*GLUCOSE-6-PHOSPHATASE DEFICIENCY CAUSED BY RADIATION-INDUCED ALLELES AT THE ALBINO LOCUS IN THE MOUSE**

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The series of multiple alleles at the albino locus in the mouse has served as a model system for studies of the nature of the relationship between allelic effects.[1] In particular, these alleles lent themselves to an attack on the problem of whether allelic effects differed from each other quantitatively or qualitatively. The four alleles at the albino locus were shown to control changes in the amounts rather than the quality of melanin produced. While all four of these were viable when homozygous, four radiation-induced albino mutations of relatively recent origin turned out to be lethal in the homozygous state, with death occurring within several hours after birth. These four behaved in breeding tests as alleles at the albino locus, producing albino offspring in combination with the old c^a (albino) allele, and interacting with the c^{ch} (chinchilla) allele to give a pigment dilution intermediate between chinchilla and albino. A large percentage of newborn homozygous for the new albino mutation had gross morphological abnormalities; however, even those which were perfectly normal morphologically failed to survive. In further search for the nature of the lethal effect of the mutations, all four types of homozygotes were discovered to be hypoglycemic shortly after birth, with a deficiency of glucose-6-phosphatase in liver and kidney. The following report deals with the genetics, the biochemistry, and the structural abnormalities of the mutants and presents a discussion of possible mechanisms of gene effects as well as the relation between the various albino alleles.

Materials and Methods.—Three of the mutations (c^{65K}, c^{112K}, c^{14CoS}) were kindly sent to us by Dr. L. B. Russell of Oak Ridge for a study of the nature of their lethal effects. An additional lethal albino allele (c^3) came from Dr. Searle at Harwell. The individual alleles induced separately by radiation had originated in hybrids from crosses of 101 × C_3H strains; they have been maintained in strains heterozygous for c^{ch} (chinchilla). Standard genetic breeding tests confirmed the allelic nature of the new and the old albino alleles. Autopsies of newborn albinos revealed the morphological abnormalities reported below. Dextrostix® were used for qualitative determinations of blood sugar. Quantitative blood sugar determinations were carried out with a micromodification of the glucose oxidase procedure. For this purpose, newborn albinos and their colored littermates were decapitated and the free-flowing blood was suctioned into lambda pipettes. The smallest volume to give reproducible results with the assay was 40 μl, and this amount was obtained from only 50% of the animals at ages of about 5–6 hr after birth.

Enzyme and glycogen assays were performed on livers removed quickly from decapitated newborn mice, wrapped in parafilm, and frozen on dry ice. The tissues were kept at −20°C until the determinations were performed. The biochemical studies failed to show significant differences between the results obtained with the individual albino alleles. All of these are therefore referred to together as "albinos" in the subsequent report of the biochemical results.

The average weight of the liver (59 mg) was the same for colored and albino newborn mice. A 1:10 homogenate in water at 0°C was prepared with a small glass homogenizer,

taking 80% of the weight as the water content of the liver. In the case of glycogen synthetase, a 1:40 homogenate was prepared with 0.25 *M* sucrose buffered with 0.1M Tris–0.004 *M* EDTA–0.002 *M* mercaptoethanol, pH 7.55. Activity measurements were at 30°C. All enzyme tests (except UDPG pyrophosphorylase) were with 0.1 ml of homogenate in a volume of 0.4–0.5 ml and were stopped by 0.5 ml of 5% perchloric acid, followed where necessary by neutralization with 0.1 ml of 2 *M* K_2CO_3 and centrifugation.

For glucose-6-phosphatase assay the incubation mixture (0.5 ml) contained 0.1 ml 0.24 *M* glucose-6-P and 0.2 ml 0.3 *M* citrate, both at pH 6.7. After 20 min of incubation inorganic P was determined by the Fiske-Subbarow method. For selective destruction of glucose-6-phosphatase 0.1 ml of homogenate was incubated with 0.1 ml of 0.05 *M* acetate pH 5 for 30 min at 37°C, followed by addition of glucose-6-P and citrate and incubation as above. Glucose-6-phosphatase activity was calculated from the difference between these two values.

