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Deficiency in plasma protein synthesis caused by x-ray-induced lethal albino alleles in mouse

([^{14}C]leucine incorporation/immunoprecipitation/hepatic albumin fraction/regulatory genes)

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ABSTRACT **Plasma protein synthesis was studied in mice bearing x-ray induced lethal mutations at the albino locus. Newborn albino mutants showed a decrease in each of the three principal plasma proteins, albumin, α-fetoprotein, and transferrin, when compared with colored littermate controls. Incorporation of [^{14}C]leucine into plasma proteins of the newborn albinos 30 min after injection was only ⅕ that of the controls, but incorporation into total liver protein was only slightly diminished. Incorporation of [^{14}C]leucine into an albumin fraction obtained by immunoprecipitation from livers incubated *in vitro* in an amino acid mixture was also strongly diminished. Thus, the liver of 18-day-old albino fetuses incorporated into this fraction ⅓ and that of newborn albinos ⅛ as much as the controls, but in both cases the incorporation into total liver protein was only 25% less than in the respective controls. These results indicate that the rather severe structural abnormalities observed in the mutants in the endoplasmic reticulum and the Golgi apparatus are not associated with a general deficiency of hepatic protein synthesis. Instead the data from this and previous work show that the progressive deficiency from fetal life to birth involves certain specific proteins represented by several perinatally developing enzymes and by plasma proteins. It is suggested that the mutational effects observed in these mice are due to deletions involving regulatory rather than structural genes at or near the albino locus.**

Studies of four x-ray induced lethal mutations at the albino locus of the mouse (linkage group I, chromosome 7) have shown the existence of ultrastructural abnormalities of the endoplasmic reticulum and Golgi apparatus in liver and kidney of newborn homozygotes associated with deficiencies of at least three enzymes (glucose-6-phosphatase, tyrosine aminotransferase, and serine dehydratase) which normally increase in these tissues shortly after birth. In addition, serum protein levels were found to be significantly lower in the newborn albino mutants than in their colored littermates (1, 2). Whether more than one serum protein was affected was not determined.

It has been established that albumin, transferrin, and α-fetoprotein are synthesized primarily on membrane-bound polysomes of liver cells (3, 4). The synthesis of albumin and transferrin increases during the neonatal period (5), while that of α-fetoprotein actually decreases, leading to the absence of this serum protein in the adult animal (6). The export route for these and other secreted proteins is generally thought to be through the cisternae of the endoplasmic reticulum and the Golgi apparatus (7), but this is still under discussion (8, 9).

A more detailed study of the behavior of the serum proteins in these mutants is presented here.

MATERIALS AND METHODS

The source of chemicals for polyacrylamide gels was Eastman Organic Chemicals. Sodium dodecyl sulfate (technical) was from Matheson, Coleman and Bell. Amido Black 10B (for electrophoresis) was purchased from E. Merck, Darmstadt, Germany. Aquasol, Protosol, and [^{14}C]leucine (310 μCi/μmol) were from New England Nuclear and amino acids from Calbiochem. Bovine serum albumin, mouse albumin (Fraction V powder, 95% albumin), and rabbit transferrin were Pentex brand from Miles Laboratories. Goat antiserum to mouse albumin and normal goat serum were obtained from ICN. GF/A glass fiber filter papers (2.4 cm) were obtained from Whatman and the vacuum filtration apparatus from Millipore.

Mice. Heterozygotes bearing lethal albino (c^{14CoS} or c^{3H}) and chinchilla alleles (c^{ch}) were mated. Newborn and fetal albinos could be detected by absence of eye pigment but heterozygotes and homozygous normals could not be distinguished easily from each other at this age and served as controls. Newborn mice were used before they had nursed and care was taken to select only those in perfect condition. This was particularly important because of the short survival of the mutants. Some experiments were also conducted with 18-day-old fetuses, the age of which was determined by the vaginal plug method.

