Protein Degradation in Normal and Beige (Chediak-Higashi) Mice

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ABSTRACT The beige mouse, C57BL/6 (bg/bg), is an animal model for the Chediak-Higashi syndrome in man, a disease characterized morphologically by giant lysosomes in most cell types. Half-lives for the turnover of [^{14}C]bicarbonate-labeled total soluble liver protein were determined in normal and beige mice. No significant differences were observed between the normal and mutant strain for both rapidly and slowly turning-over classes of proteins. Glucagon treatment during the time-course of protein degradation had similar effects on both normal and mutant strains and led to the conclusion that the rate of turnover of endogenous intracellular protein in the beige mouse liver does not differ from normal.

The rates of uptake and degradation of an exogenous protein were determined in normal and beige mice by intravenously injecting ^{125}I-bovine serum albumin and following, in peripheral blood, the loss with time of phosphotungstic acid-insoluble bovine serum albumin and the parallel appearance of phosphotungstic acid-soluble (degraded) material. No significant differences were observed between beige and normal mice in the uptake by liver lysosomes of ^{125}I-bovine serum albumin ($t_{1/2}$ = 3.9 and 2.8 h, respectively). However, it was found that lysosomes from livers of beige mice released phosphotungstic acid-soluble radioactivity at a rate significantly slower than normal ($t_{1/2}$ = 6.8 and 3.1 h, respectively). This defect in beige mice could be corrected by chronic administration of carbamyl choline ($t_{1/2}$ = 3.5 h), a cholinergic agonist which raises intracellular cyclic GMP levels. However, no significant differences between normal and beige mice were observed either in the ability of soluble extracts of liver and kidney to bind [^{3}H]cyclic GMP in vitro or in the basal levels of cyclic AMP in both tissues. The relevance of these observations to the presumed biochemical defect underlying the Chediak-Higashi syndrome is discussed.

INTRODUCTION

The beige mouse is an animal model for the human Chediak-Higashi (CH)[1] syndrome, a rare autosomal recessively inherited disease. The disease is characterized by large granules (lysosomes) in most cell types, partial albinism, increased susceptibility to pyogenic infections, and several functional abnormalities of leukocytes including defective lysosomal degranulation and impaired chemotaxis. Thus far, the disease has been described in man, mink, cattle, and mice (2–7).

Although the exact biochemical defect is not known, recent reports have indicated that lysosomes may accumulate in the beige mouse because of defective exocytosis resulting either from decreased intracellular motility of lysosomes or from their improper fusion with the plasma membrane. Brandt et al. (8) have reported that beige mice treated with androgen had significantly higher levels of three hydrolytic lysosomal enzymes in kidney than the normal parent strain (C57BL/6). Excretion into the urine is a major mechanism of removal of lysosomal enzymes from normal mouse kidney. Brandt et al. proposed that this process is impaired in beige mice because of an abnormal fusion of lysosomes with the plasma membrane of the kidney proximal tubule cells.

Oliver and co-workers (9, 10) studied the mobility of concanavalin A receptor complexes on polymorpho-

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; cAMP, cyclic AMP; cGMP, cyclic GMP; CH, Chediak-Higashi; PMN, polymorphonuclear leukocyte; PTA, phosphotungstic acid; S_3, 105,000 *g* supernate of tissue homogenate.

nuclear leukocyte (PMN) membranes as a test of microtubule integrity. They reported that normal PMNs showed uniform distribution of membrane-bound concanavalin A. By contrast, concanavalin A was aggregated into surface "caps" on both colchicine-treated normal PMNs and untreated PMNs from either beige mice or human CH patients.

The spontaneous capping response of PMNs of the beige mouse in vitro was inhibited by cyclic (c)GMP and by cholinergic agonists such as carbamyl choline that raise intracellular cGMP levels. Oliver et al. also reported that these drugs and cGMP itself prevented the formation of giant lysosomes in cultured embryonic fibroblasts from beige mice (11).

These responses can also be reproduced in vivo in the beige mouse (9). Animals treated with low levels of cholinergic agonists show normal granule morphology and a normal degree of concanavalin A-induced cap formation in peripheral blood PMN. These findings led to a hypothesis that defective cGMP generation underlies all characteristic changes seen in the disease.

