Tertiary time much of the western part of the continent was organized into large units that permitted formation of extensive bodies of lake water. The latter part of the Tertiary saw development of a more rugged and diversified topography analogous to that now in existence. Comparison of Fig. 1 with the map showing Pleistocene lakes (*1*) indicates that many of the basins occupied by lakes in Pliocene time were also the sites of Pleistocene lakes. Thus, in many places throughout the West, the landscapes we know today have been in existence for more than a million years in something approaching their present form.

The distribution of lake deposits shown in Fig. 1 is taken from reports in the literature. The sources of information are given on page 109 and keyed by numbers to the map.

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Metabolic Deficiencies in Protozoa Induced by Thalidomide

Abstract. *Thalidomide inhibits growth of some protozoa. This inhibition was counteracted by nicotinic acid, nicotinamide, nicotinamide adenine dinucleotide, and vitamin K₁. The mechanism of toxicity may be an interference of cellular oxidation. A protozoan test system is useful for studying the potential "side actions" of drugs in higher animals and man.*

How thalidomide induces congenital malformations is unknown. Since similar teratogenic effects have been produced in animals by metabolic interferences (*1, 2*), it seemed appropriate to find out whether thalidomide acts as an antimetabolite. The development of peripheral neuritis (*3*) and congenital malformations (*4, 5*) that followed its use has focused attention on the need for additional methods to screen drugs for potential toxicity. For this purpose, a protozoan test system which disclosed the metabolic deficiencies induced by primidone (*6*), thyroactive compounds (*7*), and triparanol (*8*) was used.

Thalidomide, in appropriate concentrations, inhibited growth of the photosynthetic flagellates *Euglena gracilis, Ochromonas malhamensis,* and *Ochromonas danica,* and the ciliate *Tetrahymena pyriformis* and was therefore judged toxic for these organisms. Methods for growing these protozoa have been described (*9*). An aqueous solution of thalidomide [α-*N*(phthalimido)glutarimide] was prepared by dissolving the drug with dilute KOH and then immediately neutralizing with dilute HCl so that the concentration was 100 mg/ml. Further dilutions were made with distilled water. All organisms were grown in chemically defined media. Thalidomide was added to the media in concentrations which inhibited protozoan growth (Table 1). Media were sterilized by autoclaving at 118° to 121°C, 16 pounds per square inch, for 30 minutes. Metabolites that were not heat resistant, such as tryptophan intermediates, nicotinamide adenine dinucleotide (NAD), and vitamin K₁ (menadione) were sterilized by passage through ultra-fine fritted glass filters and added aseptically in appropriate dilution with distilled water. The toxic effects of thalidomide were not altered by autoclaving. Growth of *Lactobacillus leichmannii, L. casei, L. arabinosus, Pediococcus cerevisiae, Escherichia coli* I, and *E. coli* 113-3, was not inhibited by thalidomide; therefore, only results obtained with protozoa are given here.

Growth was inhibited by thalidomide at concentrations of 2.0 mg/ml for *Ochromonas danica,* 3.0 mg/ml for *Euglena gracilis* and *Tetrahymena pyriformis,* and 6.0 mg/ml for *Ochromonas malhamensis* (Table 1.). This toxicity was not altered by purines or pyrimidines (adenine, adenosine, guanine, guanosine, uracil, uridine, cytosine, cytidine, thymine, thymidine, xanthine, xanthosine, inosine), or amino acids (alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine), or a metals mixture (Fe, Zn, Mn, Cu, Mo, Co, V, I, Se), alone or in combinations.

A vitamin mixture containing thiamine, nicotinic acid, pantothenate, pyridoxamine, riboflavin, choline, inositol, thymine, orotic acid, *p*-aminobenzoic acid, vitamin B_{12}, biotin, thioctic acid, and folic acid reversed the toxicity of thalidomide for all organisms. Nicotinic acid proved to be the active agent.