The phosphorylase assay was based on the liberation of inorganic P from 0.025 *M* glucose-1-P at pH 6.2 in the presence of 0.025 *M* NaF, 0.001 *M* 5′-AMP, and glycogen. In the phosphoglucomutase assay the above Tris buffer (without sucrose) contained catalytic amounts of glucose-1,6-diP, 0.01 *M* glucose-1-P, and 0.01 *M* Mg^{++}. The glucose-6-P formed was measured with glucose-6-P dehydrogenase and TPN. In the assay for glycogen synthetase incubation was with 0.2 ml 0.01 *M* UDPG–1% glycogen, 0.1 ml of the above Tris buffer, and, where added, 0.1 ml 0.01 *M* glucose-6-P. The UDP formed was measured with phosphoenolpyruvate, pyruvate kinase, lactate dehydrogenase, and DPNH. Appropriate blanks were run with each of these enzyme tests. In the UDPG pyrophosphorylase test the glucose-1-P formed from UDPG and inorganic pyrophosphate was measured in Tris buffer at pH 7.55 in a coupled assay with Mg^{++}, phosphoglucomutase, glucose-6-P dehydrogenase, and TPN. The homogenate was centrifuged at high speed and 0.025 ml was added per ml of reaction mixture. After a short lag period linear reaction rates were measured over a period of 6 min ir a Zeiss spectrophotometer with a jacketed cell holder.

For glycogen (actually total carbohydrate) determination, 0.1 ml of homogenate was hydrolyzed for 3 hr in 1 *N* NCl, followed by neutralization and determination of glucose by means of hexokinase, ATP-Mg^{++}, glucose-6-P dehydrogenase, and TPN. Free glucose in the liver was determined in neutralized perchlorate filtrates with the same enzyme system.

Results.—Tests of the radiation-induced albino alleles against the old c^a allele showed the heterozygotes to be albino and completely viable. Table 1 presents a summary of breeding tests of the alleles in a variety of combinations. Crosses of the individual albino alleles with each other show a significant deficiency of homozygotes at birth, as evidenced by experiments 1–4. This deficiency may be attributed to perinatal death since ratios of colored to albino fetuses dissected from pregnant mothers agree with those expected in the case of all four alleles (Table 2, expts. 1–4). Experiments 5–10 in Table 1 were designed to test the various alleles for complementation with each other. All combinations produced albino offspring, but the perinatal viability of animals heterozygous for two different albino alleles was increased beyond that of the corresponding homozygotes in several of the intercrosses, particularly those in which the allele c^{14CoS} was involved (Table 1, expts. 8, 9, and 10). In addition to the decrease of perinatal death, newborn albino mice heterozygous for two different albino alleles showed increased postnatal viability and sometimes lived up to eight or ten hours after birth.

Dissections of newborn albinos revealed abnormalities of the thymus in about 50 per cent of c^{65K} homozygotes. These abnormalities ranged from complete absence of the gland in about one quarter of the abnormal to the absence of one

TABLE 1. *Results of breeding tests and of dissections of mice carrying the alleles c^{65K}, c^{112K}, c^{14CoS}, and c^{3}.*

Parents			Offspring at Birth: Colored	Offspring at Birth: Albino	Offspring at Birth: Total	Dissections (Albinos): No. dissect.	Dissections (Albinos): No. having abnormal thymus	Dissections (Albinos): No. having abnormal kidneys
Intraline crosses								
(1)	$c^{ch}/c^{65K} \times c^{ch}/c^{65K}$	Obs.	1300	228 (c^{65K}/c^{65K})	1528	93	45	20
		Exp.	1146	382	$p < 0.001$			
(2)	$c^{ch}/c^{112K} \times c^{ch}/c^{112K}$	Obs.	1699	214 (c^{112K}/c^{112K})	1913	42	27	24
		Exp.	1434.75	478.25	$p < 0.001$			
(3)	$c^{ch}/c^{14CoS} \times c^{ch}/c^{14CoS}$	Obs.	2213	510 (c^{14CoS}/c^{14CoS})	2723	128	1	2
		Exp.	2042.25	680.75	$p < 0.001$			
(4)	$c^{ch}/c^{3} \times c^{ch}/c^{3}$	Obs.	782	173(c^{3}/c^{3})	955	42	4	36
		Exp.	716.25	238.75	$p < 0.001$			
Interline crosses excluding c^{14CoS}								
(5)	$c^{ch}/c^{65K} \times c^{ch}/c^{112K}$	Obs.	137	11(c^{65K}/c^{112K})	148	9	2	2
		Exp.	111	37	$p < 0.001$			
(6)	$c^{ch}/c^{65K} \times c^{ch}/c^{3}$	Obs.	92	15(c^{65K}/c^{3})	107	10	2	2
		Exp.	80.25	26.75	$p = 0.006$			
(7)	$c^{ch}/c^{112K} \times c^{ch}/c^{3}$	Obs.	80	15(c^{112K}/c^{3})	95	4	2	2
		Exp.	71.25	23.75	$p = 0.04$			
Interline crosses including c^{14CoS}								
(8)	$c^{ch}/c^{65K} \times c^{ch}/c^{14CoS}$	Obs.	99	23(c^{65K}/c^{14CoS})	122	8	0	0
		Exp.	91.50	30.50	$p = 0.13$			
(9)	$c^{ch}/c^{112K} \times c^{ch}/c^{14CoS}$	Obs.	110	35(c^{112K}/c^{14CoS})	145	14	0	0
		Exp.	108.75	36.25	$p = 0.86$			
(10)	$c^{ch}/c^{14CoS} \times c^{ch}/c^{3}$	Obs.	369	102(c^{14CoS}/c^{3})	471	14	0	0
		Exp.	353.25	117.75	$p = 0.06$			

