Chromosomenpaar 10 gebildet wurde. In der Gruppe von Kulturen, die mit Mitomycin C behandelt wurde, fanden sich 37 Klasse-I- und 32 Klasse-II-Quadriradiale. Von diesen 69 Quadriradialfiguren wurden 19 vom Chromosomenpaar 10 gebildet (Tabelle). Das bedeutet, dass 26,2% dieser mit Mitomycin-C induzierten Quadriradiale nur 1 bestimmtes Chromosomenpaar betreffen.

Die Quadriradialbildungen und Homologenassoziationen der 1656 Metaphasen der ersten Versuchsreihe und die 3 Figuren in den 300 Metaphasen der Kontrollgruppe dürften als spontane Ereignisse innerhalb dieses In-vitro-Kultursystems zu betrachten sein, die eine gerade noch erkennbare Frequenz zeigen, nämlich 25 Ereignisse in 1956 Metaphasen. Mit Mitomycin-C wurde die Frequenz der Quadriradialbildungen in Schweinelymphozytenkulturen wesentlich erhöht. In 300 Metaphasen finden sich 69 solcher Figuren. Aus diesen Ergebnissen ist unschwer abzuleiten, dass das Chromosomenpaar 10 des Schweines mit seinem grossen achromatischen Bereich eine Disposition für solche Austauschereignisse hat. Ähnliche Dispositionen für chromosomale Austauschereignisse in der Mitose wurden für bestimmte Humanchromosomen beschrieben[6,8,9].

Nach einem Bruch in homologen Abschnitten von Nichtschwesterchromatiden treten 3 Reunionsfiguren auf[6]. Es handelt sich dabei um die völlige Restitution dieser Chromatiden und um Klasse-I- und Klasse-II-Quadriradiale. Die Wahrscheinlichkeit für das Auftreten einer dieser Figurtypen sollte gleich gross sein. Da Restitutionsfiguren in der Metaphase nicht erkennbar sind, verbleiben nur Quadriradialfiguren der Klasse I und II als zytologisch sichtbare Austauschereignisse zwischen homologen Nichtschwesterchromatidabschnitten. Sowohl in der Reihe der ersten 1656 Metaphasen kann in den 3 Klasse-I- und 5 Klasse-II-Quadriradialen, als auch in der Reihe der Mitomycin-C-induzierten 37 Klasse-I- und 32 Klasse-II-Typen annähernd ein 1:1-Verhältnis gesehen werden, das auf einen mitotischen Materialaustausch an Homologen hindeutet. Eine Erklärungsmöglichkeit dafür bietet mitotisches Crossing over, wie es mehrfach beschrieben wurde[10,11]. Demnach könnten die beschriebenen Quadriradialbildungen als mitotische Chiasmata verstanden werden, wie das für ähnliche Situationen an Human- und Primatenchromosomen in Betracht gezogen wird[1,12]. Zu dieser Interpretationsmöglichkeit würden die Berichte über erhöhtes Vorkommen von mitotischem Crossing over im zentromerische Heterochromatinbereich von *Drosophila melanogaster*[10,13,14] nahtlos in Einklang zu bringen sein. Der heterochromatische zentromernahe Bereich des Chromosoms 10 beim Schwein könnte in dieser Weise als eventuelle Ursache für die erkennbare Disposition dieses Schweinechromosoms für Quadriradialbildungen gewertet werden.

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Genetic polymorphisms and intrauterine development. Evidence of decreased heterozygosity in light-for-dates human newborn babies

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Summary. In 2 independent samples of low-birth-weight infants the proportion of females and homozygotes for a series of polymorphic systems was higher in light-for-dates than in preterm babies. The observation seems to give support to the hypothesis that homozygosity for 'normal' polymorphisms may decrease in general intrauterine growth rate. Since it is known that survival rate is strongly related to birth weight, a correlation between growth retardation and homozygosity may have a major role in the maintenance of such polimorphisms.

It has been suggested that enzyme polymorphisms have a regulatory function on metabolism, and that heterozygotes may have a selective advantage since they could modulate reaction rate in order to compensate for variation in biochemical conditions[1]. The hypothesis has also been put forward that even if the allozymes of a locus were functionally identical, heterozygotes for dissimilar sequences of transcription-initiating sensors should be advantaged since their mean time of activation is lower than that of homozygotes[2].

Considering that most of enzymatic systems are activated during intrauterine development when body growth is very

Table 1. Distribution (%) of sex, blood groups and enzyme phenotypes in the sample from Rome (PT, preterm; LFD, light-for-dates)

	Sex	ABO				Rh(D)	PGM_1			ACP_1					ADA			No. of
	Females	A	B	AB	O	negative	1	2-1	2	A	B	BA	CB	CA	1	2-1	2	infants
PT	59.6	41.8	10.9	-	47.3	3.6	50.0	42.3	7.7	-	61.8	27.3	9.1	1.8	92.2	7.8	-	57
LFD	69.4	25.0	16.7	2.8	55.6	22.2	52.8	30.6	16.7	9.1	60.6	27.3	3.0	-	90.3	9.7	-	36
Adults	49.1	41.7	8.6	3.0	46.7	16.0	51.3	39.9	8.8	8.6	43.9	31.7	12.2	3.4	82.5	17.2	0.3	

Significance of the difference in proportions of females and of (detectable) homozygotes for blood groups and enzyme polymorphisms (ADA 2 has been excluded). Wilcoxon test; one tail probability. LFD vs PT: $p < 0.02$.

rapid, the degree of the heterozygosity could affect significantly growth rate before birth. As a corollary one will expect an increased proportion of homozygotes among growth retarded newborn infants.

