alleles have been recognized so far. All populations proved polymorphic for at least 2 *Pept*-1 alleles. The average degree of heterozygosity of the *Pept*-1 gene (=44.6) is clearly above the average for all loci calculated for this species by Pinsker and Sperlich[7] (13.7; 17 loci in 7 populations) and Marinkovic et al.[11] (11.4; 28 loci in 1 population). Hence, the *Pept*-1 gene must be considered one of the most polymorphic in *D. subobscura* (like *Aph*-3, *Hk*-1, and *Lap*-4[7]).

From the table it is further seen that a general and quite strong cline exists for the frequencies of the *Pept*-1 alleles 0.40 and 1.00 moving from north to south. A frequency difference of about 50% is visible if the 2 most peripheral populations, Sunne and Bizerte, are compared. The correlation between the frequencies of the allele 0.40 and the geographic latitudes is statistically highly significant ($r = -0.874$ with 6 d.f., $p < 0.01$).

The cline in *Pept*-1 alleles of *D. subobscura* which we observed in the present survey appears to be of special interest since such clinal changes of allozyme allele frequencies are not common in natural populations of other *Drosophila* species[12]; yet some cases have been reported[13]. Our finding can hardly be explained by genetic drift between geographically separated populations alone. It might rather be connected with the strong inversion polymorphism present in this species. Since gene arrangements are not thought to be selectively neutral and since the frequencies of a number of inversions on chromosome 0 of *D. subobscura* follow geographically a quite similar pattern to that observed for the alleles of the *Pept*-1 locus, coselection due to tight linkage seems a plausible explanation. This assumption is sustained directly by our data and the inversion frequency data if the results from neighbouring 'continental' populations and 'island' populations (populations 1 vs 2, 5 vs 6, and 8 vs 7) are compared. The frequencies of the *Pept*-1 alleles in the 'island' populations always differ somewhat from the 'continental' populations. Such a differentiation is also found for the frequencies of the various chromosomal arrangements of chromosome 0 of *D. subobscura*[14].

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Effects of prednisolone and butyrate on agglutinability of HeLa cells by concanavalin A[1]

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Summary. Agglutinability by concanavalin A was measured with $HeLa_{65}$ cells grown with prednisolone or sodium butyrate, 2 compounds that increase the activity of the carcinoplacental form of alkaline phosphatase, an enzyme localized in membranes. Prednisolone enhanced concanavalin A agglutination approximately 3-fold while sodium butyrate had no effect.

HeLa, a cervical carcinoma cell line, has been extensively characterized and provides a good model for studies of gene expression. Both prednisolone (Δ^1-hydrocortisone), a steroid hormone with potent glucocorticoid activity, and sodium butyrate, a 4-carbon aliphatic monocarboxylate, alter gene expression in $HeLa_{65}$ cells. Both increase the activity of the carcinoplacental form of alkaline phosphatase[2-6], an enzyme localized in membranes. Butyrate induces synthesis of human chorionic gonadotropin[7-10] whereas prednisolone does not[10]. Prednisolone also causes a morphological alteration with flattening of $HeLa_{65}$ cells and an increase in the amount of cytoplasm[2,5] whereas butyrate causes $HeLa_{65}$ cells to become more spindle shaped and fibroblastic[5].

In the present study, $HeLa_{65}$ cells were grown with either prednisolone or butyrate and the effect on agglutinability

Agglutinability of $HeLa_{65}$ cells by concanavalin A: The effects of prednisolone and butyrate[a]

Experiment	Rate of agglutination (ΔA_{546}/min) Control EDTA	EDTA + trypsin	Prednisolone EDTA	EDTA + trypsin	Butyrate EDTA	EDTA + trypsin
1	0.015		0.057			
2	0.016		0.094			
3	0.021		0.043			
4	0.019				0.020	
5	0.024	0.028				
6	0.017	0.026				
7	0.019	0.032				
8	0.016	0.031				
9		0.024		0.066		0.015
10		0.024		0.067		0.037