Because ascorbic acid potentiates chemotaxis of normal leukocytes, Boxer et al. (12) examined the effects of ascorbate on PMNs from Chediak-Higashi patients. They reported that cAMP levels in PMNs of such patients were an order of magnitude higher than normal. Ascorbic acid treatment, both in vitro and in vivo, reduced cAMP levels and corrected functional defects in PMNs from patients with CH syndrome. They hypothesized that cGMP, which also appeared to correct CH leukocyte function in vitro, may act by antagonizing the effect of excessive cAMP. Thus cAMP may inhibit both microtubular assembly (13) and chemotaxis (14) while cGMP potentiates these processes in normal leukocytes.

The lysosome is widely believed to be involved in the degradation of proteins of intracellular (15–18), serum (19, 20), and foreign (15, 21) origin. Because the beige mouse exhibits abnormally enlarged lysosomes in most cell types, we have compared the turnover of endogenous and exogenous proteins in normal and beige mice. In addition, we have examined both the levels of cAMP and the binding of [^{3}H]cGMP in normal and beige mouse tissue extracts. These assays were performed because the uptake of materials by lysosomes may involve cAMP and microtubule integrity (22, 23). Defective microtubule formation in the beige mouse may in turn be the result of abnormal cyclic nucleotide metabolism.

METHODS

Animals. Male inbred C57BL/6 mice were purchased from Sprague-Dawley (Madison, Wis.); male C57BL/6 (bg/bg) mice were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were bred at the McArdle Laboratory. Both strains were utilized at 3–4 mo of age. The animals were maintained on a 12-h light–12-h dark schedule, and were fed *ad libitum* on Purina chow (Ralston Purina Co., St. Louis, Mo.).

Turnover of endogenous liver proteins. Where indicated, glucagon (200 μg/day per mouse) was administered subcutaneously as a 1:1 emulsion with trioctanoin (24). All mice were injected with 100 μCi $NaH^{14}CO_3$ (60 mCi/mmol) and were killed by cervical dislocation at times indicated. Livers were homogenized in 4 ml of 0.25 M sucrose–0.05 M Tris, pH 7.8–0.025 M KCl–0.005 M $MgCl_2$ and were centrifuged for 10 min at 10,000 rpm (Sorvall SS-34 rotor, DuPont Co., Instrument Products Div., Wilmington, Del.). The resulting supernates were then recentrifuged for 1 h at 45,000 rpm (Beckman 50 Ti rotor, Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). These final supernatant (S_3) fractions were stored frozen at −20°C.

50-μl aliquots of S_3 were pipetted in duplicate on glass fiber discs (Whatman GF/C, 2.4 cm, Fisher Scientific Co., Pittsburgh, Pa.), the proteins were precipitated in 10% trichloroacetic acid, and the discs were washed as described previously (24). Radioactivity was determined with toluene-2,5-diphenyloxazole on a Nuclear Chicago scintillation counter at about 25% efficiency.

A second set of duplicate discs containing 50-μl aliquots of S_3 was washed by the same procedure and was used for protein determinations. These washed discs were incubated overnight in scintillation vials containing 2 ml of 1 N NaOH to dissolve the protein. 100-μl aliquots were removed for protein determination by the method of Lowry et al. (25).

Uptake and degradation of ^{125}I-BSA. All mice received 0.01% NaI (wt/vol) in their drinking water for at least 1 wk before use in these experiments to minimize uptake of iodide by the thyroid gland. Where indicated, some groups also received 0.02% (wt/vol) carbamyl choline for at least 1 wk in their drinking water (9), which was changed daily. Animals were lightly anesthetized with Nembutal and were injected via the tail vein with 50 μl of a solution containing 20 μCi ^{125}I-bovine serum albumin (BSA) with nonradioactive carrier BSA to a total of 100 μg protein. A Hamilton microliter syringe was used when precise measurements of uptake were desired, as described by Davidson et al. (26). At suitable intervals, duplicate 20-μl blood samples were taken in heparinized disposable micropipets by slicing 1 mm of tissue from the end of the tail. Blood samples were immediately added to 1-ml aliquots of phosphotungstic acid (PTA) reagent (0.375% PTA in 0.5% HCl), vortexed and allowed to stand at least 15 min on ice. The tubes were then centrifuged for 30 min at 2,800 rpm in a Sorvall GLC-1 centrifuge (DuPont Co., Instrument Products Div.) located in a cold room. The precipitates were washed twice in 1-ml aliquots of cold PTA reagent; tubes containing these precipitates were placed in plastic tissue culture tubes (no. 3033, Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). The three supernates (original plus two washes) were combined in other culture tubes, and the radioactivity was determined with a gamma counter (Amersham/Searle Corp., Arlington Heights, Ill.) at 75% efficiency for ^{125}I.