Preparation of Samples. Animals were decapitated 1–3 hr after birth. Blood was collected in heparin-coated capillary tubes and centrifuged at 200 × *g* for 10 min. The plasma was separated from the packed erythrocytes by breaking the capillaries and the tubes were resealed. Livers were dissected and flash frozen on dry ice. Plasma and livers were stored at −80°.

Electrophoresis. For the polyacrylamide gel experiments, 5 μl of plasma was diluted to 0.2 ml with water. One-tenth milliliter, representing approximately 50 μg of protein, was electrophoresed 4.5 hr at 3 mA per gel at room temperature in 0.05 M Na barbital, 0.035 M EDTA, pH 8.6. Gels, 80 × 3 mm, were 7.5% in acrylamide [2.2 g of monomer, 110 mg of bisacrylamide, 0.014 ml of *N,N,N′,N′*-tetramethylethylenediamine (TEMED), and 10 mg of ammonium persulfate for 30 ml of gel solution]. The glass tubes were first coated with a 1% solution of Photo-Flo 200. Gels were fixed overnight in 10% trichloroacetic acid and stained with 0.1% Amido Black 10B in 5% (vol/vol) acetic acid for 2 hr. They were destained in several changes of 7% acetic acid in capped tubes on a rotator. Two days were allowed for complete removal of background staining, as that had proved satisfactory in a control experiment with bovine serum albumin. The three prominent protein bands were sufficiently narrow and separated so that they could be removed in gel pieces exactly 7 mm long cut with a razor. These pieces were eluted for 24 hr in capped tubes containing 1 ml of a solution of 0.2 M NaOH, 0.05 M EDTA, and 50% (vol/vol) ethanol. The absorbance of the dye was read at 630 nm against the extract from an unstained piece of gel and compared with

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standard curves based on bands eluted from gels electrophoresed with 25 and 50 μg of mouse serum albumin or rabbit transferrin.[§] The absorbance of the standard Amido-Black extracts was proportional to the amount of protein added to the gels. Transferrin/Amido-Black extracts gave an absorbance which was 79% of that for mouse albumin on a mg of protein basis. The concentration of α-fetoprotein was determined by comparison with the mouse albumin standards. Since our main interest was to obtain comparative values between controls and mutants, it seemed acceptable not to have homologous standards for transferrin and α-fetoprotein. Total protein was determined by the Lowry method (10) using bovine serum albumin as standard.

Some stained gels were scanned for absorbance at 630 nm with a Gilford spectrophotometer model 240 equipped with a linear transport device and recorder. Scans were made before and after turning the gel 180° and the peak areas were cut out, weighed, and averaged.

[^{14}C]Leucine Incorporation. Newborn mice were injected intraperitoneally with 0.05 ml of 0.9% saline containing 0.5 or 1.0 μCi of [^{14}C]leucine. A Hamilton syringe of 0.1-ml capacity with a spring release was used. Any animals that showed leakage of fluids were discarded. The animals were killed 30 min after injection. Livers were weighed, homogenized in 1 ml of ice-cold 0.9% saline in glass Potter-Elvehjem homogenizers, and sonicated. To each of two 0.25 ml aliquots were added with mixing 1.55 ml of H_2O and 0.2 ml of 50% trichloroacetic acid. After 20 min the suspension was filtered with suction through GF/A glass fiber filters in a Millipore filtration apparatus and the precipitate was washed in place three times with 3-ml portions of 5% trichloroacetic acid. The filter containing the precipitate was placed in a glass vial and 2 ml of Protosol were added. The vial was tightly capped and incubated at 55° for 30 min to dissolve the protein. Three milliliters of Aquasol scintillation fluid were added and mixed by which time the glass fibers were translucent. The radioactivity of the contents was determined after 4 days, when the counts in the controls (Aquasol-Protosol) had declined to background levels due to the slow decay of chemiluminescence. Averages of five 1-min counts were made except for samples from plasma with less than 200 cpm for which ten 1-min counts were averaged. Plasma samples, 4–15 μl, were diluted to 1 ml and made 5% in trichloroacetic acid after removal of an aliquot for protein determination. The remaining procedure was identical to that for the liver samples.