2 series of low birth weight (LBW, $\leqslant 2.5$ kg) Caucasian infants were studied. A prospective sample of 93 singleton babies, whose parents were not related, was collected during the years 1976–1977 from the population of Rome. Phosphoglucomutase locus 1 (PGM_1), adenosinedeaminase (ADA), erythrocyte acid phosphatase (ACP_1), ABO and Rh (D) phenotypes were determined by standard methods[3]. Babies were subdivided into preterm (PT-36 weeks or less) and mature (37 weeks or more). The last group includes the majority of growth retarded (light-for-dates, LFD) infants[4]. A sample of 98 LBW infants, collected during the years 1968–1972 from the white population of New Haven (Connecticut, USA) was also studied. These infants were partially consecutive and partially selected for ABO and/or Rh incompatibility. Phosphoglucomutase locus 1 (PGM_1) and locus 3 (PGM_3), ABO and Rh (D) phenotypes were determined by standard methods[3]. Data on inbreeding had not been recorded in this series.

Table 1 describes the distribution of sex, blood groups and enzyme phenotypes in LBW infants and adult population of Rome. LFD babies show an increase in the proportion of females and homozygotes (at least of detectable ones) for all systems investigated as compared to adult population. PT babies show an increase of proportion of females and homozygotes for ACP_1 and ADA as compared to adults. The proportion of females and homozygotes among LFD is higher than among PT babies, with the exception of ADA which shows a marked increase of homozygotes in both classes of infants as compared to adults. The difference between LFD and PT is statistically significant. Discriminant analysis[5] on sex, ABO, Rh, PGM_1 and ACP_1 is reported in table 2. The separation between LFD and PT babies is highly significant ($p < 0.005$). The contribution of the single variables to discrimination is moderate and rather uniform. Table 3 shows the proportion of heterozygotes for the polymorphic systems studied and the mean value of heterozygosity for PT, LFD and normal adults from Rome. LFD show a reduction of mean heterozygosity as compared to both PT and adults. Only a slight difference is observed between PT and adults.

Table 4 shows the distribution of sex, blood groups and enzyme phenotypes in LBW infants from New Haven. Also in this sample, the proportion of females and homozygotes among LFD is higher than among PT babies for all systems investigated.

The pattern of differences between PT and LFD infants appears very similar in 2 different populations. Therefore the possibility that ethnic stratification may be the cause of the observed differences can be reasonably excluded. Erythrocytic developmental changes for enzymes and antigens might be another potential source of error. The comparison of light-for-dates with preterm infants excludes also this possibility. In the sample from Rome, inbreed families were excluded. Therefore, since the higher proportion of homozygotes in LFD infants is observed in both samples, this cannot be due only to a higher degree of inbreeding. Moreover, the very low sex ratio among LFD argues against this possibility. Inbreeding in fact has been found generally associated with an increase of sex ratio[6].

Although the sample of polymorphic systems is relatively small to allow definite conclusions, both the regularity of the pattern in LFD babies (table 2) and its similarity in 2 different populations are highly suggestive and seem to give support to the hypothesis that homozygosity for 'normal' polymorphisms may decrease, in general, intrauterine growth rate. It is very likely, however, that different homo-

Table 2. Discriminant analysis on PGM_1, ACP_1, sex, ABO and Rh to distinguish between PT and LFD. LBW infants from the population of Rome. Direct analysis by Wilks' method

Standardized discriminant function coefficients	
PGM_1	−0.437
ACP_1	−0.525
Sex	−0.285
ABO	−0.353
Rh	−0.555
Eigenvalue	0.251
Canonical correlation	0.448
Wilks' lambda	0.799
χ^2	18.235
DF	5
p	< 0.005

Table 3. Percent of heterozygotes for polymorphic systems in the sample from Rome. ABO and Rh heterozygosity among adults has been estimated assuming Hardy-Weinberg equilibrium. For infants the proportion of heterozygotes among A, B and Rh (+) has been assumed equal to that of adults

	ABO	Rh	PGM_1	ACP_1	ADA	Mean value of heterozygosity
PT	45.4	53.8	42.3	38.2	7.8	37.5
LFD	39.5	43.4	30.6	30.3	9.7	30.7
Adults	46.6	48.0	39.9	47.3	17.2	39.8

Significance (t-test for coupled data; 1-tail probability). LFD vs PT: $p < 0.025$.

