and size. It indicates that there is both a genetic and functional relation between light and heavy chains, which is probably related to their role in antibody function. Furthermore, despite the variation in sequence to which the NH_2-terminal portions of light and heavy chains are subject, portions of the tertiary structure may be rather stable since certain sequence regions as well as the disulfide bridges are similar from species to species and within antigenic types and classes.

Most theories of hypermutation are not compatible with these findings. Indeed, these structural relationships are difficult to explain by any mechanism other than an accumulation of mutations through many separate genes for κ- and λ-light chains, and probably also for γ-, α-, and μ-heavy chains. As in the myoglobin-hemoglobin example, this structural homology can be accounted for by the hypothesis of common ancestry of light and heavy chains from a single primitive gene that gave rise to light and heavy genes through a process of duplication and independent mutation as proposed by Hill *et al.* (*17*). Our results suggest that κ- and λ-chain specialization preceded interspecies differentiation and probably occurred on an evolutionary time scale at about the same period as the separation of the genes for α- and β-hemoglobin chains.

Note added in proof. Hood *et al.* (*20*) have demonstrated variation in the sequence of the NH_2-terminal octadecapeptide of human λ-chains analogous to that known for κ-chains. On this basis they, too, have suggested that the genes coding for κ-chains and λ-chains have evolved from a common ancestral gene.

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15. Abbreviations for amino acid residues: Lys, lysine; His, histidine, Arg, Arginine; Asp, aspartic acid; Asn, asparagine; Asx, aspartic acid or asparagine, identity not established; Thr, threonine; Ser, serine; Glu, glutamic acid; Gln, glutamine; Glx, glutamic acid or glutamine, identity not established; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ilu, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Trp, tryptophan; CyS, half-cystine.
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Leukocyte Oxidase: Defective Activity in Chronic Granulomatous Disease

Abstract. *The intact leukocytes of two children with chronic granulomatous disease fail to reduce nitroblue tetrazolium during phagocytosis. This is due to defective operation of an oxidase of reduced nicotinamide adenine dinucleotide that is insensitive to cyanide and that indirectly stimulates the oxidation of glucose-6-phosphate in leukocytes. Such leukocytes undergo no increase in oxygen consumption or in activity of the hexose monophosphate shunt during phagocytosis, although lactate production is normal. The addition of nitroblue tetrazolium to a leukocyte suspension appears to provide a sensitive diagnostic screening test for this disease.*

Chronic granulomatous disease observed in childhood is a fatal disorder characterized by increased susceptibility to infection, which begins during the first year of life (*1*). Although many types of organisms have been isolated from the affected patients during the later phases of their illness, the initial series of infections is usually caused by staphylococci. The patients frequently develop granulomas in nodes, lungs, liver, and spleen.

Studies by Holmes and her co-workers (*2*) suggest that the circulating granulocytes of these patients ingest but do not destroy bacteria. These workers have also stated that the respiration of such granulocytes does not increase during phagocytosis (*3*).

We have recently observed two unrelated male children with chronic granulomatous disease, one of whom has succumbed to this disorder. We studied the metabolic response of their granulocytes to the phagocytosis of polystyrene spheres in an attempt to define a defect of direct oxidation of glucose-6-phosphate in these leukocytes. Some of the metabolic responses of guinea pig granulocytes to the phagocytosis of inert particles include, in addition to increased lactate production, stimulation of the activity of a reduced nicotinamide adenine dinucleotide (NADH) oxidase, increased oxygen consumption, and marked increase in the activity of the hexose monophosphate shunt (*4*).

Leukocytes were isolated from the peripheral blood of these patients and from infected controls with similar distributions of granulocytes by means of fibrinogen sedimentation and differential centrifugation. The isolated cells were thrice washed in tubes coated with silicon with Krebs Hensleit buffer (*p*H 7.4) to which glucose (11 m*M*) was added. Erythrocyte contamination of the leukocyte suspension was less than 10 percent. Phagocytosis was initiated by the addition of 0.1 ml of a dilution (one part in ten) of dialyzed polystyrene spheres (*5*) to the leukocyte concentrate which numbered 2 to 4×10^7 leukocytes per milliliter. We assessed ingestion of spheres by observing cells with Nomarski optics. The percentage of leukocytes containing more than two spheres was enumerated. Activity of the hexose monophosphate shunt in leukocytes was determined in the resting state and following ½ hour of phagocytosis in stoppered 15-ml serum bottles. The $C^{14}O_2$ was trapped on filter paper, soaked with KOH, which was suspended from the serum cap, and the C^{14} activity of the dried paper was determined in a liquid scintillation counter. Lactate production was determined by measurement of the lactate concentration (*6*) of a 3 percent perchloric acid filtrate of the leukocyte suspension. The oxidase activity manifested during phagocytosis was de-

Table 1. Lactate production by resting leukocytes and by phagocytosing leukocytes after 30 minutes of phagocytosis at 37°C. Values expressed as micromoles of lactate produced per ½ hour per 1.0 × 10^8 cells.

