JA-116

EXPERIMENTAL AND MOLECULAR PATHOLOGY 58, 40–52 (1993)

Studies of Adenine Nucleotide Biochemistry in the Chediak–Higashi Syndrome

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Received January 31, 1992, and in revised form December 11, 1992

The Chediak–Higashi syndrome (CHS) is an inherited disorder of humans and of several animal species, characterized by partial albinism, pseudohemophilia, increased susceptibility to disease, and large inclusions in all granule-forming cells. In this study, various parameters of adenine nucleotide biochemistry were examined in beige mouse kidney tissue and in peripheral blood leukocytes from CHS mink. There were no differences in the total protein content, total ATPase activity or the magnesium (Mg^{2+}) ATPase or the sodium–potassium (Na^+–K^+) ATPase activities, the concentrations of ATP, ADP, and AMP, or the adenylate energy charge (AEC) in kidney extracts from beige and normal mice. In studies of leukocytes, there were no differences in the concentrations of ATP, ADP, AMP, and cAMP or the AECs in total leukocyte preparations and in extracts from granulocytes or nongranulocytes. These results can be explained by any one of several hypotheses: no storage pool of adenine nucleotides exists in the tissues examined; or the alleged storage pool is not affected by CHS; or the quantity of nucleotides in the alleged storage pool is too minute to be evaluated by current techniques; or the CHS defect might cause a shift from the storage pool to the metabolic pool. © 1993 Academic Press, Inc.

INTRODUCTION

The Chediak–Higashi syndrome (CHS) is an autosomal recessive genetic disorder, characterized by partial oculocutaneous albinism, bleeding tendencies, increased susceptibility to disease, and large intracytoplasmic inclusions in all granule-forming cells (Beguez-Cesar, 1943; Padgett *et al.*, 1964; Lutzner *et al.*, 1967; Taylor and Farrell, 1973; Kramer *et al.*, 1977). The disease was first described in humans (Beguez-Cesar, 1943) and has since been reported in several animal species including mink (Padgett *et al.*, 1964), cattle (Padgett *et al.*, 1964), mice (Lutzner *et al.*, 1967), cats (Kramer *et al.*, 1977), and a killer whale (Taylor and Farrell, 1973).

CHS is a genetic disorder that affects nearly every organ system and cell type in the body. The basic biochemical defect responsible for the various abnormalities of CHS has not been defined; however, the autosomal recessive nature of the syndrome mandates the formation of hypotheses which are developed around a common denominator for all affected cell types.

It is well documented that the pseudohemophilia of CHS is due to a thrombopathia which involves a virtual absence of dense granules from affected platelets and defective storage of the substances normally contained within those granules (Bell *et al.*, 1976; Buchanan and Handin, 1976; Corash *et al.*, 1977; Meyers *et al.*, 1978; Meyers *et al.*, 1979a,b,c; Meyers *et al.*, 1981; Rendu *et al.*, 1983). Among the compounds included in this storage pool and found at reduced levels in CHS platelets are ATP, ADP, and serotonin (Bell *et al.*, 1976; Buchanan and Handin,

0014-4800/93 $5.00
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1976; Corash *et al.*, 1977; Meyers *et al.*, 1978; Meyers *et al.*, 1979a,b,c; Meyers *et al.*, 1981; Rendu *et al.*, 1983). Furthermore, it has been shown that the decreased amounts of these compounds are due almost exclusively to the absence of this storage pool, justifying the classification of the bleeding problem of CHS as a platelet storage pool defect (Bell *et al.*, 1976; Buchanan and Handin, 1976; Corash *et al.*, 1977; Meyers *et al.*, 1979a,b; Meyers *et al.*, 1981).

Functional and morphologic defects have been described in most cell types in affected individuals, including peripheral blood leukocytes and renal tubular epithelial cells. The CHS inclusions are a consistent morphologic feature in leukocytes as well as in renal tubular epithelial cells from affected species (Beguez-Cesar, 1943; Sato, 1955; Donohue and Bain, 1957; Padgett *et al.*, 1964, Lutzner *et al.*, 1966; Lutzner *et al.*, 1967; Prieur *et al.*, 1972; Taylor and Farrell, 1973; Essner and Oliver, 1973; Oliver, 1973; Essner *et al.*, 1974; Kramer *et al.*, 1977; White and Clawson, 1979; Eguchi *et al.*, 1982). CHS leukocytes suffer from various functional abnormalities which undoubtedly include decreased bactericidal activity and defective antibody-dependent cellular cytotoxicity. These may also include diminished chemotactic and phagocytic capacities (Clark and Kimball, 1970; Davis, 1970; Clawson *et al.*, 1971; Gallin *et al.*, 1974; Renshaw *et al.*, 1974; Clawson *et al.*, 1979).