Based on control experiments, the livers of fetal and newborn mice were found to be small enough to be incubated *in vitro* without slicing.[¶] Incorporation of [^{14}C]leucine was carried out as follows. After weighing, the livers were rinsed with 1 ml of cold 0.9% NaCl to remove adhering blood. They were incubated in 5 ml beakers in 1 ml of Krebs-Henseleit salt solution (11) containing bicarbonate equilibrated with a 5:95 gas mixture of CO_2 and O_2 and containing a mixture of 21 amino acids at 0.1–0.4 mM concentration. Incubation was in a moisture-saturated oven at 37° with gentle shaking and gassing with the above mixture. After 10 min, incorporation was started by adding [^{14}C]leucine, 0.25 μCi for the livers of newborn animals and 0.5 μCi for the livers of 18-day-old fetuses. After 2 hr the contents of each beaker were transferred to ice-cold conical glass homogenizer tubes of 5-ml capacity and the beaker was rinsed with 1 ml of ice-cold 0.9% NaCl/1% deoxycholate, followed by homogenization. The homogenates were kept frozen until analyzed.

The homogenates were centrifuged for 30 min at 165,000 × *g* and the supernatant was collected by suction. One-tenth milliliter aliquots were used for duplicate protein determination by the Lowry method. Two-tenth milliliter aliquots were precipitated with 10% trichloroacetic acid and the precipitates were collected on filters, washed, and prepared for counting as described above. For immune precipitation (based on previous trials) 0.2 ml of centrifuged homogenate was mixed with 0.125 ml of goat antiserum to mouse albumin in 1-ml conical centrifuge tubes. A similar tube to which normal goat serum was added served as control. The tubes were incubated for 1.5 hr at 37° and then stored overnight in the cold. The precipitates were collected by centrifugation and washed four times with 0.5 ml of 0.9% NaCl. The third wash generally had only a few cpm above background. The precipitates were then dissolved in 0.1 ml of 1 M NaOH plus 0.005 ml of 20% sodium dodecyl sulfate. After standing overnight, aliquots were taken for protein analysis and for counting in 3 ml of Aquasol. Background counts were obtained with 0.1 ml of 1 M NaOH plus 0.005 ml of 20% sodium dodecyl sulfate, treated in the same way as the samples. Counting was done in a Packard scintillation counter model 3320. All counts were corrected for background. In the case of the immune precipitate they were also corrected for the radioactive material precipitated by normal goat serum.

Some of the immune precipitates were subjected to acrylamide gel electrophoresis in the presence of dodecyl sulfate. The stained protein bands were cut out, dissolved, and bleached in 1 ml of 30% (wt/vol) H_2O_2 at 55° for 2 hr and counted after the addition of 10 ml of Aquasol.

Control procedures consisted of the following. It was first determined that most of the incorporated labeled material was found in the supernatant of the detergent-treated and centrifuged homogenate as described above. No difference in the incorporation at 30, 60, and 120 min was found when incubation of the whole liver was compared with incubation of separate liver lobes. In order to investigate whether the immune precipitation was complete with some surplus of antibody remaining, 2 μg of carrier mouse albumin was added to the supernatant after removal of the immune precipitate by centrifugation. The supernatants of the samples precipitated with normal goat serum were similarly treated. The precipitate which formed was then analyzed in the same manner as the first. On an average, the second immune precipitate corrected for the precipitate with normal goat serum yielded 14 cpm/ml of homogenate for the newborn and somewhat higher values for the fetuses. This control also tests for the completeness of removal of the first immune precipitate by the centrifugation.

RESULTS

***In Vivo* Experiments.** In 11 experiments (only five of which were analyzed for individual proteins as in Table 1), the mutant albinos showed lower total plasma proteins than their littermate controls, with an average decrease of 22%. The difference between albinos and colored littermates was statistically significant ($P < 0.01$). Previous results were obtained with serum rather than plasma and the analysis was by the Weichselbaum (12)

[§] Because of a tendency for mouse albumin to aggregate, some stain was found at the origin in gels of albumin controls and plasma samples. The dye at the origin was extracted from all gels and the absorbance was added to that assigned to the albumin band (compare Table 1). No dye was found at the origin of transferrin or bovine serum albumin control gels.