In experiments designed to measure the organ distribution of injected ^{125}I-BSA, animals were perfused through the heart with cold physiological saline, as described by Davidson et al. (26), until livers and kidneys had blanched free of blood.

Cyclic GMP binding assay. The cGMP binding assay was

performed with a variation of the method of Lincoln et al. (27). Male mice were killed by cervical dislocation, and the tissues were quickly excised and homogenized in 2 vol of 25 mM Na phosphate, pH 7.4, containing 2 mM EDTA and 10 mM dithiothreitol. The homogenates were centrifuged at 10,000 rpm for 15 min (Sorvall SS-34 rotor), and the resulting postmitochondrial supernate was centrifuged at 45,000 rpm for 90 min (Beckman 50 Ti rotor). Aliquots of the resulting high-speed supernate (S_3) were used for both cGMP binding and protein determination by the biuret method (28).

The binding assay was performed in a total volume of 0.12 ml and contained, in order of addition: 0.03 ml of 25 mM Na phosphate, pH 7.4; 2 mM EDTA; 0.02 ml of 2.5 mM 3-isobutyl 1-methylxanthine; 0.02 ml of [^{3}H]cGMP (0.1–5 pmol); and 0.05 ml S_3. To estimate nonspecific binding of radioactivity, blank discs were assayed with aliquots of BSA (30 mg/ml) added in place of S_3. Blank values were routinely subtracted from the radioactivity values of the assay discs. 3-isobutyl 1-methylxanthine, an inhibitor of cGMP phosphodiesterase (29), was first dissolved in 20% ethanol with phosphate-EDTA buffer (same as above) and then added to volume. The assay mixture was incubated in 3-ml glass centrifuge tubes for 60 min on ice. At the end of this time period, 2 ml of cold phosphate-EDTA buffer was added to each tube, and the contents were quickly filtered through Millipore filters (2.5-cm diameter, type HA, 0.45-μm pore size) with a Millipore sampling manifold (Millipore Corp., Bedford, Mass., no. 2702550). The tubes and filters were washed four times; twice with 2-ml aliquots of phosphate-EDTA buffer and then twice with 2-ml aliquots of 10 mM $MgCl_2$. The filters were placed in scintillation vials and then air dried at 90°C. After adding 5 ml of toluene-2,5-diphenyloxazole, bound radioactivity was determined in a Nuclear-Chicago scintillation counter at about 25% efficiency.

Glucagon was purchased from Eli Lilly and Co., Indianapolis, Ind.; trioctanoin from Eastman Chemical Products, Inc., Kingsport, Tenn.; phosphotungstic acid from Mallinckrodt Chemical Works, St. Louis, Mo.; and 3-isobutyl-1-methylxanthine from Aldrich Chemical Co., Milwaukee, Wis. Carbamyl choline and Trizma base were products of the Sigma Chemical Co., St. Louis, Mo. ^{125}I-BSA (6–60 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. Both $NaH^{14}CO_3$ (60 mCi/mmol) and [8-^{3}H]cGMP (21 Ci/mmol) were purchased from Amersham/Searle Corp.

RESULTS

To determine whether or not the abnormally large lysosomes found in all tissues of the beige mouse are associated with any aberrations in the overall rate of intracellular protein degradation, half-lives for [^{14}C]bicarbonate-labeled soluble liver proteins were determined. Mice were each injected with 100 μCi of $NaH^{14}CO_3$ (30) and then killed at specified intervals. Radioactivity per milligram protein was determined in aliquots of liver cytosol as described under Methods.

The results of a 137-h experiment designed to measure the mean half-life of a slowly turning-over class of liver proteins are shown in Fig. 1. A similar experiment that measures the mean half-life of more labile liver proteins by following an 8-h time-course is shown in Fig. 2. Table I summarizes the results of a series of such protein degradation studies.