TABLE 2. *Classification of fetuses 15-19 days of age.*

Expt.	Parents		Offspring: Colored	Albino	Total
(1)	$c^{ch}/c^{65K} \times c^{ch}/c^{65K}$	Obs.	42	12	54
		Exp.	40.5	13.5	
(2)	$c^{ch}/c^{112K} \times c^{ch}/c^{112K}$	Obs.	93	21	114
		Exp.	85.5	28.5	
(3)	$c^{ch}/c^{14CoS} \times c^{ch}/c^{14CoS}$	Obs.	38	11	49
		Exp.	36.75	12.25	
(4)	$c^{ch}/c^{3} \times c^{ch}/c^{3}$	Obs.	14	7	21
		Exp.	15.75	5.25	
(5)	$c^{ch}/c^{14CoS} \times c^{ch}/c^{112K}$	Obs.	14	5	19
		Exp.	14.25	4.75	
(6)	$c^{ch}/c^{14CoS} \times c^{ch}/c^{3}$	Obs.	12	3	15
		Exp.	11.25	3.75	

lobe in another quarter and to severe reduction in thymus size in the remaining half of the abnormals. In addition, about 20 per cent of c^{65K} homozygotes had very small kidneys and 3 of the 93 newborn dissected had only one kidney. About 64 per cent of newborn albino mice homozygous for c^{112K} had thymus abnormalities which consisted in significant size reduction and only three animals had no thymus whatsoever. Approximately one half of the c^{112K} homozygotes had kidneys significantly smaller than normal. Sporadic cases only were found to have thymus or kidney abnormalities in c^{14CoS} homozygotes. c^{3} homozygotes had a high proportion (about 86 %) of kidneys severely reduced in size but very few thymus abnormalities. Although only small numbers of newborn heterozygous for two different alleles were dissected, the absence of abnormalities in all albinos with one c^{14CoS} allele (Table 1, expts. 8, 9, and 10) reflects their low frequency in c^{14CoS} homozygotes (expt. 3).

Since the death of albino mice within a few hours after birth could not be ascribed to gross malformations, tests of possible biochemical abnormalities were carried out. Tests with Dextrostix® gave no detectable reaction in the blood of 107 albinos obtained from the four mutant strains and one intercross. Controls consisted of the blood from 522 colored littermates which showed a wide range of blood glucose levels, but included only 13.5 per cent without detectable reaction. The results of quantitative glucose determinations confirmed the qualitative data. Blood sugar values in 28 albinos ranged from 5–40 mg% with an average of 17 mg%, whereas the values of 63 colored littermates ranged from 20–100 mg% with an average of 64 mg% glucose.

The injection of 10 mg of glucose given every six hours subcutaneously was sufficient to prolong life of the albino newborn for as long as 30 hours. The eventual death of the injected mice and their littermates might have been due to maternal neglect induced by the frequent disturbances of the cage. Injections of epinephrine and glucagon at a variety of dosages up to 5 mg/kg, D-mannoheptulose up to 5 gm/kg, and hydrocortisone (1 gm/kg) had no detectable effect in prolonging survival.