[¶] In Table 3 the average liver weight of the 18-day-old fetuses was 31 mg for the albinos and 30 mg for the controls. The average liver weight of newborn in Table 2 was 46 mg for the albinos and 44 mg for the controls. The total protein contents of the livers were 5.1 and 4.4 mg for the newborn albino and controls, respectively.

Table 1. Plasma protein concentrations in newborn albino and colored littermate controls

Litter no.	Lowry protein (mg/ml)		Amido black protein (mg/ml)							
			Albino				Colored			
	Albino	Colored	T	F	A	Sum	T	F	A	Sum
7629	15.6	23.6	2.4	3.1	8.3	13.8	4.7	4.8	12.0	21.5
7631	17.6	22.1	3.0	5.1	8.8	16.9	5.2	5.2	13.7	24.1
7650	19.7	29.0	3.1	4.0	9.9	17.0	6.0	6.3	16.9	29.2
7752	17.6	21.6	2.8	4.4	9.8	17.0	4.3	4.9	14.2	23.4
8582	16.0	20.1	2.7	3.5	9.2	15.5	4.0	4.2	14.3	22.5
Average	17.3	23.3	2.8	4.0	9.2	16.0	4.8	5.1	14.2	24.1
(±SEM)	±0.72	±1.53				±0.63				±1.34

Total protein was determined by the Lowry method and the concentrations of individual proteins, by extraction of bound Amido Black 10B from polyacrylamide gels. T, transferrin; F, α-fetoprotein; A, albumin. Plasmas of each type of mouse within a litter were pooled. The Amido Black albumin includes protein that remained at the origin, which was on an average 3.38 mg/ml in the controls and 2.26 mg/ml in the albinos.

method with a correction for contamination of the serum by hemoglobin.‖ In that series, the average decrease in serum proteins in four different strains of albino mutants varied between 20 and 27%, with a grand average for 18 litters of 22.4% (2).

The analysis of plasma samples on polyacrylamide gels showed three major bands corresponding to albumin, α-fetoprotein, and transferrin (13). Typical scans of acrylamide gels are shown in Fig. 1. A minor band just behind α-fetoprotein and two faint bands approximately 1.5 and 2.0 cm from the origin were more prominent in the gels from control than albino plasma, but they were not identified. It can be seen that the area of each of the three major bands is decreased in the albinos. On a weight basis of cut-out areas from scans (average of two pairs) the albumin, α-fetoprotein, and transferrin were diminished 34.0, 23.9, and 43.5%, respectively. This may be compared with the experiments in Table 1 in which the amounts of the individual proteins were determined spectrophotometrically by extraction of the Amido Black bands from acrylamide gels. On an average the albino plasma contained 35.2, 21.6, and 41.6% less albumin, α-fetoprotein, and transferrin, respectively, than the plasma of control littermates.

In Table 1 and Fig. 1 the three proteins measured constitute a large percentage—perhaps as much as 85%—of the total plasma proteins. This is mentioned here because in the incorporation experiments with [^{14}C]leucine the total rather than individual plasma proteins were precipitated and counted (Table 2). The rate of incorporation per mg of plasma protein was greatly diminished in the albinos, being on an average only 1/5 as much as in the controls. On the other hand, the incorporation of label into liver proteins was nearly the same in the two groups, the albinos showing on an average 82% as much incorporation as the controls. The plasma protein synthesis in the liver represents a relatively small percentage of the total hepatic protein synthesis (see below). The results suggest that there is a specific rather than a general disturbance in protein synthesis in the albino mutants. Size or protein content of the liver could not be a factor in the lower production of labeled plasma protein in albinos, since they were nearly the same as in the controls (see¶ footnote).