Leukocytes from patient with	Resting	Phagocytosing
Asthma	3.15	4.48
Pneumonia	3.65	5.60
Peritonitis	4.27	5.29
Congestive heart failure	2.07	4.79
Pneumonia	1.73	2.90
Chronic granulomatous disease, Patient 1	2.80 3.71	4.03 4.57
Chronic granulomatous disease, Patient 2	1.67	3.15

tected after the addition of 0.2 percent oxidized nitroblue tetrazolium and 1m*M* KCN to the reaction mixture. Reduction of the dye produces a deep blue color.

The results observed in leukocytes derived from other infected and healthy individuals confirmed some of the observations of Karnovsky and his coworkers (*4*). We refer to these as "normal" leukocytes. During phagocytosis, normal leukocytes reduced nitroblue tetrazolium to a deep blue color within 5 minutes, whereas in the resting state they required up to 30 minutes to bring about an equal degree of dye reduction. There was increased lactate production during phagocytosis by normal cells (Table 1). In addition, there was a marked increase in the activity of the hexose monophosphate shunt, as manifested by the production of $C^{14}O_2$ from glucose-1-C^{14} (Table 2). There was only slight increase in production of $C^{14}O_2$ from glucose-6-C^{14}. The metabolic response of the intact leukocytes of the two patients with chronic granulomatous disease during phagocytosis was abnormal. As expected from the data of Holmes and co-workers (*2*), these cells ingested polystyrene spheres. On the other hand, they did not reduce nitroblue tetrazolium at a normal rate whether resting or phagocytosing. During phagocytosis they produced lactate at an increased rate (Table 1) but failed to generate an increase in the hexose monophosphate shunt (Table 2). When these leukocytes were exposed to methylene blue in the absence of polystyrene spheres, their hexose monophosphate shunt activity increased even more markedly than did that of normal cells (Table 3). In contrast to normal cells, oxygen consumption of the leukocytes, measured only in the second patient, did not increase during phagocytosis (*7*).

Table 2. Effect of phagocytosis on stimulation of the leukocyte hexose monophosphate shunt in resting (A) and phogocytosing (B) cells.

Leukocytes from patient with*	$C^{14}O_2$ (count/min per 10^8 leukocytes)		
	A	B	B/A
Pneumonia	377	2099	5.6
Pulmonary infarction	304	1270	4.2
Pneumonia	65	1056	16.3
Pneumonia	155	1640	10.6
Pyelonephritis	81	1226	15.1
Wilson's disease	192	7232	37.7
Chronic granulomatous disease, Patient 1	43	84	1.9
	155	160	1.0
	29	22	0.7
Chronic granulomatous disease, Patient 2	321	381	1.1
	409	125	0.3

* The leukocytes were incubated at 37°C for 30 minutes in Krebs Hensleit buffer at *p*H 7.4 with 11 m*M* glucose and glucose-1-C^{14} (2.5 × 10^5 count/min).

Table 3. Effect of methylene blue on stimulation of the hexose monophosphate shunt. Without methylene blue, A; with methylene blue, B.

Leukocytes from patient with*	$C^{14}O_2$ (count/min per 10^8 leukocytes)		
	A	B	B/A
Pneumonia	297	2427	8.2
Asthma	459	2511	5.5
Chronic granulomatous disease, Patient 1	85	3245	38.1
Chronic granulomatous disease, Patient 2	148	3462	23.3

* The leukocytes were incubated at 37°C for 30 minutes in Krebs Hensleit buffer at *p*H 7.4 with 11 m*M* glucose, 2.0 m*M* methylene blue, and glucose-1C^{14} (2.5 × 10^5 count/min).

Lysis by freezing and thawing of the granulocytes of both patients following the addition of either NADH or NADPH caused greater reduction of nitroblue tetrazolium than occurred when the cells were intact. This reaction was not inhibited by cyanide and was potentiated to a greater extent by NADH than by NADPH.

Our studies indicate that the intact phagocytosing leukocytes of the patients with chronic granulomatous disease fail to evince a cyanide-insensitive NADH oxidase which is apparently not essential to the ingestion process. The results of studies with methylene blue suggest that the failure of these cells to exhibit stimulation of the hexose monophosphate shunt during phagocytosis is not due to any loss of one or more of the enzyme activities usually associated with the operation of the hexose monophosphate shunt. Rather, it may be due to the lack of the NADH oxidase activity which stimulates the shunt in normal phagocytosing cells. Exactly how the lack of oxidase activity in intact leukocytes is related to the inability of these cells to destroy ingested bacteria is not established at this time, nor is the cellular site of the enzyme released during lysis by freezing and thawing. Of clinical significance is the fact that the addition of nitroblue tetrazolium to a suspension of leukocytes in a glucose-rich buffer containing cyanide appears to provide a sensitive, diagnostic, screening test for this condition.

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Relative Brain Size: A New Measure

Abstract. *The relation of the volume of the endocranial cavity to the area of the foramen magnum is a measure of relative brain size in mammals. The outstanding advantage of this method is that only a skull is required for a set of measurements.*

The method most commonly used to investigate relative brain size in mammals involves a comparison of brain weight and body weight (*1*). However, that method is limited because body weight varies greatly within a species. Furthermore, it is difficult to obtain statistically significant data for common species and any data at all for rare mammals. The ratio of brain weight to spinal cord weight has been proposed as an index of relative brain size (*2*), but that approach, while alleviating the first problem, makes it