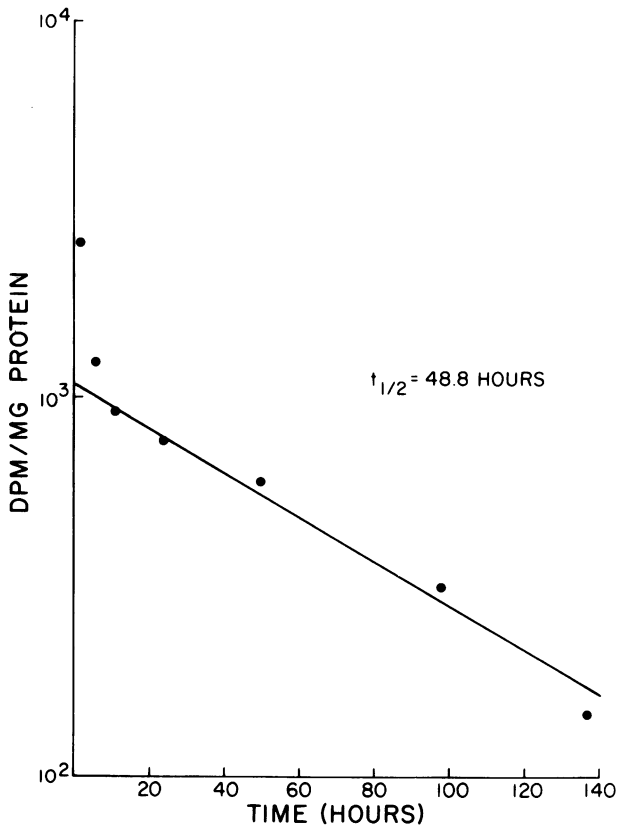

FIGURE 1 Turnover of total soluble liver protein in normal C57BL/6 mice. Mice were injected with 100 μCi $NaH^{14}CO_3$ and killed in groups of three at the times indicated. Pooled liver homogenates were centrifuged as described under Methods to obtain postmicrosomal supernatant fractions. Aliquots of these supernatants were pipetted onto glass fiber discs, the proteins were precipitated with cold 10% trichloroacetic acid, and nonprotein-bound radioactivity was removed by sequential washings as described previously (24). Radioactivity per milligram protein on each disc was determined as described under Methods.

No significant differences were observed between normal and beige mice in the turnover of either class of soluble liver proteins. The effect of treatment of mice with glucagon was tested inasmuch as it has been reported that this hormone induces autophagic vacuole formation and alters several physical properties of lysosomes (31–33). A single intraperitoneal injection of 100 μg of glucagon per day slowed the degradation of the more rapidly turning-over class of proteins somewhat, but had only a slight effect on the half-life of the more stable class (Table I). Again, no significant differences between normal and beige mice were observed.

Because it was apparent that the degradation of endogenous intracellular liver protein was not impaired in beige mice, the ability of these animals to degrade exogenous protein was examined. ^{125}I-BSA

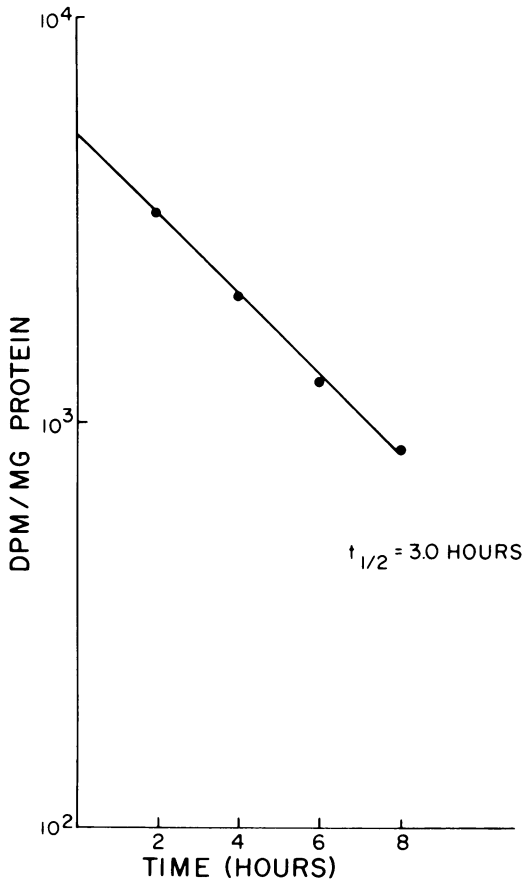

FIGURE 2 Turnover of total soluble liver protein in normal C57BL/6 mice. The protocol was exactly the same as in Fig. 1, except that groups of three mice were killed at 2-h intervals up to only 8 h; thus more rapidly turning-over proteins were examined.

(100 μg protein containing 16 μCi) was injected into the tail vein of anesthetized mice. Duplicate blood samples were taken from the tail at 15-min intervals, and the native proteins were precipitated immediately with cold PTA reagent and washed as described under Methods. The PTA-insoluble precipitate represented native ^{125}I-BSA, while the PTA-soluble supernate contained degraded fragments of this protein (26). A typical time-course for the disappearance of native ^{125}I-BSA and the parallel appearance of degraded fragments in the peripheral blood is shown in Fig. 3. These processes were essentially linear for at least 4 h when plotted semilogarithmically.