Evidence of defective kidney function has also been reported in CHS. The clearance of exogenous substances, such as horseradish peroxidase, by affected kidneys occurs at a rate much lower than normal (Brandt *et al.*, 1975; Lyons and Pitot, 1976). This phenomenon was traced to the accumulation of these substances within CHS megagranules in renal proximal tubular epithelial cells.

Another interesting finding is that the enlarged CHS granules have a tendency to leak their contents into the cytosol (Kimball and Ford, 1970; Kimball *et al.*, 1975). This might conceivably expose compounds, which normally would be protected by cell membranes, to excessive catabolism.

In this study, we attempted to ascertain whether or not the defect in storage of adenine nucleotides, seen in platelets in CHS, might also exist in other cells. Currently, it is generally accepted that cells, other than platelets, do not store adenine nucleotides; however, our examination of the literature has uncovered no scientific evidence to support this belief. In fact, several authors have documented that ATP and 5-hydroxytryptamine have a propensity to form micelles in artificial mixtures and in storage organelles (Bernies *et al.*, 1969), and they have shown that this phenomenon occurs not only in platelets, but also in megakaryocytes (Tranzer *et al.*, 1972) and in cells of the adrenal medulla (Smith, 1968). Serotonin storage has also been documented in neurons (Inversen, 1967) and some authors have suggested that the neuronal amine storage resembles that of platelets enough to warrant using platelets as a model for aminergic neurons. This would tend to support a hypothesis that other cells might also store amine nucleotide complexes. Interestingly, it has been shown that treatment of CHS neutrophils with serotonin achieved partial correction of the bactericidal defect (Kaplan *et al.*, 1978). Also, our initial studies showed that leukocytes of CHS mink and cattle had significantly lower levels of ATP and ADP than normal (Jamison *et al.*, 1980). It was hypothesized that, if an undefined storage pool for adenine nucleotides existed in cells, the CHS defect might be manifested, similarly to platelets, by defective storage of these nucleotides. Adenine nucleotides were measured in beige mouse kidneys and in CHS mink peripheral blood leukocytes.

MATERIALS AND METHODS

Mouse Renal Studies

Three breeding pairs each of CHS mice (C57 Bl/6J bg/bg) and control mice (C57 Bl/6J) were purchased from Jackson Laboratory (Bar Harbor, ME). The colonies were established from these breeding pairs and they were housed in the Laboratory Animal Care Service Center at Michigan State University. Age- and sex-matched pairs were selected for the experiments.

In the mouse renal nucleotide studies, the mice were anesthetized with pentobarbital and the kidneys were surgically removed. Immediately after being removed, the kidneys were placed in labeled polyethylene freezer bags and were quickly frozen to −196°C by freeze-clamping as described by Giesy and Dickson (1981). Extraction of the nucleotides was accomplished as described by Geisy and Dickson (1981).

The nucleotides were measured with high-pressure liquid chromatography (HPLC). The mobile phase was driven by two LDC constametric HPLC pumps. Samples were injected in precise 10.0-μl volumes and the nucleotides were separated with a Partisil-10 strong anion exchange column. The nucleotides were detected with an ultraviolet ISCO V-4 variable wavelength detector at a wavelength of 258 nm. A Hewlett–Packard recording integrator was used to record the data. A gradient system, controlled by an LDC gradient master, was used for the mobile phase. The flow rate was maintained at 1.0 ml per min. The initial conditions were 100% solution A and the gradient proceeded nonlinearly (mode 3) to 85% solution B in 45 min. The final conditions were maintained for 15 min and then the gradient was automatically reset. The column was allowed to reequilibrate between runs by making injections at precisely 1.5-hr intervals. Nucleotide values were corrected based on the dry weight of the tissue pellets.