Table 4. Distribution (%) of sex, blood groups and enzyme phenotypes in the sample of LBW infants from New Haven. The number of infants for whom the information had been recorded is reported in parenthesis

	Sex Females	ABO O	Rh (D) negative	PGM_1 homozygotes	PGM_3 homozygotes
PT	57.4 (47)	31.1 (45)	4.4 (45)	54.2 (48)	55.0 (20)
LFD	65.3 (49)	32.7 (49)	12.2 (49)	64.7 (34)	57.1 (28)

Significance (sign-test; 1-tail probability). $p = 0.0312$.

zygotes might show a diverse susceptibility towards growth retardation. For PGM_1 and ACP_1, in fact, the excess of PGM_1-2 and B in LFD (compared to adults) appears much stronger than that of other homozygous phenotypes.

Some 'normal' polymorphisms may also influence the duration of gestation. The distorsions of ACP_1 and ADA distributions, observed in PT infants, may be expression of these interactions.

It is well known that survival rate is strongly related to birth weight. Assuming that the proportion of LFD is equal to 0.05 and that their probability of survival as compared to other babies is equal to 0.7[7], we have calculated in the sample from Rome an approximate mean relative fitness of 0.993 for homozygotes. However, small differences of fitness among homozygotes would influence the polymorphic frequencies considerably. The study of a consecutive series, including 'normal' and 'heavy weight' infants, could allow a more precise evaluation of selection intensity.

A correlation between growth retardation and homozygosity for allelic types of enzymes and antigens may have a major role in the maintenance of 'normal' polymorphisms.

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5-Methylangelicin: a new highly photosensitizing angular furocoumarin

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Summary. 5-Methylangelicin, a new highly photosensitizing angular furocoumarin, was studied in 2 different biological systems, the T_2 phage and Ehrlich ascites tumor cells; in comparison with angelicin, the parent compound, it was several times more active.

Furocoumarins are well-known photosensitizing drugs which on irradiation with long wave UV-light react with the pyrimidine bases of DNA[2]. 2 different classes of furocoumarins have been described; psoralen derivatives, which have a linear molecular structure, and are able to form both monofunctional adducts and inter-strand cross-links with DNA, and angelicin derivatives, which have an angular structure and can form only monoadducts. The biological consequences of these types of damage have been widely studied[3]; in particular, cross-links produce heavy genetic damage with a high killing effect. Angelicin, the parent angular compound, is not very useful for biochemical and medical applications, even though it is an interesting monofunctional reactive, because of its poor photobinding ability towards DNA and therefore its low sensitizing activity.

Studying several angelicin derivatives we have already described the interesting properties of 4,5′-dimethylangelicin[4]; in this paper we deal with another new angular furocoumarin showing high photosensitizing activity, i.e. 5-methylangelicin.

Materials and methods. 5-Methylangelicin was prepared by chemical synthesis, which will be described elsewhere; angelicin was a gift of the Franco-Indian Pharmaceutical Co. (Bombay). ^{3}H-thymidine (21 Ci/mM) and ^{3}H-uridine (25 Ci/mM) were purchased from the Radiochemical Centre (Amersham, England). All irradiations were performed, as already described[4], in Hanks' solution containing the furocoumarin, using Petri dishes (5 cm in diameter; 5 ml aliquots) and a Philips HPW 125 lamp (365 nm; irradiation intensity 2×10^6 quanta/sec). T_2 bacteriophage was grown in brain-heart infusion (Difco Laboratories) using *E. coli* B_{48} as host bacteria; irradiations were performed at concentrations of 10^9 phages and 4 µg of drug per ml. Virus titers were determined according to Adams[5]. Ehrlich ascites tumor was routinely transferred by i.p. injection of 2×10^6 cells into Swiss mice; the 50% lethal dose corresponded to the injection of 5×10^2 cells/mouse. After irradiation (2×10^7 cells/ml), DNA and RNA syntheses were studied as previously described[4], by incubating cells (samples of 2×10^6) at 37 °C for 15 min in Hanks' solution (0.5 ml) in the presence of labelled nucleoside (1 µCi). The trichloroacetic-precipitable radioactivity was then determined using a Beckman LS 150 liquid scintillation spectrometer. The DNA and RNA contents were determined by the diphenylamine[6] and orcinol[7] reactions respectively. The results, calculated as a percentage of the radioactivity

Effect of irradiation (365 nm) in the presence of 5-methylangelicin or of angelicin on the tumor transmitting capacity of Ehrlich ascites cells

Furocoumarins	µg/ml	Irradiation time (min) 8	16	32
5-Methylangelicin	4	100 (9.2±0.13)	40 (9.4±0.26)	0 (60)
Angelicin	4	100 (9.2±1.3)	100 (9.4±0.26)	100 (11.2±0.32)
	20	100 (11.2±0.7)	100 (14±1.7)	30 (29.6±3.5)

After irradiation the tumor cells were injected i.p. into Swiss mice (5×10^6/animal) which were then observed for 60 days. The mortality percentage (due to the tumor growth) and, in brackets, the mean survival time (days), calculated excluding the survivors, and the S.E., are reported. Ehrlich cells incubated in the dark in the presence of the drugs, or irradiated in their absence, when injected in the same amount into healthy mice produced a 100% mortality and a mean survival time equal to that of the untreated cells (about 7±0.5 days).