The inability of the albino mice to maintain an adequate blood glucose level after birth can be explained by the low level or even total absence of glucose-6-phosphatase in the liver (Table 3). (The average difference between the readings

TABLE 3. *Glucose-6-phosphatase, glucose, and glycogen content of the liver and kidney of newborn and three-week-old mice.*

Type of mouse	Glucose-6-Phosphatase Activity (μmoles/gm/min) Liver	Kidney	Glucose content of liver (mg/100 gm)	Liver glycogen (%)
Newborn				
Albinos*	(18) <0.1	(6) 0.33 ± 0.04	(6) 83 ± 33	(12) 1.9 ± 0.9
Controls†	(16) 3.9 ± 1.2	(7) 0.92 ± 0.17	(9) 444 ± 149	(13) 5.9 ± 3.1
3 Weeks Old				
Homozygous (c^{ch}/c^{ch})	(7) 14.9 ± 2.0	(4) 9.5 ± 1.4		(3) 1.9 ± 0.2
Heterozygous (c^{ch}/c)	(7) 16.3 ± 0.7	(4) 9.4 ± 1.4		(3) 2.2 ± 0.1

The number of samples analyzed is given in parentheses. In the case of the kidney of newborn mice each sample consisted of 4 to 6 pooled kidneys. The mean ± the average deviation from the mean are recorded.

* Not included are two cases in which the glucose-6-phosphatase activity in "albino" livers was 1.0 and 2.2 μmole/gm/min, respectively (see text).

† These include homozygous normal and heterozygous mice, since it is not possible to distinguish them at birth on the basis of fur color.

before and after destruction of glucose-6-phosphatase was 1.9 Klett units for the albinos and 85 for the controls. Thus, the albino livers contained at the most 2% of the enzyme content of the normal livers. Two exceptions are noted in Table 3. In one of these the free glucose content of the liver was 57 mg per 100 gm, thus confirming the fact that the liver was from an albino.) No inhibition of glucose-6-phosphatase was detected when liver homogenates of albinos and controls were mixed 1:1 or 2:1. Glucose-6-phosphatase of the kidney was also diminished in albinos as compared to controls. The hydrolysis of glucose-6-P by nonspecific liver phosphatases (mostly acid phosphatase which is not destroyed at pH 5) was the same in albinos and controls and amounted to 1.0 and 1.1 μmoles/gm/min, respectively.

Two groups of workers [2, 3] have produced evidence that the microsomal glucose-6-phosphatase of the rat liver also has pyrophosphatase activity. In confirmation of this idea it has been found[4] that liver homogenates of children with glucose-6-phosphatase deficiency are unable to hydrolyze inorganic pyrophosphate. The liver homogenates of the albino mice also failed to do so, whereas the homogenates of controls hydrolyzed this substrate at pH 5.5 in the absence of Mg^{++} at a somewhat faster rate than glucose-6-phosphate.

An attempt was made to induce enzyme formation in albinos by injecting them *in utero* with glucagon on the 18th to 19th day of gestation following a technique described by Greengard and Dewey.[5] The results were negative in albinos and positive in controls four to five hours after injection (Table 4). Similarly, albinos kept alive by glucose injections up to 26 hours did not develop glucose-6-phosphatase activity in the liver. A parallel observation has been made in two children with congenital hepatic glucose-6-phosphatase deficiency, where over a period of six years no recovery from an extremely low enzyme level could be detected.[6]

Glucose-6-phosphatase activity is low in the embryonal liver and kidney of guinea pigs and rises rapidly after birth.[7] A corresponding effect is seen in mice

(Table 3). It is noteworthy that no difference in enzyme level could be detected between homozygous normal and heterozygous three-week-old mice. Another characteristic feature of embryonal development is a sharp rise in liver glycogen shortly before term, followed by a rapid fall after birth.[8, 9] The latter has been associated with the increase in glucose-6-phosphatase activity. Owing to the fact that the different animals are not synchronous in their development, even within the same litter, one finds large variations in individual glycogen values and enzyme levels (cf. the average deviations from the mean shown in Tables 3, 4, and 5. It is interesting that the liver glycogen falls also in the albinos at the time of

