shown that feeding habits play an important controlling part[6]. In man, fasting has been shown not to eliminate the rhythm. Kleitman found that diurnal temperature curves may differ in individual subjects with extremes of morning or early evening peaks[7], and also that after waking the morning rise in temperature may be followed by a 'plateau' effect for the remainder of the day and early evening[8]. In monkeys, the temperature rhythm has been reversed by reversal of lighting, while gradual extinction occurred after one week in darkness[9]. The diurnal rhythm has been reversed in man by reversal of daily routine[7], though resistance to change has been noted. Individuals may vary greatly in their adaptability, some failing to adapt after three weeks on night work, and others become adapted in a few days. Burton[10] found that his own temperature rhythm required 3–4 days to become accommodated with English time after a flight from Canada. This involves a time-shift of 5 hours in the daily routine.

In Fig. 1 selected periods are used to illustrate the diurnal variation, the intensity of the morning rise and the adaptation to a reversed routine. Reversal of the diurnal temperature curve occurred in 3–4 days and all the subjects showed the same general pattern. The normal temperature observed on waking on the third morning after reversal of routine is indicative that the rhythms were becoming stabilized on the new routine while the typical post-waking rise was present four days after reversal. Throughout the test periods no variations occurred in environmental temperatures sufficient to invalidate any comparison between periods. The temperature variations are clearly not directly dependent on physical activity or food intake because reversal did not occur immediately when the activity-sleep routine was reversed. They depend ultimately on habit and environment, with light as the most likely controlling stimulus. The time of adaptation reported here agrees well with Burton's observation of 3–4 days to become adjusted to a 5-hr. time-shift.

One further point of interest is that etiocholanolone causes a rise of body temperature after injection which may last for several hours[11]. In view of the diurnal variation of ketosteroid output which is greater by day than by night it was thought that this might be influencing the temperature rhythm. In 4 of the male subjects of this work, a simultaneous study of keto- and ketogenic-steroid excretory patterns was made. Reversal of the keto- and ketogenic-steroid pattern occurred in 2 and 6–8 days respectively. Because the temperature rhythm was reversed in 3–4 days it seems unlikely that these steroids play an important part in the control of body-temperature rhythms though this does not exclude the possibility that they exert some effect upon temperature-levels.

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TWO LINKED GENES SHOWING A SIMILAR TIMING OF EXPRESSION IN MICE

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TWO single gene mutations in the mouse show similar timing of expression during post-natal development. The two mutant alleles were found to produce nearly normal phenotypes until some critical time after birth following which normal differentiation is arrested. The first of these genes, originally reported by Morrow, Greenspan and Carrol[1] in *C3H* mice is expressed in the recessive state by a deficient production of the enzyme β-glucuronidase[2,3]. It has recently been demonstrated that homozygous mutant animals (*gg*) produce the enzyme in some tissues, notably liver, only until a certain post-natal stage, after which a progressive decline sets in and the enzyme ceases to accumulate further. Wild-type (*GG*) stocks produce the enzyme throughout growth[4]. A representative experiment is shown in Fig. 1.

The second mutation is that responsible for a recessive hereditary visual cell degeneration recently described from several laboratories[5–8]. At birth, the retinæ of mice homozygous for the recessive allele (*rd*) do not differ significantly from those of control animals, and post-natal differentiation at first proceeds normally. Beginning, however, at the onset of the most rapid phase of visual cell differentiation on the tenth day, and just after the visual cells have become minimally responsive to light stimulation, the cells die. By the fourteenth day, 50 per cent of the visual cells have disintegrated; at three weeks of age practically none remain, and the animals are blind. This process has been described in detail[8].

Both mutations are characteristic of *C3H/Ha* mice, and the experiments to be described show that in addition to sharing a similar pattern of expression, they are closely linked on the same chromosome. They do not, however, appear to control the same physiological process.

In these experiments β-glucuronidase was assayed by a modification of the phenolphthalein glucuronide procedure of Talalay *et al.*[9]. Activity is expressed as μgm. of phenolphthalein formed per hour when assayed at 56° C. Presence or absence of visual cell degeneration was ascertained by the electrical response to light stimulation (electroretinogram) and histological examination[8].