In the enzyme assays, the mice were euthanatized by cervical dislocation and both kidneys were immediately removed and placed in a beaker on ice. A 15-ml Corning glass tissue grinder was placed on ice and about 5.0 ml of 0.01 *M* Tris–HCl was pipetted into the tissue grinder. A mouse was then selected and euthanatized and both kidneys were immediately removed and placed on ice. One kidney from each mouse was weighed and assayed for its protein content using a Sigma Diagnostics Total Protein Kit. The other kidney was placed in the tissue grinder and the grinder was shaken. The 0.01 *M* Tris HCl was poured off, retaining the kidney, and ice-cold 0.01 *M* Tris–HCl was added to a final volume of 8.0 ml. The kidney–Tris–HCl mixture was then ground thoroughly while holding the tissue grinder in ice. The homogenate from each mouse was assayed for protein content with a Sigma Diagnostics total protein kit and was then adjusted to 1.0 mg protein/ml.

The ATPase assays were completed as described by Akera and Cheng (1977).

Mink Leukocyte Studies

The mink were purchased from farms with no recent history of Aleutian disease and were kept for a period known to exceed the incubation period for Aleutian disease. They were housed out of doors at the Poultry–Fur Bearing Animal Farm at Michigan State University. Forty adult males, half with the CHS genotype (aa) homozygous recessive and half phenotypically normal mink with either genotype (aA) or (AA) for the Aleutian gene were used for the experiments.

In the leukocyte studies, white blood cells were isolated from samples of peripheral blood. The mink were anesthetized by ether inhalation and, following appropriate cleansing and hair removal, whole blood was extracted via jugular venipuncture. A 12-ml syringe containing 1.0 ml of 0.18 *M* (K_3) EDTA was used to extract 9.0 ml of blood, achieving an approximate volume of 10.0 ml. Either three control mink or three affected mink were bled each time, using the same procedure for each.

White blood cell and platelet counts, as well as smears for differential counts were prepared from each sample. Unopette WBC/platelet dilution systems were used and the counts were completed manually. The smears were stained with Wright's stain and differentials were also done.

While centrifuging the whole blood, the tubes for the white blood cell wash were prepared by pipetting 4.0 ml of distilled water into one tube and 4.0 ml of 2× EPS (double isotonic strength, EDTA–phosphate-buffered saline solution) into another tube. This was duplicated for each sample and the tubes were placed in a 37°C water bath.

The blood samples were centrifuged at 3000g for 15 min and the plasma portion was discarded. The buffy coat was carefully removed and transferred to another labeled 15-ml centrifuge tube. In a two-step procedure, the red blood cells (RBC) in the buffy coat were lysed and the white blood cells were washed. The RBC lysis was accomplished by hypotonic shock by adding 4.0 ml of distilled water and vortexing immediately at a low setting. After approximately 15 sec, the 2× EPS was added and the sample was vortexed again, thus restoring the cell suspension medium to an isotonic state. The sample was then returned to the water bath until each buffy coat was similarly handled. These steps were repeated for each buffy coat. The samples were recentrifuged at 900g for 8 min and the supernatants were removed and discarded. The lysing steps were repeated, the samples recentrifuged at 900g for 8 min, and the supernatants removed and discarded. Approximately 1.0 ml of the EPS was then added to each pellet and the samples were vortexed. The three samples were then combined in a polycarbonate centrifuge tube and each tube was washed with approximately 1.0 ml of the EPS which was then also added to the combined sample. The sample was brought to a final volume of 10.0 ml using EPS and the cells and platelets were counted.

The counts were done manually in duplicate using Unopette WBC/platelet dilution chambers and were completed within 1 hr. Exactly 100 μl of the cell sample was added to an equal volume of 15% bovine serum albumin and direct smears were made for completing differentials. The differential counts ranged as follows: 70–90% neutrophils, 8–16% lymphocytes, 3–5% monocytes, and 0–6% eosinophils. The sample was then centrifuged at 900g for 8 min and the supernatants were removed and discarded. After 250 μl of ice-cold 7% perchloric acid was added to the pellet, it was then vortexed and immediately placed in an ice-water bath. The sample was centrifuged at 12,000g at 1.0°C for 20 min and the supernatant was collected. It was brought to a final volume of 0.5 ml and maintained in an ice-water bath until measured. In order to maintain the highest possible concentrations of nucleotides in the samples, the pH of the samples was not altered. This allowed for much smaller sample volumes, but since the samples could not be stored in the acid, they were measured the same day.

