using the *opaque-2* values as numerators and the normal values as denominators. The ratios follow. Lysine: acid-soluble, 3.2; zein, 3.0; glutelin, 1.0. Histidine: acid-soluble, 3.4; zein, 1.2; glutelin, 0.9. Arginine: acid-soluble, 2.3; zein, 1.2; glutelin, 1.1. Amide ammonia: acid-soluble, 0.6; zein, 1.8; glutelin, 0.9.

These data suggest that the acid-soluble and zein fractions are quite unlike those of the normal endosperms, whereas the glutelin fractions of the two endosperms are similar with respect to the basic amino acids and amide ammonia. On the basis of these preliminary findings, the increased content of lysine in the *opaque-2* endosperm can be attributed to three factors: (i) increased lysine in the acid-soluble fraction, (ii) increased lysine in the zein fraction, and (iii) reduction in the ratio of zein to glutelin.

The zein fraction of *opaque-2* endosperm contained 0.9 g of lysine per 100 g of protein ($N \times 6.25$), which is more than ten times the amount (0.08 g) found in a composite of zein fractions from U.S. and Guatemalan endosperms (*4*), and three times the amount found in the normal endosperms from the same ear. This finding, together with the 1.8-fold increase in amide ammonia (probably from glutamine) supports the conclusion that *opaque-2* endosperm contains a type of zein that is chemically, and perhaps physically, unlike any described heretofore. Complete amino acid analysis of the copper fractions will be published later.

This is the first demonstration of a radical change in the protein composition of maize endosperm, and is caused by a single mutant gene. In addition to its possible usefulness in studies on protein synthesis, the *opaque-2* gene should permit the development of commercial strains of corn with much higher lysine contents. The value of such corn in human and animal nutrition has been emphasized in previous publications (*4*, *7*).

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Nicotinamide Adenine Dinucleotidase Activity in Cells of Tuberculous Animals

Abstract. *There is an increase in the nicotinamide adenine dinucleotidase activity of polymorphonuclear leukocytes, monocytes, and lymphoid cells from tuberculous animals, compared with that of cells from normal animals. There seems to be a correlation between the ability of the animal to develop a progressive disease and its ability to react with an increase in the activity of the enzyme.*

There is an increased activity of nicotinamide adenine dinucleotidase in organs of tuberculous mice with a concomitant reduction in nicotinamide adenine dinucleotide concentration (*1*). These biochemical changes could be elicited by administration of a lipid fraction derived from the tubercle bacilli (cord factor), and the toxicity of this factor could be alleviated by administration of nicotinamide (*2*). Furthermore during the tuberculous infection in mice, soluble dinucleotidase can be detected in their livers and lungs. On the basis of this observation, the enzyme in its soluble form was assumed to come into contact with nicotinamide adenine dinucleotide of the cytoplasm and mitochondria and cause its splitting (*3*).

In the guinea pig, an animal extremely sensitive to infection with tubercle bacilli, the activity of the enzyme is dramatically increased during the infection, whereas in the tuberculous mouse, an animal relatively resistant to infection with tubercle bacilli, such is not the case (*4*). The activity of the enzyme of liver microsomes of infected guinea pigs increases 36-fold while that of liver microsomes of the infected mice is elevated twofold, compared with liver microsomes from normal animals (*3*, *4*).

This report deals with the nicotinamide adenine dinucleotidase activity of polymorphonuclear leukocytes, monocytes, and lymphoid cells from tuberculous and normal guinea pigs, strain C57 black mice, and albino mice.

Albino mice and the C57 strain of black mice, weighing about 20 g, and guinea pigs with an average weight of 400 g, were used as the source of peritoneal polymorphonuclear leukocytes and monocytes, as well as of lymphoid cells from mesenteric and tracheobronchial lymph nodes.

Guinea pigs were infected by intramuscular injection, and the mice by intravenous injection of tubercle bacilli, strain $H_{37}Rv$, grown on Lowenstein-Jensen medium. The amount injected was about 0.1 mg (dry weight).

The establishment of the tuberculous infection was determined by the presence of characteristic lesions in the organs of the animal.

Cells were obtained from mice about 2 weeks after infection and from guinea pigs about 3 weeks after infection.

Polymorphonuclear leukocytes from normal mice were obtained as follows: 3 ml of 0.1 percent glycogen in saline was injected intraperitoneally. Four hours later the mice were killed by decapitation. Cells were collected by washing the peritoneal cavity with Krebs-Ringer phosphate solution supplemented with heparin (2 units/ml). Usually cells from three mice were pooled in siliconized tubes and differential counts showed that 75 percent were polymorphonuclear and 25 percent were mononuclear cells. Polymorphonuclear cells from infected mice were obtained by the same procedure but collected about 16 hours after injection of the irritant.

To obtain polymorphonuclear cells from normal and tuberculous guinea pigs, about 15 ml of 0.1 percent glycogen in saline was injected intraperitoneally. Four hours later 30 ml of heparinized Krebs-Ringer phosphate solution was introduced into the peritoneal cavity, and the exudate was col-

lected by gravity drainage into siliconized centrifuge tubes. Differential counts usually showed that about 90 percent of the collected cells were polymorphonuclear leukocytes.