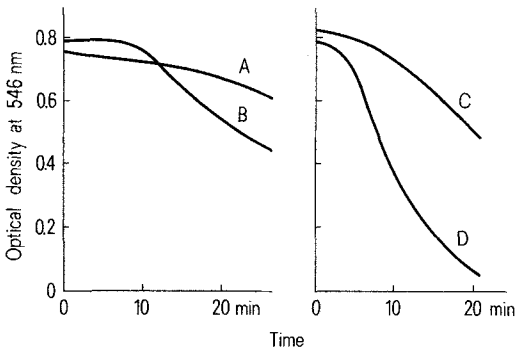
[a] Replicate cultures were grown for 120 h in medium with or without 3 μM prednisolone or 1 mM butyrate. Cells were harvested with 0.02% EDTA alone or with 0.02 EDTA + 0.04% trypsin in Puck's saline A. Each agglutination mixture contained 1 mg con A and 1.25×10^6 cells in a total volume of 1 ml. The agglutination rate without con A (< 0.008 for control cells, < 0.011 for butyrate-treated cells, < 0.013 for prednisolone-treated cells) was subtracted in each case.

by concanavalin A (con A) was measured. Plant lectins that bind to specific carbohydrate-containing sites on cell surfaces often are used as membrane probes to detect surface alterations. Con A, a plant metalloprotein, causes agglutination of fetal cells[11,12] as well as mammalian cells transformed by oncogenic viruses[13,14] and chemical carcinogens[15]. Normal cells, although possessing binding sites, can only be agglutinated following treatment with proteolytic enzymes[16,17]. Moreover, both normal and transformed cells bind equal amounts of the lectin at room temperature, indicating that the alteration of cell membranes which promotes agglutination is not simply the unmasking of cryptic receptors[17,18].

Materials and methods. $HeLa_{65}$ cells were grown in monolayer culture in Waymouth's medium containing 10% fetal calf serum and antibiotics (50 units penicillin, 50 μg streptomycin and 35 μg kanamycin per ml). The methods of maintaining cultures and subculturing have been previously described[3]. Agglutination of $HeLa_{65}$ cells was measured by the sedimentation kinetics of cell clusters as determined spectrophotometrically at 546 nm by the method of Hwang et al.[19]. Replicate flasks of $HeLa_{65}$ cells were grown for 120 h in complete medium without (controls) or with either 3 μM prednisolone of 1 mM sodium butyrate. Cells were removed from glass surfaces by 2 methods. Monolayer cultures were incubated for 3 min at 37 °C with 0.02% EDTA alone or with 0.02% EDTA and 0.04% trypsin in Puck's saline A. Cell suspensions were counted in a hemocytometer, washed by centrifugation in 0.15 M saline, and suspended in 0.15 M saline. In all experiments, agglutination mixtures contained 1.25×10^6 cells with or without 1 mg con A (Sigma, Grade IV) in a final volume of 1 ml 0.15 M saline in a $4 \times 1 \times 1$ cm quartz cuvette. Recordings of the decrease in A_{546} over time in the presence of con A showed 3 phases: a) a lag phase with no change; b) a linear phase of rapid change; and c) a final phase of much slower change. The measure of agglutinability was taken to be the change in absorbance per time (ΔA_{546}/min) in phase b after subtracting the agglutination rate without con A in the same time period. $HeLa_{65}$ cells had a finite rate of sedimentation in the absence of con A which increased from zero time and reached a maximum between 10 and 20 min of about 0.010 ΔA_{546}/min with control and butyrate-treated cells and 0.025 ΔA_{546}/min with prednisolone-treated cells. This natural sedimentation rate appeared in experiments with con A as phase c. Phase c continued until A_{546} reached blank values. Therefore, in experiments with con A the final level of con A-mediated agglutination was taken as the total difference in A_{546} at the end of phase b between cells incubated with and without con A. We were able to demonstrate 90% inhibition of agglutination by addition of 2.5 mM α-methyl-D-mannopyranoside to the mixture of con A and $HeLa_{65}$ cells indicating that agglutination was mediated by interaction between con A and its specific glycosyl receptor[19]. Agglutination measured by the quantitative spectrophotometric technique was corroborated by observing the formation of clusters by light microscopy.

Results. The rate of agglutination with prednisolone-treated $HeLa_{65}$ cells was 3-4-fold higher than control cells (table, experiments 1-4). Butyrate did not increase agglutination. Other parameters of agglutination with con A were also affected by prednisolone. The duration of the lag phase before agglutination (phase a) was inversely proportional to agglutinability, e.g. 8 min with control and butyrate-treated cells and 2 min with prednisolone-treated cells (fig.). The final level of agglutination (total ΔA_{546} calculated at the end of phase b) was directly proportional to the rate (ΔA_{546}/min) of agglutinability. For example, in a typical experiment the final level of agglutination was approximately 0.220 for control and butyrate-treated cells and 0.470 for prednisolone-treated cells.