Tissue sampling in the granulocyte and the nongranulocyte studies were basically the same as in the total leukocyte studies with a few differences. A centrifuge

tube containing 3.0 ml of Ficoll was prepared for each sample and placed in a 37°C water bath. Following the second RBC lysis step, the samples were carefully layered on the Ficoll before centrifugation. These samples were centrifuged at 1800g for 10 min and the supernants were removed and discarded. The nongranulocytes from each sample were harvested from the upper aspect of the Ficoll and pooled in a polycarbonate centrifuge tube. The central portion of the Ficoll gradient was discarded and the granulocytes from each sample were harvested from the pellet and pooled in a polycarbonate centrifuge tube. Both the granulocytes and nongranulocytes were diluted to 10 ml with EPS. Cell counts and differentials were then completed. The nongranulocyte samples consisted of approximately 80–90% lymphocytes, 5–10% monocytes, and occasional granulocytes. The granulocyte samples consisted of approximately 90–95% granulocytes and 3–5% lymphocytes. The samples were centrifuged at 900g for 8 min and the supernatants were discarded. Approximately 250 μl of ice water-maintained 7% perchloric acid was added to each sample and the sample was placed in an ice-water bath. From this point on the procedure was the same as described in the total leukocyte studies.

In the leukocyte studies, the nucleotides were measured with the same HPLC procedure as described in the mouse renal studies, except that a 50-μl injection loop was used instead of the 10-μl loop employed in the mouse renal studies.

Formula for Calculating the AEC

The formula used for calculating the AEC is the concentration of ATP plus one-half the concentration of ADP divided by the sum of the concentrations of ATP, ADP, and AMP.

Statistical Analysis of Data

The data sets with even sample numbers were evaluated statistically with a two-tailed *t* test for paired samples with even sample numbers. The data sets with uneven sample numbers were evaluated statistically with a two-tailed *t* test for data with uneven sample numbers.

RESULTS

Mouse Kidney Studies

Various parameters related to adenine nucleotide biochemistry were examined in extracts of whole kidneys from both normal and CHS mice. These included total protein content, total ATPase activity, magnesium ATPase activity, sodium–potassium ATPase activity, the concentrations of ATP, ADP, and AMP, and the AEC values. There were no differences between the control and affected groups in any of these tests. The results of these studies are depicted in Table I.

Mink Leukocyte Studies

Phosphoadenylate concentrations and AEC values were assessed in preparations of total leukocytes, as well as granulocytes and nongranulocytes, from normal and CHS mink. The differences between control and affected groups were not statistically significant for each of the parameters measured. The results of these studies are depicted in Tables II, III, and IV.

TABLE I
Biochemical and Adenine Nucleotide Values of the Mouse Kidney Studies

Parameter	Normal	CHS
Total protein (mg protein/mg kidney)	0.263 ± 0.03[a] (n = 6)	0.254 ± 0.025[a] (n = 6)
Total ATPase (μM activity/mg protein/hr)	95.20 ± 6.90 (n = 6)	97.30 ± 19.77 (n = 6)
Magnesium ATPase (μM activity/mg protein/hr)	61.25 ± 7.52 (n = 6)	68.47 ± 18.50 (n = 6)
Sodium–Potassium ATPase (μM activity/mg protein/hr)	32.17 ± 10.66 (n = 6)	26.05 ± 7.57 (n = 6)
ATP (μM/g dry wt)	3.140 ± 2.414 (n = 8)	3.441 ± 1.856 (n = 7)
ADP (μM/g dry wt)	2.714 ± 0.907 (n = 8)	2.436 ± 0.720 (n = 7)
AMP (μM/g dry wt)	2.013 ± 1.464 (n = 8)	1.485 ± 0.926 (n = 7)
AEC (see formula in text)	0.579 ± 0.099 (n = 8)	0.625 ± 0.094 (n = 7)

[a] Values represent means and standard deviations.

DISCUSSION

The hypothesis for this study was developed based on the proven defect in the storage of ATP, ADP, serotonin, and other secretable compounds in CHS platelets. Furthermore, our initial studies showed that leukocytes from CHS mink and cattle had significantly lower concentrations of ATP and ADP than normal. These experiments were designed to test the hypothesis that the CHS defect was characterized by defective storage of nucleotides in other tissues as well.