The removal of ^{125}I-BSA from the blood is accomplished in vivo by pinocytosis and results in the formation of pinocytotic vacuoles in liver and kidney cells (34–36). These vacuoles fuse with primary lysosomes, resulting in the formation of secondary lysosomes. These bodies are the site of degradation of exogenous proteins (15, 21, 26, 37). The secondary lysosomes may then migrate to and fuse with the plasma membrane where the degraded fragments are discharged into the extracellular compartment by exocytosis. Tables II and III summarize the apparent half-lives for the processing of (^{125}I)-BSA by these mechanisms, as determined from the slopes of the logarithmic plots.

It was observed (Table II) that untreated beige mice had a small but significant ($P < 0.005$) impairment in the rate of uptake of ^{125}I-BSA compared with normal. When mice were treated with 100 μg of glucagon 1 h before ^{125}I-BSA injection, there was a small increase in the half-life of circulating native BSA in both strains. This increase was significant ($P < 0.0025$) for normal mice but not significant for the mutant group. When animals were treated with low levels of the cholinergic agonist, carbamyl choline, in their drinking water, the rate of BSA uptake for each strain was not significantly different from that of untreated controls.

The half-life for the appearance of circulating PTA-soluble fragments of BSA is shown in Table III. The half-life for this process in beige mice was more than twice that observed in normal mice. Glucagon treatment caused almost a fourfold increase in this half-life for normal mice with no change in this parameter in beige mice. Carbamyl choline administration reversed this defect in the mutant mice in that the release of PTA-soluble radioactivity under these conditions occurred at a rate not significantly different from normal.

To determine which organ took up most of the injected ^{125}I-BSA, groups of normal mice were killed at

TABLE I
Turnover of Total Soluble Intracellular Protein in Normal and Beige Mouse Liver

Condition	Half-life	
	Normal	Beige
	h	
(A) Untreated		
Short-term	3.0	3.7
Long-term	48.8	46.0
(B) Glucagon-treated		
Short-term	4.2	4.1
Long-term	46.4	45.1

The protocol was the same as described under Figs. 1 and 2. Groups of mice receiving glucagon were injected once daily with 200 μg glucagon subcutaneously as a 1:1 emulsion in trioctanoin. The degradative rate constants (K) were determined from the linear regression of ln (dpm/mg) against time, and half-lives were then calculated from the relationship $t_{1/2} = \ln 2/K$.

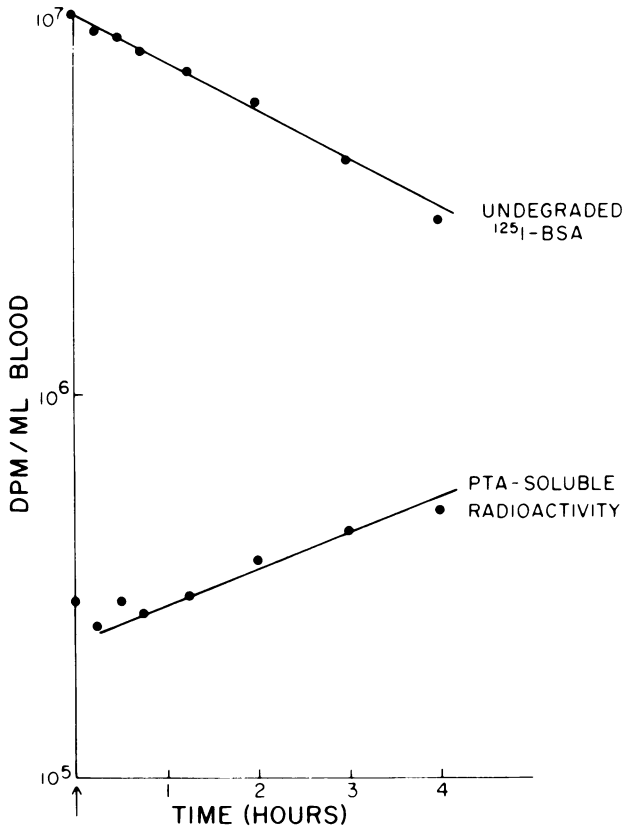

FIGURE 3 Degradation of ^{125}I-BSA in a normal C57BL/6 mouse. ^{125}I-BSA (100 μg protein containing 16 μCi) was injected into the tail vein. Blood samples were taken at intervals shown and were immediately precipitated in cold PTA reagent. Precipitates were isolated and washed by centrifugation as described under Methods. Radioactivity in PTA-insoluble precipitates represents undegraded native BSA; PTA-soluble radioactivity represents degraded fragments (26).