TABLE 4. *Effect of injection of fetuses near term with glucagon.*

Type of mouse	Time after injection (hr)	Glucose-6-phosphatase activity (μmoles/gm/min)	Glycogen (%)
Control	1–5 (S)	(8) 1.6 ± 0.6	(11) 8.7 ± 2.7
Albino	1–5 (S)	(3) 0.0	(4) 10.1 ± 4.7
Control	1 (G)	(4) 1.3 ± 0.2	(2) 11.9
Control	4–5 (G)	(4) 3.0 ± 1.2	
Albino	1–2 (G)	(4) 0.0	(2) 6.6
Albino	4–5 (G)	(2) 0.0	

The details are given in the text. (G) refers to the intraperitoneal injection of 0.05 ml containing 0.05 mg of glucagon and (S) to a similar injection of saline. The number of samples analyzed is given in parentheses. The mean ± the average deviation from the mean are recorded.

TABLE 5. *Activity of various enzymes in the liver of newborn mice.*

Type of mouse	Phosphorylase	Phospho-glucomutase	Glycogen Synthetase −G-6-P	Glycogen Synthetase +G-6-P	UDPG Pyro-phosphorylase
Albinos	(4) 8.5 ± 2.3	(4) 6.1 ± 3.6	(5) 0.25 ± 0.05	(5) 0.41 ± 0.03	(5) 7.2 ± 3.0
Controls	(4) 7.3 ± 1.7	(4) 4.1 ± 1.6	(6) 0.30 ± 0.08	(6) 0.79 ± 0.3	(5) 11.2 ± 2.6

The number of samples analyzed is given in parentheses. Enzyme activities are given in μmoles/gm/min. The mean ± the average deviation from the mean are recorded.

birth (cf. Table 4 versus Table 3), although this cannot be attributed to glucose-6-phosphatase activity. For this reason other enzymes connected with glycogen metabolism were determined (Table 5). Although glycogen synthetase activity measured in the presence of glucose-6-P appeared to be low in albinos, they were able to form liver glycogen when injected with glucose. Thus, two mice which survived eight hours had 2.7 and 4.3 per cent liver glycogen and one which survived 25 hours had 3.1 per cent.

In Table 3 the glucose content of the liver has been determined by a specific enzymatic method. One would expect the free glucose in the liver to be in equilibrium with blood glucose, but since it is difficult to freeze the liver rapidly enough to prevent postmortem glycogenolysis, the liver values are generally higher. It seems clear that in comparison to the controls the livers of albinos did not form glucose very rapidly. The liver contains α-glucosidases which can form glucose from glycogen and from oligosaccharides, and glucose can also be formed from glucose-6-P by unspecific phosphatases. In order to determine the rate of these processes, homogenates from albino livers were incubated with 5 mM inorganic P, pH 6.7. Glucose was formed at a rate of 0.25 μmole/gm/min. In addition, glucose-6-phosphate accumulated at a rate of 1.1 μmoles/gm/min. Lactate formation was not measured, but would undoubtedly account for addi-

tional glycogen breakdown. It thus seems possible for glycogen to decrease in the liver of albinos, even though glucose-6-phosphatase activity is very low, but one would not expect this to be greater than in controls if glycogen synthesis were entirely normal. In children with hepatic glucose-6-phosphatase deficiency, liver glycogen is generally between 7 and 12 per cent, compared to a normal value of less than 5 per cent.

Discussion.—The four radiation-induced alleles at the albino locus in the house mouse have been shown to have a variety of effects on the morphological as well as on the biochemical level. The existence of these pleiotropic effects must be taken into consideration in attempts at further identification of the nature of the genetic defect. The first question concerns the possible causal relation between morphological and biochemical abnormalities. If the genetic defect could in fact be ascribed to a point mutation, the various effects observed should trace back to a single deviation from the normal gene product, and all subsequent defects should be causally related to each other. It is not likely that the morphological defects of thymus and kidney are responsible for the deficiency of glucose-6-phosphatase and the subsequent hypoglycemia of the albino mutants. The reverse sequence, namely the causal role of disturbances of carbohydrate metabolism in developmental abnormalities, cannot be excluded at this time. As a matter of fact, the work of Landauer[10] and of Zwilling [11] provides strong indication for the association of hypoglycemia and developmental abnormalities in chick embryos. In the interpretation of the mutant mouse embryo, however, the maternal supply of blood sugar must be kept in mind.