Treatment with trypsin is known to enhance agglutinability of cells with con A[19]. $HeLa_{65}$ cells harvested by incubation for 3 min at 37 °C with 0.02% EDTA and 0.04% trypsin had an average agglutinability of 150% of control (table, experiments 5-8). However, the differential effects of predni-



Con A-induced agglutination of $HeLa_{65}$ cells measured by quantitative spectrophotometry[19]. Cultures were grown for 120 h in medium without (control) or with 3 μM prednisolone. Cells were harvested with 0.02% EDTA in Puck's saline A. Each curve shows changes in A_{546} with time for 1.25×10^6 $HeLa_{65}$ cells with or without 1 mg con A in a total volume of 1 ml. *A* control cells alone; *B* control cells with con A; *C* predisolone-treated cells alone; *D* prednisolone-treated cells with con A.

solone and butyrate on $HeLa_{65}$ cell agglutinability were still observed with trypsin-treated cells (table, experiments 9 and 10).

Discussion. The quantiative spectrophotometric agglutination assay of Hwang et al.[19] revealed surface changes in $HeLa_{65}$ cells mediated by growth in prednisolone. Three parameters of agglutination kinetics were affected by prednisolone; the lag phase was decreased and both the rate of agglutination and total agglutination were increased. Cortisol has been reported to produce a variable increase in con A agglutination of 3T3 cells[20]. Growth of HeLa cells in medium containing prednisolone has been shown to increase agglutination by what germ agglutinin[21], to alter the phospholipids of membranes[22] and to render the cells more resistant to deoxycholate lysis[23].

Agglutination by plant lectins such as con A has been considered a marker for the level of dedifferentiation in neoplasia[11]. The failure of butyrate to stimulate con A-mediated agglutination may be an example of its activity as a naturally occurring reverse transformation agent[24,25]. Butyrate has been shown to increase cell surface fibronectin in CHO cells[26]. Some of the morphological changes of transformation (cell rounding, loss of cell alignment, increased microvilli and blebbing) may be caused by decreased adhesion secondary to the loss of fibronectin from the cell surface. Salvato et al.[21] hypothesized that increased agglutination of prednisolone-treated HeLa cells by wheat germ agglutinin is caused by increased sialopeptides at the cell surface. More studies will be needed to distinguish among alternative interpretations of these agglutination data. Characterization of the pleiotypic effects of differentially acting inducing agents as butyrate[27] and prednisolone may help to establish causal relationships between different biochemical events in gene expression in HeLa cells.

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Estimation of the odorous steroid, 5α-androst-16-en-3-one, in human saliva[1]

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Summary. The concentration of the urine-smelling steroid, 5α-androst-16-en-3-one, has been measured by radioimmunoassay in the saliva of 9 men and 4 women. The lower limit of detection was estimated to be 0.725 nmoles/l. In six of the men the range of concentrations of the odorous steroid was 0.8–1.8 nmoles/l saliva (3 men had less than the estimated lower limit of detection). In only one of the women studied could the 5α-androst-16-en-3-one be measured (0.83 nmoles/l) in the saliva.

Considerable interest has been aroused recently in the urine-smelling steroid, 5α-androst-16-en-3-one (5α-androstenone) and in the closely-related musk-smelling 5α-androst-16-en-3α-ol (3α-androstenol) because of the possibility of these being human pheromones. It is well-known that both compounds are formed in boar testis[4] and that they induce the female pig, in oestrus, to take up the 'mating stance'[5]. The odorous 3α-androstenol (as glucuronide) occurs in the urine of men and women[6] while 5α-androstenone has been found in axillary secretions of men but only to a limited extent in women[7,8]. In freshly-collected apocrine secretions, 5α-androstenone is either absent or present only in minute quantities[8,9] and this, together with the fact that washing the axillae with a bacteriocidal solution markedly reduces the 5α-androstenone content of 24-h collections[10], strongly suggests that the steroid may be formed on the skin surface from a precursor which is secreted from the apocrine glands and then modified by skin micro-organisms[11].

During the past few years, evidence has been provided to indicate that, when men and women are subjected to the smell of 3α-androstenol, their judgements may be altered[12,13]. Kirk-Smith and Booth[14] have further shown that the smell of 5α-androstenone may affect choice of location in others' presence. There has also been much discussion of sex difference in the ability to smell 5α-androstenone[15] and