Although there was considerable inter-litter variation in the amount of label incorporated into liver protein, the agreement for the different animals within the same litter was fairly close. It seems possible that the age after birth—the hour is in many cases not exactly known—has something to do with this variability.** Food intake was not a factor, since only animals with empty stomachs were used. Based on the ratio, plasma cpm/liver cpm (Table 2), there is also a large variability in the amount of plasma protein that gets labeled per mg of labeled liver protein. Here again the intra-litter variability (compare two colored controls in two of the litters) is much smaller than that between different litters. The main conclusion that the albinos, for nearly equal incorporation into liver protein, incorporate significantly less label into the plasma proteins is not affected by this inherent variability.

A rough estimate of the plasma proteins made in the liver in percent of total protein synthesis may be arrived at as follows. Assuming that the plasma volume of a newborn mouse is about 0.05 ml (15,††), the total quantity of plasma proteins would be about 1.2 mg (0.05 ml × 24 mg/ml). Then, taking the average count per mg of plasma protein for the controls in Table 2 (2550 cpm), we arrive at a total incorporation of 3060 cpm. The total cpm incorporated into liver protein (average of 4.4 mg) for the same animals was 5330 × 4.4 or 23,450 cpm. Thus, the plasma represents about 13% of the total [^{14}C]leucine incorporated into the liver in 30 min. The same calculation for the albinos in Table 2 yields only 2% of the total [^{14}C]leucine incorporated into plasma protein.

***In Vitro* Experiments.** Since the lesions of the endoplasmic reticulum of the mutants begin to develop in fetuses around the 17th day of gestation and are fully developed in the newborn (gestation = 20 days), it was of interest to compare animals at these two stages in the ability to synthesize plasma proteins. For this purpose isolated livers were incubated in an amino acid mixture containing [^{14}C]leucine and the incorporation into liver protein and a liver albumin fraction isolated by immune precipitation was measured (Table 3). The albumin fraction consists of albumin from the blood remaining in the liver, proalbumin (16), and finished albumin made in the liver. This

‖ With the heparin-coated capillaries described under *Materials and Methods* there was little, if any, contamination of the plasma by hemoglobin.

** In fact, Yeoh (14) compared incorporation of [^{14}C]leucine in rat littermates at different times after surgical delivery. The rats immediately after delivery incorporated leucine into liver and plasma proteins several times less rapidly than the rats 5 hr after delivery. Similar results were obtained when the transferrin and albumin fractions of the liver were studied.

††Ref. 15 gives an average of 40.4 μl/g for the plasma volume of *Rattus norvegicus*. Newborn mice average slightly over 1 g in body weight.

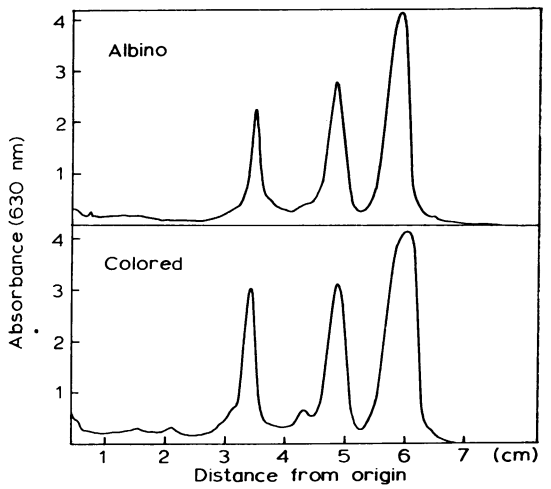

FIG. 1. Scans of acrylamide gels stained with Amido Black. Equivalent plasma samples from albino and colored mice from litter no. 7631 (Table 1) are shown. The absorbance at 630 nm is given in arbitrary units, with the base line set at the absorbance of the clear gel. From left to right, transferrin, α-fetoprotein, and albumin.

fraction also includes the goat immunoglobulins. Normal goat serum under the same conditions produced only a small precipitate including a few cpm and was used as correction factor for the experiments in Table 3. The incorporation of label into the immune fraction shows quite clearly that although it is deficient in all albinos, it is much more so in the newborn than in the 18-day-old fetuses. Thus, the albino fetuses incorporated ⅓ as much into the liver albumin fraction as did their littermate controls, whereas in the newborn the difference was nearly 8-fold. In confirmation of the above, acrylamide gel electrophoresis of some immune precipitates and counting of the cut-out albumin fraction showed an 8-fold difference in incorporation between newborn albinos and controls and a smaller difference between the respective 18-day-old fetuses. In all gels only the band identified as albumin by a control gel contained significant radioactive material.