A correlation between the different biochemical effects of the mutations, i.e., those resulting in the albino phenotype and those interfering with normal glycogen metabolism, is by no means obvious. Albinism in c^a homozygotes has been ascribed to a tyrosinase deficiency,[12] but a metabolic connection between tyrosinase and glucose-6-phosphatase, the enzyme deficient in the radiation-induced albinos, is not easily found. The present evidence favors the interpretation that the effects of the radiation-induced albino mutations differ qualitatively from those of the original *c* alleles; these mutations may therefore not be true alleles at the *C* locus.

The existence of roughly three types of effects, first on the morphological level, secondly on pigmentation, and finally on glycogen metabolism seems to indicate that the radiation-induced albino mutations represent chromosomal deficiencies of an area which includes the albino locus. This locus is in chromosome I of the mouse and the upper limits of the chromosomal deficiencies can only be determined by the exclusion of the markers taupe (2% recombination) on one side and shaker-1 (4% recombination) on the other side of the albino locus, which has not been done as yet. It is conceivable that the deficiency comprises various loci which control development of thymus and kidney and the enzyme glucose-6-phosphatase.

The four alleles differ somewhat in their effects and may, therefore, be overlapping deficiencies of various sizes. c^{65K} and c^{112K} show the greatest similarities morphologically and biochemically. A possible difference between them may be found in the thymus defects which consist of absence of thymus in c^{65K} and reduc-

tion in size in the case of c^{112K}. c^3 affects kidney development primarily, but is biochemically indistinguishable from c^{65K} and c^{112K}. c^{14CoS} may well be the least extensive deficiency since it has no effect on kidney and thymus, and shows some degree of complementation with the other alleles by increasing survival of newborn heterozygous for it and another of the albino alleles.

The pigment differences which distinguish normal homozygotes from heterozygotes at three weeks of age are not paralleled by similar differences of glucose-6-phosphatase activity in these two groups. There exists, therefore, a dosage phenomenon on the phenotypic level in terms of one of the mutational effects but not of the other. The present evidence does not give any indication of whether the mutation affects the structural gene for glucose-6-phosphatase or one regulating its activity; equally, the fine structure of the genetic region controlling enzymes involved in pigment formation and in glycogen metabolism remains the object of future analysis. Only one other inherited defect in glycogen metabolism has been described in mice of the *I* strain. This is a deficiency in phosphorylase *b* kinase caused by a sex-linked recessive gene.[13]

Summary.—Four radiation-induced alleles at the albino locus of the mouse produce, in homozygotes, in addition to absence of pigmentation, abnormalities of thymus and kidneys and deficiency of glucose-6-phosphatase. The resulting hypoglycemia accounts for death of newborn mice within a few hours after birth. The mutations are considered to be chromosomal deficiencies including a genetic region which controls enzymes involved in melanin formation and in glycogen metabolism.

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Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate; UDPG, uridine diphosphate glucose; 5′-AMP, adenosine 5′-phosphate; TPN, triphosphopyridine nucleotide; DPNH, diphosphopyridine nucleotide, reduced form.

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[1] Dunn, L. C., *J. Gen.*, **33**, 443 (1936).
[2] Stetten, M. R., and H. L. Taft, *J. Biol. Chem.*, **239**, 4041 (1964).
[3] Nordlie, R. C., and W. J. Arion, *J. Biol. Chem.*, **239**, 1680 (1964).
[4] Illingworth, B., and C. F. Cori, *Biochem. Biophys. Res. Commun.*, **19**, 10 (1965).
[5] Greengard, O., and H. K. Dewey, *J. Biol. Chem.*, **242**, 2986 (1967).
[6] Illingworth, B., *Am. J. Clin. Nutr.*, **9**, 683 (1961).
[7] Nemeth, A. N., *J. Biol. Chem.*, **208**, 773 (1954).
[8] Kornfeld, R., and D. H. Brown, *J. Biol. Chem.*, **238**, 1604 (1962).
[9] Lea, M. A., and D. G. Walker, *Develop. Biol.*, **15**, 51 (1967).
[10] Landauer, W., *J. Exptl. Zool.*, **109**, 283 (1948).
[11] Zwilling, E., *J. Exptl. Zool.*, **109**, 197 (1948).
[12] Foster, M., *J. Exptl. Zool.*, **117**, 211 (1951).
[13] Lyon, J. B., Jr., J. Poster, and M. Robertson, *Science*, **155**, 1550 (1967).