intervals after injection, and their livers and kidneys were blanched *in situ* with physiological saline as described in Methods. This perfusion removed most of the radioactive blood from these organs and permitted an estimate of bound intracellular ^{125}I-BSA. As shown in Fig. 4, 34% of the injected radioactivity remained in the extracellular (blood) compartment after 5 min, with 18% and 6% in the liver and kidney, respectively. After 30 or 60 min, the liver still held over three times the radioactivity found in the kidney. Thus the liver appears to be the primary site of ^{125}I-BSA degradation. When this experiment was repeated with beige mice, essentially identical results were obtained (data not shown).

Oliver and Zurier (9) have reported that cGMP and two analogs of acetylcholine (carbamyl choline and carbamyl-β-methylcholine) which elevate intracellular cGMP levels (38) can reverse the abnormal

TABLE II
Uptake of ^{125}I-Bovine Serum Albumin from Peripheral Blood

Treatment	Half-life	
	Normal	Beige
	h	
None (n = 7)	2.8±0.3	3.9±0.2
Glucagon (n = 6)	4.4±0.2	4.6±0.4
Carbamyl choline (n = 4)	2.6±0.2	3.5±0.3

The protocol is described under Methods. Half-lives were calculated as described under Table I. Values for each group represent the mean±SEM of the number of animals shown in parentheses. The statistical significance of the differences between group means was tested by using a one-tailed Student's t test, and the results are reported in the text.

lysosomal morphology seen in PMNs from human CH patients. In addition, we have just shown that carbamyl choline can reverse a functional defect in beige mice. It therefore seemed possible that the recessive mutation which causes the CH syndrome may involve an alteration of cGMP binding proteins. To test this hypothesis, an assay for cGMP binding proteins in mouse tissues was performed as outlined under Methods. The results shown in Table IV demonstrate the specificity of the binding assay under the conditions employed. Binding was assayed at pH 7.4 because cGMP is reported to bind to type I cAMP-dependent protein kinase at pH 4 (39). We observed that in the presence of a 20-fold molar excess of cAMP over cGMP, 87% of [^{3}H]cGMP bound to the proteins compared with the control without cAMP. Excess nonradioactive cGMP competed almost completely with [^{3}H]cGMP as expected. GMP caused some enhanced binding of [^{3}H]cGMP, an observation similar to that reported by Lincoln et al. (27).

TABLE III
Release of PTA-Soluble ^{125}I into Peripheral Blood

Treatment	Half-life	
	Normal	Beige
	h	
None (n = 5)	3.1±0.2	6.8±0.4
Glucagon (n = 5)	11.8±1.1	6.7±0.5
Carbamyl choline (n = 4)	3.4±0.4	3.5±0.5

The protocol is described under Methods. Half-lives were calculated as described under Table I. Values for each group represent the mean±SEM of the number of animals shown in parentheses. The statistical significance of the differences between group means was tested by using a one-tailed Student's t test, and the results are reported in the text.

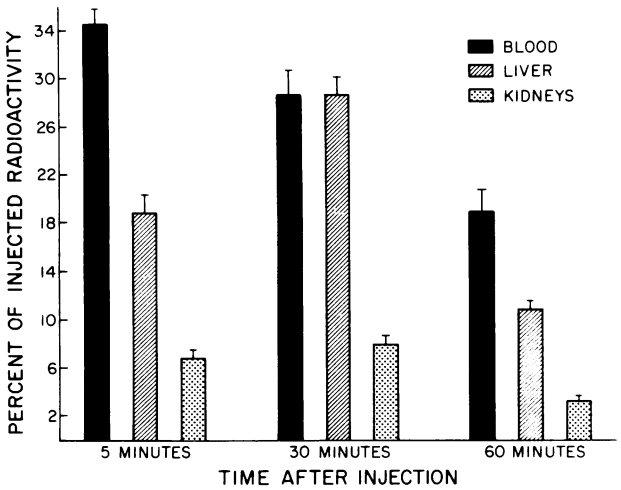

FIGURE 4 Distribution of radioactivity after intravenous injection of ^{125}I-BSA into normal C57BL/6 mice. Mice were injected with 8 μCi of ^{125}I-BSA, killed at intervals shown, and their organs were perfused *in situ* with cold physiological saline as described by Davidson et al. (26). Blood samples were taken with a 100-μl capillary pipette from chest cavity bleeding immediately after cervical dislocation. Whole organs and blood samples were placed in plastic culture tubes (Falcon Plastics), and the radioactivity was counted in a gamma counter as described under Methods. Values for each time point represent the mean±SEM of three animals.