Table 2. Incorporation of [^{14}C]leucine into livers and plasma of newborn albino (c^{14CoS}/c^{14CoS}) and colored controls (c^{ch}/c^{14CoS} or c^{ch}/c^{ch})

Litter no.	Phenotype	cpm/mg of protein		Ratio of specific activities plasma/liver
		Liver	Plasma	
9508	Albino	1771	298	0.17
	Colored	2119	1224	0.58
9509	Albino	6051	718	0.12
	Colored	6209	2823	0.46
	Colored	5626	2369	0.42
1259	Albino	6264	1221	0.19
	Colored	6610	4995	0.76
	Colored	5704	3718	0.65
1376	Albino	3728	148	0.04
	Colored	4760	1621	0.34
1386	Albino	4124	359	0.09
	Colored	6300	1085	0.17
Average	Albino	4390	550	0.12
	Colored	5334	2550	0.66

Animals in the first two litters were injected intraperitoneally with 0.5 μCi and those in the last three litters with 1.0 μCi of [^{14}C]leucine. The animals were killed 30 min after injection. Liver counts are the average from 2 aliquots. In two litters, two colored controls were studied.

Table 3. Incorporation of [^{14}C]leucine in isolated liver of 18-day-old fetuses and newborn albino and colored littermate controls

Donor animals	Homogenate		Immune precipitate		
	Protein, mg/ml	cpm/mg of protein	Protein, mg/ml of homogenate	cpm/mg of protein	cpm, %
18-Day-old fetuses					
5 albino	1.34	5500	0.22 (0.04)	175	3.2
5 colored	1.29	7340	0.34 (0.03)	482	6.5
Newborn					
5 albino	2.29	2160	0.35 (0.01)	20	0.9
5 colored	2.70	2910	0.59 (0.01)	158	5.4

Mutants of the c^{14CoS} and c^{3H} strains were used as donor animals. After 2 hr of incubation with 0.5 μCi for the fetuses and 0.25 μCi for the newborn, the livers were homogenized and analyzed as described under *Materials and Methods*. Protein in the immune precipitate refers to that obtained per ml of homogenate with goat antiserum against mouse albumin, corrected for the precipitate obtained with normal goat serum. The values in parentheses show the amounts of protein precipitated per ml of homogenate by normal goat serum. The cpm in the immune precipitate is calculated per mg of protein in the homogenate. In the last column the ^{14}C in the immune precipitate is given in percent of the ^{14}C in the homogenate.

It is possible that the difference in plasma protein synthesis between fetuses and newborn is related to the progressive nature of the lesions of the endoplasmic reticulum. However, such a difference is not apparent when incorporation into total liver proteins is measured. In Table 3 both the albino fetuses and newborn incorporated about 25% less label into total liver proteins than their respective controls. The time course of incorporation was also studied. There was an increase in rate with time, the incorporation during the second hour being considerably faster than during the first hour, but no essential difference in the kinetics between albinos and controls could be detected. The data as a whole show that the deficiency in hepatic protein synthesis in albinos is not a general one, but is restricted to specific proteins represented by certain perinatally developing enzymes (2) and by plasma proteins.

The last column in Table 3 shows that the colored fetuses incorporated a larger percentage of the total counts into the albumin fraction than did the colored newborn. The albino fetuses were still synthesizing albumin, although at a diminished rate, and it was mainly the albino newborn that were strongly deficient. This agrees with the data shown in Table 2, where it was calculated that the newborn albinos incorporated only 2% of the [^{14}C]leucine found in the liver proteins into the plasma proteins as compared to 13% for the colored controls. This situation probably explains why the mutant mice are born with a relatively slight plasma protein deficiency (compare Table 1). The half-life of albumin in adult rats has been reported to be 2.66 days (17) and the regulation of the plasma level has been shown to depend not only on the rate of synthesis but also on the rate of destruction (18).