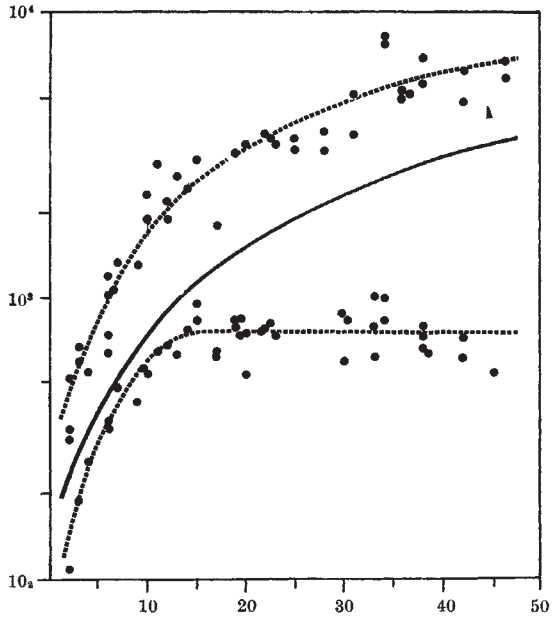

Fig. 1. The total enzyme content of liver plotted as a function of days of age in a population of mice segregating for the *g-G* alleles (that is, the progeny of a *gg* × *Gg* cross). Each point represents a single mouse and enzyme activity is expressed as total units per liver. The solid line is the growth curve of liver in this population expressed as mgm. × 10 dry weight. The dashed lines are drawn to indicate the growth curves of the enzyme in the two classes of progeny

It is apparent from Table 1, which lists the results obtained with a number of mouse strains, that *C3H/Ha* mice show both mutations. In *AKR*, *C3H/St* and *CFW* lines the *g-G* and *rd-Rd* allelic pairs are expressed independently of each other. The *C3H/St* and *CFW* are blind, but have high liver glucuronidase, whereas the *AKR* animals show no visual cell degeneration, but have low glucuronidase-levels. Evidence that the two loci do not affect a common function was also obtained from examination of *Ha/ICR* Swiss mice. The *Ha/ICR* Swiss are a deliberately non-inbred population. Among mice of this stock approximately 13 per cent (59/444) were

Table 1. Glucuronidase Content and Sightedness of Mouse Strains

Strain	No. of animals	Liver glucuronidase (units/mgm. wet wt.)	Sightedness
LaA	3	5·4	+
IPBR	3	6·7	+
I	3	6·7	+
5-PRUNT	3	6·8	+
H	3	7·0	+
CBA/St	6	7·4	+
CFW	3	7·6	0
C/St	3	7·8	+
B1⁺	3	7·9	+
CHI	3	8·0	+
C3H/St	9	8·3	0
JK	3	8·8	+
PBR	6	9·2	+
A	3	9·2	+
BrS	3	9·4	+
PIN	3	9·5	+
N	3	9·9	+
F	3	10·3	+
C57	3	10·3	+
2-PRUNT	3	10·5	+
DBA/2	10	11·6	+
LOW	3	12·2	+
AK	6	0·9	+
C3H/Ha	10	1·6	0

Table 2. Linkage of Glucuronidase and Hereditary Visual Cell Degeneration

gG rd Rd × *gg rd rd*

	Blind	Sighted
High enzyme	1	33
Low enzyme	23	2

The crosses used were (*C3H/Ha* × *DBA/2*) × *C3H/Ha* and (*DBA/2* × *C3H/Ha*) × *C3H/Ha*. In crosses of the first type, the *C3H* carried the milk factor; in those of the second type, they did not.

blind and all (47/47) had high enzyme. That the genetic factor responsible for the visual defect in Swiss mice involves the same locus as that in the *C3H* is indicated by the failure (0/102) to observe sighted offspring of *C3H/Ha* × Swiss matings. Also, the behaviour of the *rd* allele in various other crosses was independent of its origin.

The F_1 animals of the cross *C3H/Ha* × *DBA/2* are heterozygous at both loci and were indistinguishable from the dominant *DBA/2* parent. Examination of the offspring of a backcross of the F_1 to the *C3H* parent showed that the two loci are linked, being approximately 5 cross-over units apart (Table 2). Other linkages of the *Rd* or *G* genes are not known. In the present experiments neither factor was linked to sex, or to the coat colour genes *A*, *B* and *D*. In addition, these loci were not linked to the H-2 genic complex[10]. It has been reported that *Rd* is not linked to pigmy[11] or silver[12], and *G* is not linked to the colour genes, *A*, *B* and *C*[3]. These facts eliminate their location on linkage groups I, II, IV, V, VIII, IX or XX[13].

For the *rd* allele, although differentiation of the visual cells is blocked and cell death ensues, no effect is observed in the other retinal cells of the same ontogenetic origin, the pigment epithelium, the bipolar cells, and the ganglion cells. Similarly, for the *g* allele, although enzyme ceases to appear in the liver after the seventeenth day, enzyme production in the spleen is maintained throughout its growth. For both of these loci expression of the recessive alleles involves a nearly normal function until in some tissues, at a time characteristic for each, functional failure occurs. Despite these similarities, the two genes are separable by crossing-over and do not appear to involve a common function.

Although the genetic linkage observed may be purely fortuitous, in view of the similarities of the two loci the possibility must be considered that their linkage reflects the functional organization of the chromosomes.