To strengthen the initial findings, it was necessary to test the hypothesis in another affected species and in another affected tissue. Renal tissue of beige mice was chosen.

In the experiments on renal tissues from beige and control mice, no differences were noted in the concentrations of ATP or ADP. These results conflicted with

TABLE II
Adenine Nucleotide Concentrations and AEC Values of Total Leukocytes from CHS and Normal Mink

Parameter	Normal	CHS
ATP (nM/10^7 cells)	6.667 ± 2.002[a] (n = 11)	6.610 ± 1.554[a] (n = 10)
ADP (nM/10^7 cells)	2.05 ± 1.27 (n = 10)	1.40 ± 0.85 (n = 10)
AMP (nM/10^7 cells)	0.89 ± 0.85 (n =9)	0.78 ± 0.86 (n = 10)
cAMP (nM/10^7 cells)	0.83 ± 0.76 (n = 11)	0.37 ± 0.32 (n = 10)
AEC (see formula in text)	0.80 ± 0.09 (n = 8)	0.83 ± 0.07 (n = 10)

[a] Values represent means and standard deviations.

TABLE III
Adenine Nucleotide Concentrations and AEC Values of Granulocytes from CHS and Normal Mink

Parameter	Normal	CHS
ATP (nM/10^7 cells)	7.25 ± 1.81[a] (n = 6)	7.30 ± 1.07[a] (n = 6)
ADP (nM/10^7 cells)	2.95 ± 2.35 (n = 6)	2.09 ± 2.09 (n = 6)
AMP (nM/10^7 cells)	0.94 ± 0.18 (n = 5)	0.55 ± 0.45 (n = 6)
cAMP (nM/10^7 cells)	1.07 ± 0.38 (n = 6)	0.52 ± 0.45 (n = 6)
AEC (see formula in text)	0.78 ± 0.07 (n = 5)	0.83 ± 0.07 (n = 5)

[a] Values represent means and standard deviations.

those of the initial leukocyte studies. There were also no differences in the AMP or cAMP concentrations.

In the present studies, the AEC was calculated using the formula described under Materials and Methods. The AEC is a measurement of the extent that the ATP–ADP–AMP system is filled with high-energy phosphate bonds and is probably the most powerful indicator of the phosphoadenylate energy status of a cell. Again there was no difference between the control and the affected values.

In measuring the nucleotides, considerable variation was experienced. Several factors were considered as the source of this variation. First, the variation may have been due to loss of nucleotides during the dissection procedure. Considering the extremely short half-life of nucleotides in tissues, this possibility was very likely. Theoretically, variations of as little as 1 or 2 sec in the period between removal and freezing of the kidney could create marked differences in the results.

A second possibility was that nucleotides were somehow being lost during extraction. This possibility was tested by spiking several of the samples at the beginning of the extraction procedure and by processing standards through the extraction procedure. In fact, nearly 98% of the nucleotides were consistently

TABLE IV
Adenine Nucleotide Concentrations and AEC Values of Nongranulocytes from CHS and Normal Mink

Parameter	Normal	CHS
ATP (nM/10^7 cells)	7.47 ± 1.67[a] (n = 6)	6.41 ± 1.34[a] (n =6)
ADP (nM/10^7 cells)	1.17 ± 0.51 (n =6)	0.89 ± 0.46 (n =6)
AMP (nM/10^7 cells)	0.13 ± 0.18 (n =4)	0.94 ± 1.13 (n =6)
cAMP (nM/10^7 cells)	0.23 ± 0.25 (n =6)	0.12 ± 0.09 (n =6)
AEC (see formula in text)	0.91 ± 0.05 (n = 4)	0.84 ± 0.12 (n =6)

[a] Values represent means and standard deviations.

recovered. This essentially eliminated the possibility that the extraction procedure was a major contributing cause of the variation.

A third possibility was that there was variation in that part of the procedure used for correcting the measured values. In the mouse renal studies, the values were corrected based on the dry weight of the pellet from the extracted tissue. The pellets were very small and weighing them accurately proved to be very difficult. Interestingly, much of the variation was not evident in the AECs. Because calculating the AEC utilizes the correcting value in both the numerator and the denominator, this variable is mathematically eliminated. This would tend to suggest that much of the variation was in fact due to inaccuracies in weighing the dry pellets.