TABLE IV
Competition Assay for Binding of [^{3}H]cGMP by Mouse Tissue Extracts

Competitor added	Molar excess	[^{3}H]cGMP bound
		% of control
None	—	100
cAMP	20	87
	100	78
	500	47
	1000	32
cGMP	20	11
	100	4
	500	2
	1000	2
GMP	20	128
	100	145
	500	158
	1000	163

Binding was measured as described under Methods. The concentration of [^{3}H]cGMP used was 40 nM.

A Scatchard plot for the binding of [^{3}H]cGMP by soluble proteins from normal mouse liver cytosol is shown in Fig. 5. The apparent equilibrium dissociation constant (K_d) was determined from the slope, and the number of picomoles of [^{3}H]cGMP bound per milligram protein was determined from the x intercept. Table V summarizes the results of a series of such Scatchard plots with soluble extracts from liver, kidney, and lung of normal and beige mice. Both the affinity of the binding sites and the number of sites per milligram protein were essentially identical for normal as compared with beige mice in the three tissues surveyed. In agreement with Lincoln et al. (27), the lung extract contained the highest level of cGMP binding protein(s).

Boxer et al. (12) recently reported abnormally

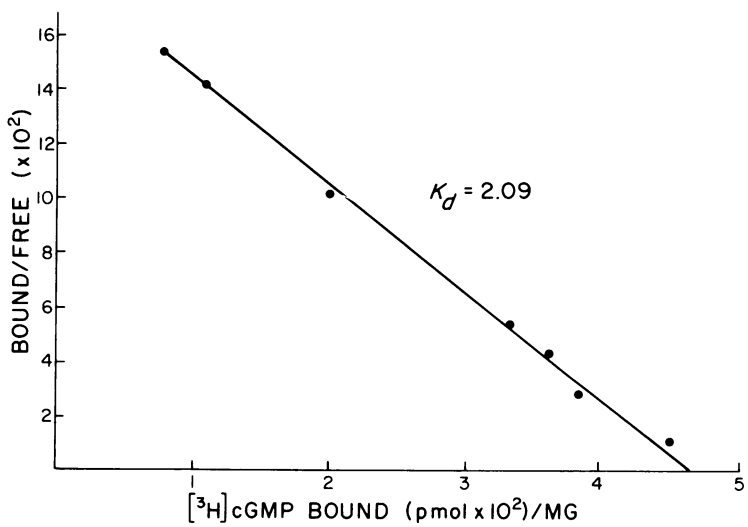

FIGURE 5 Scatchard plot for [^{3}H]cGMP binding to normal mouse liver cytosol proteins. Binding was determined as described under Methods.

TABLE V
Summary of Scatchard Plots for [^{3}H]cGMP Binding by Mouse Tissue Extracts

Tissue	Strain	K_d	[^{3}H]cGMP bound per milligram protein
		nM	*pmol*
Liver	Normal	2.28	0.045
	Beige	2.10	0.045
Kidney	Normal	5.25	0.127
	Beige	5.38	0.134
Lung	Normal	25.57	1.607
	Beige	24.33	1.555

[^{3}H]cGMP binding data were determined as described under Methods, and Scatchard plots were drawn as shown in Fig. 5. The apparent equilibrium dissociation constants (K_d) were determined from the slopes, and the numbers of moles of [^{3}H]cGMP bound per milligram protein were determined from the × intercepts of these plots.

high levels of cAMP in PMNs from a human CH patient. Both the high levels of cAMP and the functional defects in these cells were corrected by ascorbic acid therapy in vivo or in vitro. Inasmuch as microtubule assembly is reported to be inhibited by cAMP and potentiated by cGMP (13), Boxer et al. hypothesized that the CH defect may be related to a defect in cAMP metabolism. To test whether or not the beige mouse also had elevated levels of cAMP, this cyclic nucleotide was assayed in both liver and kidney of normal and beige mice. As shown in Table VI, cAMP levels in both organs were essentially identical for the two mouse strains.

DISCUSSION

The results of this investigation show that normal and beige mice have essentially identical rates of degradation of total soluble liver proteins (Table IA). This suggests that if lysosomes are involved in intracellular protein degradation, they do not represent the rate-limiting step. Despite the presence of morphologically abnormal lysosomes in CH disease, there are contradictory reports concerning lysosomal enzyme levels. Some researchers have found altered lysosomal enzyme activity (40), but these reports have not been confirmed by other investigators (3, 41).