A number of reports, stemming from the original observation of Beerman[14], have demonstrated that characteristic chromosomal regions swell and then shrink in the cells of particular tissues at certain stages in development, and the experiments of Briggs and King[15] suggest that differentiation may involve a concomitant loss of genetic totipotency among tissue nuclei. In the light of these findings several investigators have again proposed that the primary control mechanism in differentiation is a nuclear process involving differential gene activation or genetic segregation among the developing cells. Strong genetic support for a nuclear control comes from the experiments of McClintock[16–18], showing that a class of genetic elements exists that control the time during development at which secondary genetic events may occur.

The report of Callan and Lloyd[19] on the heterozygosity of puffing suggests that different alleles of the same gene can react differently to such control mechanisms during development, as appears to be

the case with glucuronidase and hereditary visual cell degeneration. They found that in the oocytes of *Triturus* neither, one, or both members of a chromosome pair may show puffs at a given locus. The finding that although cells from different individuals differed widely, all oocytes from the same animal showed identical configurations at all sites, indicated that pairs of alleles were involved in which one member forms puffs and the other does not.

If the findings reported here are considered in relation to these cytogenetic phenomena, they suggest that particular chromosomal regions may be specialized to control the timing of expression of the loci within them, and thus provide a nuclear regulation of differentiation. More serious consideration of this hypothesis must await the finding of other cases of close genetic linkage between genes showing similar patterns of physiological expression.

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AMINOGLYCOLIPIDS AND GLYCOPROTEINS OF HUMAN BRAIN

I. Quantitative Fractionation of Brain Glycolipids by Silicic Acid Chromatography

WHILE there are a number of methods by which the glycolipids of mammalian brain may be individually isolated and purified, a method would be desirable whereby all the glycolipids of brain could be quantitatively separated and recovered, and their concentrations and other properties compared between individual brains. This is of interest not only with reference to the pathological lipodystrophies[1], but also with reference to the possibilities for chemical individuality of these substances in normal brain[2].

Since silicic acid chromatography has been found to be useful in the separation of both synthetic and native lipid mixtures[3], it was applied to the present problem. Whereas gradient elution of selected lipids from silicic acid columns had been found effective in the quantitative separation of a selected group of brain lipids[4], there was no evidence to indicate that a total hot alcoholic extract of brain lipids might be resolved quantitatively by these methods. With the desire to obtain quantitatively reproducible fractions which might then be compared in brain specimens from different individuals, a stepwise elution procedure was adopted, with quantitative exhaustion at each step of all the solids which could be removed from the column with a given solvent mixture.

Fresh whole bovine cerebral hemispheres, freed of investing meninges and blood vessels, were extracted with hot methanol as previously described for the isolation of brain ganglioside[5]. This procedure has been used by numerous investigators[6] for the preliminary extraction of cerebrosides and related glycolipids. In the course of preparation of brain ganglioside the filtered hot methanolic extract is allowed to stand at 4° C. for 48 hr., during which time a precipitate forms. The precipitate is filtered in the cold, then taken up at room temperature with chloroform – methanol (2 : 1), and partitioned twice with water. The upper phase contains the bulk of the brain ganglioside and other hexosamine-rich material present in the precipitate (and discussed in the next communication), and the resultant lower phase contains the other glycolipids and phospholipids. This lower phase material is taken to dryness by vacuum distillation at 60° C., taken up with minimal amounts of chloroform – methanol (2 : 1), allowed to dry at room temperature, and stored in a vacuum desiccator.

Silicic acid (Baker's reagent grade) was dried overnight at 105° C. To columns 40 mm. × 600 mm. with a fritted disk of coarse porosity was added a thoroughly mixed suspension of 250 gm. of silicic acid and 500 c.c. of chloroform – methanol (2 : 1) and additional chloroform – methanol added until a total of 1,250 c.c. were used. When the chloroform – methanol had run through to the level of the silicic acid, 1,000 c.c. of chloroform was added until the column was translucent and free of opacities[4]. The column was charged with 1,000 or 2,000 mgm. of lower-phase material in chloroform. The solvents utilized for elution were first chloroform, then mixtures of chloroform – methanol with increasing amounts of methanol, and finally pure methanol. The entire fractionation was done in a constant-temperature room at 25 ± 2° C. Fourteen fractions were thereby obtained (Fig. 1). In addition to the good general separation previously observed with more limited mixtures between glycolipids and phospholipids[4], excellent quantitative recoveries in terms of dry weight, hexose[2], phosphorus[2], and nitrogen[2] were achieved (Table 1). This quantitative result provides a possibility for a more reliable comparison of absolute amounts of these separate constituents in both human and animal brain preparations.

Fig. 1 shows that fractions III–VI are completely phosphorus-free, yet high in content of hexose. That these are distinct fractions is demonstrated: (1) by the fact that each fraction is exhaustively removed from the column by its particular solvent mixture