Monocytes were also obtained by injections of 0.1 percent glycogen except that in the mice the cells were collected 24 hours later and in guinea pigs four days later. The differential counts showed, respectively, about 65 and 85 percent of monocytes.

Lymphoid cells were collected from the mesenteric and tracheobronchial lymph nodes by first placing the nodes in Krebs-Ringer phosphate solution and then teasing out the cells with a needle into the suspending medium. A homogeneous suspension of lymphoid cells was obtained by passing the suspension through two layers of gauze.

Cells after collection were counted in a hemocytometer; the suspension was centrifuged in a refrigerated centrifuge at low speed, the supernatant was decanted, and the cells were resuspended in the desired volume of phosphate buffer (0.1*M*, *p*H 7.1) and homogenized with a teflon grinder in a glass tube.

The enzyme was measured by the method based on the cyanide reaction of nicotinamide adenine dinucleotide (*5*). The reaction mixture contained 0.5 parts of cell homogenate, 0.3 parts of distilled water, 0.2 parts of 0.003*M* of the dinucleotide (*6*). The mixtures were incubated in a water bath at 37°C. To 1-ml samples withdrawn after various time intervals 3-ml of 1.0*M* KCN solution was added and the absorption at 325 mμ was determined (*6*). Reaction mixtures from which the dinucleotide was withheld were sampled into KCN and served as blanks. The amount of enzyme which hydrolyzes 0.1 μmole of nicotinamide adenine dinucleotide in 5 minutes was defined as one unit. Specific activity of the enzyme preparation was expressed in units per 10^6 cells.

There is an increase in the activity of the enzyme in all cells from tuberculous animals compared with those from normal controls (Table 1). From an initial uniform value in albino mice, 0.05 to 0.07 units per 10^6 cells, there is an average increase in the enzyme activity of about 90 percent above that of normal cells. In cells from black mice, this increase is much more pronounced, especially in the polymorphonuclear leukocytes and tracheobronchial lymphoid cells, being 200 and 350 percent, respectively, above that of the normal cells.

Table 1. Nicotinamide adenine dinucleotidase activity of cells from normal and tuburculous animals. The results are given as the means. The figures in parentheses indicate the number of animals.

Albino mice			Black mice C57			Guinea pigs		
Normal (units/ 10^6 cells)	Tuberculous (units/ 10^6 cells)	Increase (% above normal)	Normal (units/ 10^6 cells)	Tuberculous (units/ 10^6 cells)	Increase (% above normal)	Normal (units/ 10^6 cells)	Tuberculous (units/ 10^6 cells)	Increase (% above normal)
Monocytes								
0.06±0.02 (9)	0.13±0.1 (13)	116	0.07±0.02 (4)	0.13±0.04 (4)	86	0.2 ±0.08 (3)	0.5 ±0.01 (3)	150
Polymorphonuclear leukocytes								
0.05±0.003 (5)	0.08±0.016 (6)	60	0.05±0.03 (4)	0.15±0.03 (4)	200	0.02±0.001 (3)	0.08±0.03 (3)	300
Mesenteric lymphoid cells								
0.07±0.01 (7)	0.13±0.03 (10)	86	0.16±0.01 (5)	0.22±0.04 (4)	37	0.21±0.11 (5)	1.20±0.59 (5)	470
Tracheobronchial lymphoid cells								
0.06±0.01 (4)	0.12±0.02 (4)	94	0.06±0.026 (4)	0.27±0.08 (3)	350	0.12±0.017 (5)	1.30±0.7 (5)	980

The most pronounced elevation can be seen, however, in cells from tuberculous guinea pigs. This increase is nearly tenfold in tracheobronchial lymphoid cells, about fivefold in mesenteric lymphoid cells, and threefold and 1.5-fold, respectively, in polymorphonuclears and monocytes. The differences in nicotinamide adenine dinucleotidase activity of mesenteric and tracheobronchial lymphoid cells of black mice and guinea pigs seem to be of interest. It is probable that the tracheobronchial are more exposed than the mesenteric cells to the action of cord factor, which is shed by the tubercle bacilli in the lung or more exposed to soluble enzyme released by the tuberculous lung tissue and filtered out by the cells through an absorption phenomenon.

Albino mice are considered to be more resistant to infection with tubercle bacilli than C57 black mice. A large number of tubercle bacilli injected intravenously is required to cause a fatal infection in the mice. The guinea pig, on the other hand, is one of the most susceptible animals to a tuberculous infection. A few tubercle bacilli suffice to cause an active disease which culminates in death of the animal.

The elevation in activity of nicotinamide adenine dinucleotidase seems to be an important factor in the pathogenesis of tuberculous infection (*2, 3*).

In view of the findings of this report there appears to be a definite correlation between susceptibility to tuberculous infection and ability to react by an increase in activity of the enzyme.

It is premature to assess the meaning and importance of the increased enzymatic activity of the cells since several questions remain to be answered. Is the increase due to an activation of the enzyme already present in the cells, or are the cells induced to increased production of the enzyme? As a third alternative, the increase in enzymatic activity of the cells may be due to absorption of soluble nicotinamide adenine dinucleotidase. If the last assumption is true it will be reasonable to regard the described fact as an expression of defense carried out by cells whose most important function is defense of the host.

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6. The nicotinamide adenine dinucleotide was purchased from Sigma.
7. Absorption was determined in a Beckman DU spectrophotometer.
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