Initially the kidneys were dissected following cervical dislocation. In those samples, the ATP concentrations were consistently low and the AMP concentrations were consistently high. Because of this, the AECs for those samples were also very low. We started removing the kidneys surgically, using barbiturate anesthesia, and there was a marked improvement in the nucleotide retentions as indicated by higher ATP concentrations, lower AMP concentrations, and higher AECs. Unfortunately, this did not generate a measurable improvement in the consistency of the results. (A report on the comparison of these two techniques is being prepared for publication.)

The renal phosphoadenylate concentrations described in these studies compare favorably with those in other studies in which adenine nucleotide concentrations have been reported (Naucler *et al.*, 1984).

Renal ATPase activities were also evaluated in these experiments. The total ATPase activity as well as the Na^+–K^+–ATPase and the Mg^{2+}–ATPase activities were examined in kidney homogenates from control and beige mice. There were no differences in the ATPase activities between the control and affected mice.

The levels of ATPase activity reported in these studies are similar to those in other reports in the literature in which renal ATPase activities were measured (Buriich, 1975; Desaiah and Ho, 1976).

Because of the observed similarity between the control and the affected nucleotide concentrations in the mouse renal studies, the leukocyte findings were reexamined. Peripheral blood leukocytes were harvested from the buffy coats of the whole blood samples as described under Materials and Methods. Adenine nucleotides were extracted and measured and the AEC was calculated. There were no differences in the concentrations of ATP, ADP, AMP, or cAMP nor in the AEC values between affected and control samples. The leukocytes were also separated into granulocytic and nongranulocytic fractions and the phosphoadenylates were measured in both fractions. Again there were no differences in the concentrations of ATP, ADP, AMP, or cAMP or the AEC values.

No previous reports of the phosphoadenylate concentrations in mink leukocytes were available in the literature. The concentrations of adenine nucleotides described in these studies compare favorably to the adenine nucleotide concentrations in other leukocyte studies in other species (Mansell *et al.*, 1982; Verity *et al.*, 1983; Fulop *et al.*, 1984; Lum *et al.*, 1984).

The results of these studies conflict with those of our initial studies. Several possible explanations exist for these conflicting results. In the initial studies, the firefly luciferase technique was used to measure the nucleotides. This technique for measuring ATP utilizes photometric evaluation of an enzymatic response to the nucleotide. Since only ATP reacts with the luciferase, ADP must be chemi-

cally converted to ATP to be measured, a process which introduces considerable variation. It is possible that the conflicting results are due to different measuring techniques.

Another possible explanation for the difference is that there was platelet contamination in the samples in the initial experiments. In the current study, additional washes were performed to assure near 100% removal of platelets from the samples. Direct smears and platelet counts were done to document their virtual absence from the samples.

Root *et al.* (1972) reported that resting granulocytes and monocytes from CHS individuals had high metabolic rates as evident in significant elevations in O_2 consumption and in the rate of oxidation of [1-^{14}C]glucose. They felt that their reported differences could be explained by elevations in the rate of peroxide production. It is still tempting to suggest that this increase in the metabolic rate in resting CHS cells might be compensatory for some abnormally wasteful process, such as excessive futile cycles or excessive catabolism of the triphosphorylated adenylates. A reasonable explanation for such phenomenon is that some normal mechanism for protecting these nucleotides is lacking in CHS, thus allowing wasteful nucleotide metabolism. With the understanding that intracellular membranes in CHS cells have a tendency to be leaky, it is reasonable to hypothesize that if an undefined, membrane-bound storage pool for tri- and diphosphorylated nucleotides exists in cells, the result would be increased nucleotide exposure and an increased rate of catabolism. This possible mechanism for the CHS defect cannot be disproven with the results of this study. Even though the total intracellular phosphoadenylate concentrations were not significantly different in the CHS and control samples, the possibility exists that there is a shift in the nucleotides from a storage pool to the metabolic pool in CHS.

A search of the literature has uncovered no other reports of ATP, ADP, or AMP concentrations or of the AEC of any CHS tissue or organ other than platelets.

