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The Prime Meridian of Mars and the Longitudes of the Viking Landers

Abstract. *A new planetwide control net of Mars has been computed by a single large-block analytical triangulation derived from 17,224 measurements of 3,037 control points on 928 Mariner 9 pictures. The computation incorporated the new Viking-determined direction of the spin axis and rotation rate of Mars. The angle* V, *measured from the vernal equinox to the prime meridian (areocentric right ascension) of Mars, was determined to be* $V = 148.368° + 350.891986° (JD - 2433282.5)$, *where JD refers to the Julian date. The prime meridian of Mars passes through the center of the small crater Airy-O. The longitudes of the Viking landers are* $\lambda_1 = 47.82° \pm 0.1°$ *and* $\lambda_2 = 225.59° \pm 0.1°$.

A new coordinate system for Mars was defined after the Mariner 9 mission (*1*). This coordinate system included a new direction for the spin axis, a new rotation rate, a new reference spheroid for cartographic purposes, and a new prime meridian defined as the meridian passing through the center of a small crater called Airy-O. The system was adopted by the International Astronomical Union at its General Assembly in Sydney in 1973 (*2*) and is used on all modern maps of Mars (*3*).

Analysis of the radio tracking data from the Viking 1 and Viking 2 landers has resulted in improved values for the direction of the spin axis and the rotation rate of Mars (*4*). The location of the lander sites was also determined very accurately relative to the vernal equinox of Mars and in terms of latitude and areocentric radii. The best method of determining the longitudes of the landers is by means of a photogrammetric tie to Airy-O. However, because it has not been possible to identify the lander locations on orbiter pictures, this approach has not been feasible. This inability to find the lander locations relative to the local topography is a shortcoming of the Viking mission, and care should be taken in the future to be sure that landers and rovers can be located with reference to the local terrain. A less direct method of determining the longitudes of the landers is to measure the angle between the prime meridian and vernal equinox by treating this angle as an unknown in the least-squares computation of the planetwide control net (*5*). This method has always been used in the past as there was no hope of obtaining independently determined accurate coordinates of such points as landers.

A new planetwide control net of Mars has been computed by a single large-block analytical triangulation derived from 17,224 measurements of 3,037 control points on 928 Mariner 9 pictures. The computation incorporated the new Viking-determined direction of the spin axis and rotation rate of Mars (*4*). The photogrammetric method has been described elsewhere (*5*). The least-squares computation resulted in improved values of latitude and longitude of the control points and orientation angles of the pictures. The spacecraft coordinates when the pictures were taken were obtained from the Jet Propulsion Laboratory Science Data Team and were assumed correct in the computation. The areocentric radii at the control points were derived from three sources: 2,163 were interpolated from occultation measurements and used in past control net computations (*5*), 22 were computed photogrammetrically (*6*), and 852 were derived from elevations on the available U.S. Geological Survey 1:5,000,000 topographic series of Mars maps (MC-4, MC-10, MC-11, MC-17, MC-18, MC-19, and MC-23). The elevation contours on these maps were derived from analysis of many sources of data (*7*). The analytical triangulation required the solution of 8,858 normal equations. The standard error of the measurements was 0.0167 mm or slightly more than one pixel (0.0144 mm).

This control net computation determined the angle from the Mars vernal equinox to the prime meridian to be

$$V = 148.368° + 350.891986° (JD - 2433282.5)$$

where JD is the Julian date and 2433282.5 is the Julian date of the reference epoch 1950 January 1.0 Ephemeris Time. On the basis of the value of V, the longitudes of the Viking landers are (*4*) $\lambda_1 = 47.82° \pm 0.1°$ and $\lambda_2 = 225.59° \pm 0.1°$.