TABLE VI
Cyclic AMP Levels of Normal and Beige Mouse Liver and Kidney

Tissue	Strain	cAMP per gram tissue
		pmol
Liver	Normal	703±55
	Beige	707±52
Kidney	Normal	743±43
	Beige	743±26

cAMP determinations were carried out with a cAMP radioimmunoassay kit (Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y., no. 0750-13), following the recommended procedures. Each group represents the mean±SEM of three tissue samples, each assayed in duplicate.

Glucagon has been reported to induce autophagic vacuole formation in rat liver (32, 42). The process involves transfer of enzyme from preexisting hydrolase-containing components to newly formed autophagic vacuoles. Morphological changes and an increase in the osmotic sensitivity of lysosomes occur during the first 50 min after glucagon injection (21, 32). Under the conditions in our studies (Table IB), glucagon treatment appeared to have no effect on the turnover of the more stable class of soluble liver proteins and to prolong somewhat the mean half-life of the more rapidly turning over group. The latter effect may be due to a hormonally-induced shift in synthesis to a slightly different class of proteins with a different mean half-life, or it may be an artefact due to the enhanced fixation of [^{14}C]bicarbonate in the presence of greater activity of pyruvate carboxylase. In either case, the conclusion remains that the turnover of endogenous soluble liver proteins was unaffected by the beige mutation.

Padgett et al. (43) reported that 48 hr after normal and beige mice were injected intravenously with horseradish peroxidase, the lysosomes of normal mice had digested almost all of the horseradish peroxidase, whereas large amounts were still present in CH mice at this time. Using ultrastructural cytochemistry, these authors did not find any gross differences between normal and beige animals in the pinocytotic uptake of this foreign protein, but rather in the lysosomal processing. Consistent with these results, the present study concludes that the uptake of another foreign protein, ^{125}I-BSA, is slightly but significantly reduced in beige mice (Table II), but that the release of PTA-soluble fragments is markedly impaired (Table III).

The half-life for the degradation of ^{125}I-BSA in normal mice reported here is about 3 h. Gabathuler and Ryser (44) reported a half-life of 90 min for the degradation of ^{131}I-albumin by Sarcoma S180 cells in culture. These values are considerably greater than the half-lives reported by Davidson (45) for the degradation of ^{125}I-ribonuclease by mouse kidney lysosomes: 14 min in vivo and 11 min in vitro. These differences may possibly be related to the reported observation that 55% of injected ^{125}I-ribonuclease was absorbed by mouse kidney after 7 min (26), whereas in this present study (Fig. 4) peak absorption of ^{125}I-BSA did not

occur until about 30 min after injection. At this time, 28% of the label was bound in liver and only 8% in kidney. The preferential uptake of BSA by liver and ribonuclease by kidney may be related to the large molecular weight difference between the two proteins (67,000 for BSA and 14,000 for ribonuclease).

The nature of the presumed defect in cyclic nucleotide metabolism in CH cells has not yet been elucidated. In addition, the precise role of cGMP in microtubule assembly is not understood. Inasmuch as microtubule assembly in vitro is promoted by reduced glutathione (46) and inhibited by calcium ions (47), cGMP may represent an intermediate in the reaction. Although we have found no significant differences in total cGMP binding proteins of normal vs. beige mice (Table V), it is still possible that the cGMP-dependent protein kinase of beige mice has an altered affinity for cGMP. If this kinase were involved in microtubule assembly, this would explain the reversal of abnormalities in lysosomal morphology and function observed in vivo and in vitro when CH cells are exposed to agents which raise intracellular cGMP levels. The cGMP-dependent protein kinases of normal and beige mice are currently being compared in this laboratory.

In the studies described herein (Table VI), no elevation of cAMP levels in beige mouse liver or kidney cells was seen. The report by Boxer et al. (12) of a 10-fold elevation of cAMP in human CH PMNs compared with normal may be a phenomenon characteristic of the human CH leukocytes only. The hypothesis by Boxer et al. that excess cAMP inhibits microtubule assembly in CH leukocytes is contrary to the results of DiPasquale et al. (48), who report that cAMP actually increases the ratio of polymerized to unpolymerized tubulin in cultivated Greene melanoma cells. A complete description of the biochemical defect underlying the CH syndrome may not be forthcoming until the mechanism of microtubule assembly is more fully understood.

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