J. M. Oliver *et al.* (1975) found that neutrophils from beige mice had a Con A-labeling pattern that resembled the Con A-labeling pattern of colchicine-treated normal neutrophils. They further characterized their finding by demonstrating that the abnormal Con A-labeling pattern in the CHS neutrophils was corrected by *in vitro* treatment of the cells with agents which elevate intracellular cyclic guanosine monophosphate (cGMP) concentrations. They concluded that the morphologic and functional problems of CHS were due to malfunctioning microtubules, possibly due to a diminished capacity to generate cGMP. Later, Boxer *et al.* (1976) measured cGMP and cAMP in CHS human neutrophils and found that cAMP concentrations were elevated and cGMP concentrations were decreased. They then extended the hypothesis to include the elevated intracellular cAMP as a possible cause of the proposed microtubular defect. This theory has been much debated in the literature. Several reports have described normal microtubule morphology (Frankel *et al.*, 1978; White, 1978; White *et al.*, 1979; Ostlund *et al.*, 1980), normal cyclic nucleotide concentrations (Yegin *et al.*, 1983; Apitz-Castro *et al.*, 1985), normal Con A capping (Newburger *et al.*, 1985), and failure of ascorbic acid treatment to correct the morphologic and functional problems of CHS (Yegin *et al.*, 1983). Still another study reported finding normal microtubule numbers and length in neutrophils but abnormal microtubule assembly that was variable among CHS patients (Pryzwansky *et al.*, 1985). The authors suggested that a problem involving a failure to respond to a surface signal existed in CHS cells. In our

study, cAMP concentrations in leukocytes from CHS mink were slightly lower than normal but were not significantly different. These results tend to strengthen the argument that no cAMP-induced defect exists in CHS.

CHS mice and mink were also evaluated for a possible defect involving storage or metabolism of adenine nucleotides. In the mice, studies of renal ATPase activities and renal phosphoadenylate concentrations were completed, and in the mink, studies of leukocyte phosphoadenylate concentrations were performed. No differences were found in either the mouse renal ATPase activities or the mouse renal phosphoadenylate concentrations. This was also true for the mink leukocyte phosphoadenylate concentrations.

It has been reported that renal tubular epithelial cells in CHS individuals have a slower clearance rate of endocytized proteins (Essner *et al.*, 1974). The protein content was measured in kidneys from beige mice and normal mice to assure that the correcting values from the enzyme studies, to that factor, would not result in the introduction of an unwanted variable. Even though the clearance of endocytized protein from renal tubular epithelial cells is much slower in CHS kidneys, there was not a significant difference in the total protein content of the beige mouse kidneys.

CONCLUSIONS

The results of these studies lead to one of several possible conclusions. One possible conclusion is that the nucleotides measured in both the control and the affected animals represent the metabolic pools and that no storage pool of adenine nucleotides exists in kidney tissue or leukocytes as exists in platelets. This would tend to coincide with the results of platelet studies that indicate that the phosphoadenylate concentrations in circulating CHS platelets are very similar to the phosphoadenylate concentrations in the metabolic pools of normal platelets. The results of these studies suggest that the nucleotides found in CHS platelets coincide with those of the normal metabolic pool. Assuming that the defect in adenine nucleotide biochemistry in CHS only involves the storage pool, the affected cells or tissues that have no storage pool of adenine nucleotides should have normal nucleotide concentrations.

A second possible conclusion is that, if a currently undefined storage pool for adenine nucleotides exists in whole cells similarly to platelets, it is not affected by the CHS defect as is the storage pool in platelets. It is also possible that the CHS defect causes a shift in the intracellular phosphoadenylates from the storage pool to the metabolic pool resulting in a difference in the nucleotide biochemistry that is not apparent in measurements of total adenine nucleotide concentrations.

In respect to the kidney, it is possible that a subpopulation of cells that store nucleotides or one that is exclusively affected by the CHS defect might exist and that by measuring whole kidney preparations, the proposed nucleotide deficiencies were masked.

It is also possible that adenine nucleotides are stored in whole cells but in such minute quantities that a defect involving this storage pool may not have been measurable by current techniques.

ACKNOWLEDGMENTS

This work was largely funded by an NIH grant (NIH P40 RR01173) entitled "Animal Model Center for Significant Human Disorders" and in part by a BRSG Research Grant entitled "An Adenine

Nucleotide Defect in the Chediak–Higashi Syndrome," BRSG Committee, Michigan State University. We also acknowledge Dr. Jim Oris for having unfolded the chromatographic technique used in measuring the nucleotides.

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