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Cholera Toxin Crystals Suitable for X-ray Diffraction

Abstract. *Large crystals of the cholera toxin were grown; their dimensions, symmetry* ($P2_1$), *order, and resistance to radiation make them ideally suited for a high-resolution x-ray structure determination. There is one molecule (approximately 84,000 daltons) per asymmetric unit, and therefore the lattice reveals no molecular symmetry. Two distinct bioassays indicate that the protein from dissolved crystals retains full biological activity.*

We report here the preparation of cholera toxin (CT) crystals which are quite suitable for a high-resolution x-ray crystallographic structure determination. Cholera toxin is a protein exotoxin which, when released by the *Vibrio cholerae* in the small bowel, produces a noninflammatory secretory diarrhea (*1*). The massive secretion of salt and water is triggered by the binding of the toxin to

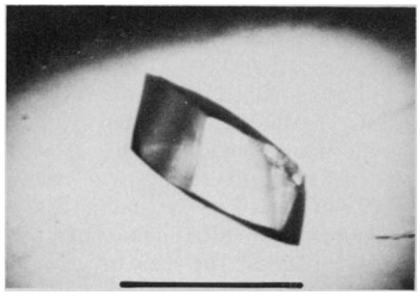

Fig. 1. Typical cholera toxin crystal. Bar is 1 mm.

specific receptors on the external surface of secretory cells in the intestinal mucosa (*2*). After van Heyningen's observation that CT interacted with mixed gangliosides, this receptor was identified by three groups of investigators as the ganglioside G_{M1} (*3*). Once bound, the toxin signals a membrane-bound adenylate cyclase to produce intracellular adenosine 3′,5′-monophosphate (cyclic AMP) which in turn stimulates the natural process for active secretion of salt and water (*2, 4*). In more general terms, the CT-G_{M1} system can be viewed as a model for a transmembranal signaling mechanism which stimulates an adenylate cyclase–cyclic AMP–sensitive process. There is intense interest in CT because of its broad spectrum of activity as an extracellular effector of a specific intracellular function, through the activation of adenylate cyclase. Attention has also begun to focus on the similarity between the CT-G_{M1} system and a wide range of receptor systems with different target functions. These include interferon (*5*), the factors related to normal and neoplastic growth (*6*), and trophic hormones such as thyroid-stimulating hormone (*7*). Cholera toxin has amino acid sequence similarities to certain mammalian hormones that also activate adenylate cyclase (*8*).

The molecular architecture of CT is a subject of intensive worldwide study. Although exact figures vary somewhat from one study to the next (*9*), qualitatively the picture is clear. Hydrodynamic studies indicate that the molecular weight of the complete toxin is approximately 84,000. There are two species of protomers called A and B. The A subunit, of which there is one per molecule, has a molecular weight of about 26,000 and is composed of two subfragments A_1 and A_2 of about 21,000 and 5,000 daltons, respectively; these are linked covalently by a disulfide bridge (*9*). The A_1 subunit (or fragment thereof), in conjunction with nicotinamide-adenine dinucleotide (NAD) and other factors, is responsible for stimulating the adenylate cyclase (*10, 11*). However, the A subunit alone cannot bind to the ganglioside receptors and therefore is severely compromised in its capacity to activate adenylate cyclase in the intact cell (*9, 11*).

The remainder of the toxin molecule is 58,000 daltons and is composed of five B subunits (*12*), each having a molecular weight of 11,600 (*8, 12*). These protomers bind avidly to G_{M1} in the cell membrane or even with the ganglioside in solution. The B subunits, however, cannot activate the adenylate cyclase; therefore, they are not active by themselves.

Table 1. The protein content of monoclinic cholera toxin crystals.

$\bar{v}$ (assumed)	ϕ_{prot}*	10^4 daltons protein/au†	Molecules CT/au‡
0.720	46.3	7.94	1
0.730	48.7	8.23	1
0.740	51.2	8.59	1
0.750	54.0	8.88	1
0.760	57.0	9.26	1

*ϕ_{prot} is the percentage of the unit cell volume occupied by the protein, given by $100 \times (\rho_{xal} - 1)/(1/\bar{v} - 1)$ where ρ_{xal} is the crystal density and $\bar{v}$ is the partial specific volume. The Ficoll gradient method permits one to assign the density of water to the interstitial fluid spaces of the crystal (*17*). †Given by $(V_{au})(\phi_{prot})(1/\bar{v})$ where V_{au} is the volume of the asymmetric unit (au) in cubic centimeters, here one-half of the unit cell; and N_o is Avogadro's number. Cell constants of the crystal in Ficoll were taken as 1.8 percent larger than in normal mother liquor on the basis of the increase in the *c*-axis observed on a Laué photograph taken of a crystal removed from the Ficoll gradient. ‡Assuming that the molecular weight is between 8.00 and 9.00 × 10^4 (see text).