Table 1. Inhibition of the toxicity of thalidomide toward protozoa by nicotinic acid, NAD, and menadione. Growth of the organisms was measured with a Welch Densichron photometer equipped with a red-sensitive probe and is expressed in optical density units (O.D.); an O.D. of 1.0 corresponds to 0.5 to 0.55 g (dry weight) of organisms per liter.

Thalidomide (mg/ml)	Inhibitory agent (mg/ml)				
	None	Nicotinic acid		NAD	Menadione
		0.001	0.01	0.1	0.0001
	Euglena gracilis				
0	2.7	2.7	2.7	2.6	2.6
1	0.7	1.4	2.5	1.7	1.8
2	0.3	1.3	2.4	1.2	1.2
3	0	0.9	1.8	1.0	0.9
	Ochromonas danica				
0	1.9	1.9	1.9	1.9	1.9
1	0.9	1.6	1.7	1.6	0
2	0	1.3	1.3	1.4	0
3	0	1.1	1.3	0.9	0
	Ochromonas malhamensis				
0	2.2	2.2	2.2	2.2	2.2
2	1.8	1.8	1.8	1.8	1.8
4	1.0	1.3	1.5	1.6	0.8
6	0	0.6	0.8	0.7	0
	Tetrahymena pyriformis				
0	0.5	1.2	1.2	1.2	0.5
3	0	0.9	1.0	0.9	0.2
6	0	0.8	0.9	0.8	0.2
9	0	0.6	0.7	0.7	0.1

None of the other vitamins diminished the toxicity, nor was any synergistic with nicotinic acid. Nicotinamide or NAD could be substituted for nicotinic acid with similar effect.

Because tryptophan is a precursor of nicotinic acid, metabolites along the pathway of synthesis to nicotinic acid were tested. 5-Hydroxytryptamine, kynurenic acid, xanthurenic acid and quinolinic acid partly inhibited the toxicity of thalidomide for *O. malhamensis*; quinolinic acid slightly inhibited the toxicity of thalidomide for *O. danica,* and none of these compounds inhibited the toxicity of thalidomide for *E. gracilis* or *T. pyriformis.*

Since NAD inhibited the toxicity of thalidomide, vitamin K₁ (menadione) was also tested for this behavior because it, too, participates in metabolic oxidations (*10*). Menadione inhibited the toxicity of thalidomide for *E. gracilis* and *T. pyriformis* but not for *O. malhamensis* and *O. danica.*

The nicotinic acid antagonist, 6-aminonicotinamide, behaved like thalidomide toward *E. gracilis* and *O. malhamensis* and, as expected, its action was overcome by nicotinic acid. *T. pyriformis* and *O. danica* were not inhibited by 6-aminonicotinamide at concentrations up to 1.0 mg/ml.

Thalidomide may possibly act at the point where nicotinic acid is synthesized

to NAD, or it may interfere with the utilization of NAD and vitamin K in cellular oxidations (*10*). If results with these microbes should apply to man, thalidomide might interfere with cellular oxidations during morphogenesis and cause abnormalities (*4, 5*). It is of interest to note that thalidomide toxicity was not reversed by riboflavin nor folic acid in the protozoan test system, although deficiencies of these two vitamins have been implicated in causing fetal malformations (*5*).

Robertson has described the development of polyneuritis and glossitis upon prolonged therapy with large doses of thalidomide in humans (*3*). These effects were controlled by the prophylactic use of vitamin B-complex. Interestingly, chick embryos injected with nicotinic acid antagonists had a high incidence of rumpleness, ectrodactylism, and ectromelia. Nicotinamide protected the embryo against these defects (*1*), and are in agreement with our results.

These investigations demonstrate the usefulness of protozoa for rapid, sensitive, and inexpensive evaluation of potential drug toxicity. Coupled with animal and clinical studies, a protozoan test system may prove useful for screening drugs (*11, 12*).