The question was raised whether there might also be a disturbance in the secretion of plasma proteins from the liver in the albino mutants. Judah and Quinn (19) have summarized experiments describing the time course of albumin synthesis

and secretion in rats after injection of [^{14}C]leucine. Labeled albumin appeared in the plasma after a delay of about 12 min. In the liver there was within 2 min a rapid labeling of a fraction precipitated with albumin antiserum, later identified as proalbumin. Conversion to albumin was followed by secretion into the blood stream via the Golgi apparatus. Dorling *et al.* (20) found that colchicine—in addition to inhibiting secretion of albumin—also decreases the rate of conversion of proalbumin into albumin and the rate of incorporation of [^{14}C]leucine into proalbumin—effects which suggest a negative feedback control system. According to Peters and Peters (21) the converse, an inhibition of albumin synthesis by cycloheximide, had no demonstrable effect on albumin secretion until the liver was nearly depleted of albumin. In a less acute situation of diminished albumin synthesis, as for example in a fasted animal, the secretion of albumin was correspondingly decreased (22). Thus, a disturbance in synthesis could be the primary cause for the diminished plasma proteins in the albinos, but a disturbance in secretion has not been excluded by the present data.

DISCUSSION

Based on complementation studies, the pleiotropic effects of albino lethal alleles have been ascribed to overlapping deletions around the albino locus (2). A further characteristic of these mutations is that heterozygotes show enzyme activities and serum protein levels identical to those of homozygous normals. This lack of gene dosage effect has led to the suggestion that regulatory rather than structural genes might be involved in these mutations.

In previous studies three hepatic enzymes, glucose-6-phosphatase, tyrosine aminotransferase, and serine dehydratase, showed activity deficiencies greater than 70–90% in albino newborn. Another enzyme studied recently, glutamine synthetase, had 56–66% lower activity in the albino liver than in that of the controls, but was at normal levels in mutant retina and brain (23). By contrast, the total plasma proteins were on an average only 22% lower in the newborn mutants than in littermate controls, but this could be accounted for by plasma proteins synthesized at a more normal rate during an earlier embryonal period. In fact, there was some support for this idea, since 18-day-old albino fetuses, while deficient in albumin synthesis in the liver, did not show the profound disturbance noted in newborn albinos. In the latter animals the incorporation of [^{14}C]leucine into the albumin fraction of the liver was only $\frac{1}{8}$ that found in the littermate controls, which brings the magnitude of the plasma protein deficiency more in line with that of the enzymes. On the other hand, incorporation into total liver proteins was not markedly diminished in the albino newborn. This was rather surprising in view of the severe structural abnormalities of the endoplasmic reticulum observed in liver and kidney of the newborn mutants. Apparently the general machinery for protein synthesis is not greatly affected.

The plasma proteins affected by the lethal albino alleles are all exported and as such presumably synthesized on membrane-bound polysomes (3, 4). Of the enzymes affected by these alleles, glucose-6-phosphatase is a tightly bound microsomal enzyme and tyrosine aminotransferase (24) and serine dehydratase (25) are synthesized to a large extent on membrane-bound polysomes. Whether the ultrastructural abnormalities in the mutants, which include the loss of some bound polysomes, can account for the decreased synthesis of exportable serum proteins and the deficiencies of membrane bound enzymes will require further investigation.

The location of the structural gene for albumin (chromosome 5, linkage group XVII) (26) and for transferrin (chromosome 9, linkage group II) (27) on chromosomes other than that of the albino locus is significant. This eliminates any possibility of ascribing the albumin or transferrin deficiency to a mutation of the structural gene. Even though the remaining proteins and enzymes have not been mapped, the respective structural genes are not likely to be included in the albino deletions. Further identification of the nature of the genome involved in the albino lethal deletions is expected from experiments in which mutant cells are hybridized with rat hepatoma cells.

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