Normal B mice	–	3/53 (5.7)	0.001[e]

[a] Cumulative histologic tumor incidence up to 18 months of age. [b] χ^2 test. [c] Weekly × 20 cell dosage according to age at time of initial cell dose as follows: 0–48 h, 10 million; 48–72 h, 20 million; 72–192 h, 30 million. There was no mortality. [d] Weekly × 2–3 cell dosage according to age at time of initial cell injection as follows: 0–48 h, 30–50 million; of 82 injected mice, 53 died of acute HVGD and 29 survived. At age 48–72 h a single dose of 75 million cells killed 1 of 5 mice and 6 weekly doses of 75 million cells beginning at this age killed 6 of 7 mice. Thus in high dose HVGD, of 94 B mice injected with $(SB)F_1$ cells, 34 survived for long-term observation. [e] Compared to both low and high dose HVGD mice.

Since in high dose HVGD F_1 cell injection into P hosts on the 2nd day of life was tumorigenic, whereas in low dose HVGD it was not, some change must have occurred in the P host mouse by the 2nd day which could be overcome by a larger dose of F_1 cells. If one assumes that HVGD is due primarily to $F_1 \rightarrow P$ reactivity[4], which is opposed by classical H-2 barrier $P \rightarrow F_1$ reactivity[3], one may be dealing with the ontogeny of the latter reaction which is as yet not detectable by GVH assay. (GVH assay of nursing B mice of various ages showed that in the B strain significant anti-F_1 reactivity developed between the 4th and 5th days of life; it increased gradually in the 1st month, and compared to 3.5 month old mice, had declined significantly at 16 months (unpublished observations).) The very late development of most lymphomas in HVGD mice may therefore bear some relation to decline in immune function of the host mouse in old age.

Although the mechanism of tumor induction remains to be elucidated, HVGD can already be added to the list of immunologic syndromes found to be lymphomagenic, e.g. the GVHR[6] and the post-thymectomy state[7]. If further research should confirm the strong suspicion of the basic similarity of HVGD and HR, it would mean that a natural lymphoma-leukemia surveillance mechanism[5] can by manipulation become tumorigenic. This is relevant to clinical bone marrow transplantation.

1 Supported by U.S.P.H.S. grant No. 15,500.
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Permeability of fresh and stored human erythrocytes to glycerol and its acylated derivatives[1]

Y. Yaeger, I. Nathan, A. Dvilansky and N. Meyerstein[2,3]

Soroka Medical Center and Faculty of Health Sciences, Ben Gurion University of the Negev, Beer-Sheva (Israel), 23 February 1979

Summary. The effect of copper ions on the permeation of glycerol and its mono-, di-, and triacetate derivatives was studied in fresh and stored erythrocytes. Permeability was unchanged with storage. Acylated glycerols permeate the cells mainly by non-facilitated mechanism, as their transport is almost unaffected by copper ions.

Glycerol permeability has been studied extensively, as a parameter for membrane function in erythrocytes of different species. Human erythrocytes have high permeability to glycerol, like those of rats, rabbits and guinea-pigs, whereas those of pigs, dogs and cats have low permeability[4,5], and those of the camel have extremely low permeability[6].

Recently, Carlsen and Wieth[7] have demonstrated by ^{14}C-glycerol exchange that glycerol transport is performed by 2 mechanisms: facilitated diffusion[8] susceptible to inhibition, and an unspecific pathway for individual molecules. In this study we investigated the permeability patterns after substitution of hydrophilic hydroxyl groups by hydrophobic moieties[9–11]. Permeability studies were also performed using these derivatives after storage, when the lipid composition and other membrane characteristics are changed[12–14].

Materials and methods. The experiments were performed on fresh erythrocytes or on cells taken out of blood units, after different periods of storage in ACD and CPD media. Glycerol lysis time (GLT) and GLT 50 were determined for glycerol and its acylated derivatives as reported previously[6,15,16]. The rate of erythrocyte lysis was followed by recording the fall in the density of the reaction mixture in a Gilford Microsample Spectrophotometer 300 – N (GLT_{50} corresponds to the time required for the OD to fall to half the initial value[15]). The determinations were performed in room temperature, pH 6.8, with or without copper ions (10^{-4} M and 10^{-5} M $CuCl_2$). Cells of fresh units were incubated for 2 h at 37 °C with or without 5 mM sodium fluoride. ATP levels and glycerol permeability were examined before and after incubation, as described above.

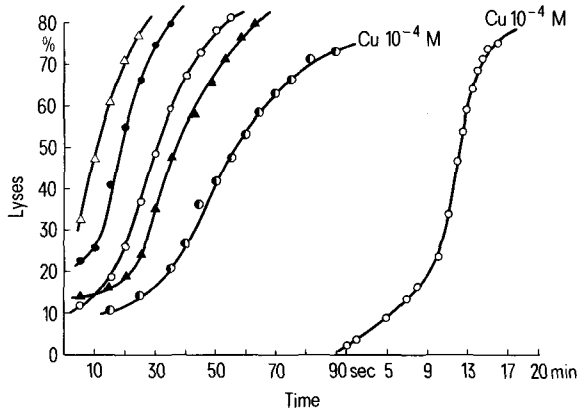
Results and discussion. The figure 1 illustrates the typical kinetics of glycerol transport and that of glycerol derivatives in the presence and absence of copper ions. On the left we see the rapid lysis curves with glycerol. The GLT_{50}-values of fresh cells varied between 30 and 60 sec[15]. The

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.

1 Portions of this work were included in the graduate thesis of Dr. Yaeger in partial fulfillment of the requirements for the M.D. degree.
2 Acknowledgement. We thank Mrs Dalia Mazor for expert technical assistance.
3 To whom reprint requests should be addressed: Faculty of Health Sciences, Ben Gurion University of the Negev, Beer-Sheva (Israel).
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The boar-pheromone steroid identified in vegetables

R. Claus and H.O. Hoppen

Institut für Physiologie, Südd. Versuchs- und Forschungsanstalt für Milchwirtschaft, Technische Universität München, D-8050 Freising-Weihenstephan, and Institut für biochemische Endokrinologie, Medizinische Hochschule, D-2400 Lübeck (Federal Republic of Germany), 13 March 1979

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.