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Ytterbium: Transition at High Pressure from Face-Centered Cubic to Body-Centered Cubic Structure

Abstract. *Pressure of 40,000 atmospheres at 25°C induces a phase transformation in ytterbium metal; the face-centered cubic structure changes to body-centered cubic. The radius of the atom changes from 1.82 to 1.75 Å. At the same time the atom's volume decreases by 11 percent and the volume, observed macroscopically, decreases 3.2 percent.*

We present here data on a pressure-induced phase transformation from a close-packed structure to a nonclose-packed arrangement of atoms. When identical spherical atoms are stacked in honeycomb-like layers in which every atom touches six neighbors in its own layer and, in addition, touches three atoms in the layer above and also three atoms in the layer below, the total space (including voids between the spheres) occupied by a large number of atoms will be at a minimum. Such arrangements in which each atom touches 12 adjacent atoms are called closest-packed structures. Identical spherical atoms arranged in any other fashion will occupy a larger total volume.

At room temperature and pressure atoms of the metal ytterbium (Yb) are arranged in a closest-packed structure known as face-centered cubic (FCC). The FCC structure is shown in Fig. 1 by the conventional "unit cell" where only the centers of the atoms (dots) are shown. A basic dimension of the FCC unit cell is the length of the cube edge which, for Yb, is 5.481 Å at 25°C, and 1 atm. This corresponds to a radius of 1.940 Å for the metallic Yb atom. We have discovered a phase transformation occurring in Yb at 25°C, 40,000 atm, wherein the FCC phase transforms into a body-centered cubic (BCC) phase (*1*). The BCC unit cell is also shown in Fig. 1. In this structure each atom touches eight surrounding atoms.

The nature of the transition was elucidated with the aid of a high-pressure x-ray diffraction apparatus which consists of a tetrahedral anvil press (*2*) to which x-ray goniometers have been attached. Primary x-rays are directed into the specimen (contained in a lithium hydride-amorphous boron tetrahedron) through a tiny axial hole in one of the triangular anvil faces. Diffracted x-rays ("powder" pattern) exit through gaskets formed between the sloping anvil shoulders and then, after passing through collimating slits, pass into the counter tubes.

The fraction of total space occupied by voids (spaces between the spheres) in FCC-closest packing is 26 percent, whereas the fraction of space taken up by voids in the non-closest-packed BCC structure is 32 percent. Offhand, therefore, it would appear impossible for pressure to affect a transformation from the FCC to the BCC structure. Unit-cell data obtained from the tetrahedral x-ray press, however, make it possible to explain what has taken place. Calculation of the radii of the atoms from these data gives $r = 1.82$ Å for the FCC modification at 40,000 atmospheres and $r = 1.75$ Å for the BCC form at the same pressure. Calculation of volume changes from these values for the radii shows that an individual, spherical Yb atom shrinks in volume by 11 percent during the transition from FCC to BCC. This is even more interesting in view of the fact that the overall, macroscopic volume change at the transition is only 3.2 percent. Of course, the change to BCC structure is responsible for the greater decrease in the volume of the individual atoms.

A look at a chart of the values for the metallic radii plotted against the

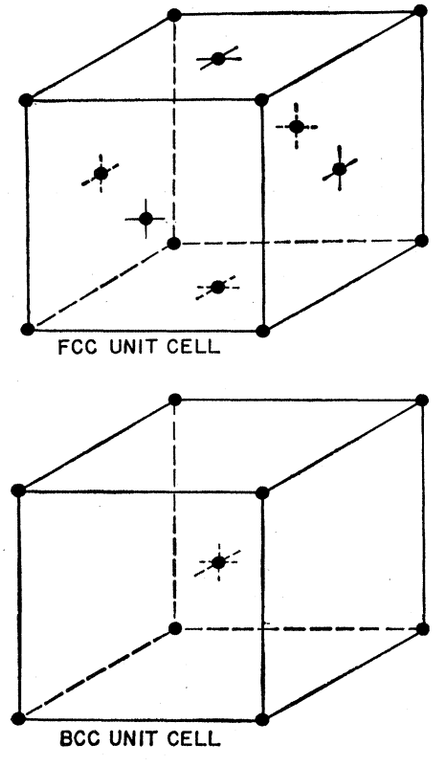

Fig. 1. Face-centered cubic (FCC) and body-centered cubic (BCC) space lattices.