The B subunits alone can apparently form a stable aggregate called choleragenoid (*13*) (as opposed to CT which is called choleragen), which binds G_{M1} and thereby competes with the same affinity as CT for the cells' binding sites (*14*). Cross-linking experiments indicate that, like the toxin, there are five B subunits per molecule (*12*). Small crystals of both choleragen and choleragenoid have been reported (*15*).

In our study, we crystallized pure CT prepared by the method of Finkelstein *et al.* (*16*). Large, well-shaped crystals were grown by vapor diffusion (*17*) at room temperature from polyethylene glycol of average molecular weight 4000 (*18*) (Fig. 1). They are monoclinic, space group is $P2_1$. Precession photographs (Fig. 2) calibrated with a silicon standard show unit cell dimensions a = 79.9 Å, b = 92.0 Å, c = 60.7 Å, and β = 106.40°. The crystal density was found to be 1.180 (± .001) g/cm³ by the Ficoll gradient method (*19*). Table 1 gives the number of daltons of protein in the asymmetric unit of the crystal as a function of the value assumed for the partial specific volume. There is clearly one molecule in the asymmetric unit and it is likely that the molecular weight is about 84,000, as indicated initially (*20*).

Figure 2 shows a 19° precession photograph of the (*h0l*) reciprocal lattice plane. The strong reflections to the edge of the photograph represent spacings of 2.4 Å. This is the second of two 42-hour 19° precession photographs taken with the same specimen. Prior to the start of this exposure, the crystal had been subjected to 50 hours of similar radiation in the course of obtaining other projections. This illustrates the remarkable degree of radiation resistance exhibited by these crystals.

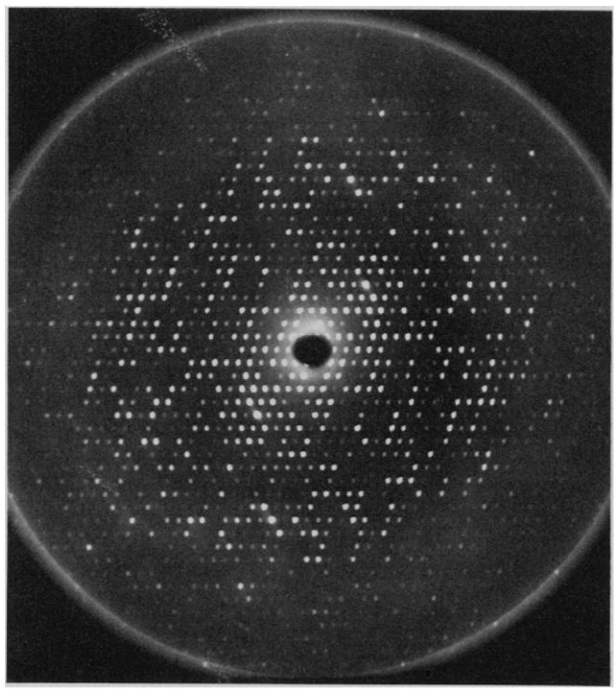

Fig. 2. Precession photograph of the (*h0l*) reciprocal lattice plane taken for 42 hours with graphite monochromatized CuKα radiation from an Elliott rotating anode generator running at 40 kv and 40 ma. This is the second such photo taken from this crystal.

Crystals of CT from the above studies were washed and redissolved, and their activity was determined in two separate bioassays. Dose-dependent activation of rabbit ileal epithelial cell adenylate cyclase (*21*) and dose-dependent induction of mouse adrenal cell morphological changes (*22*) were compared with the same phenomena induced by CT from the NIH Cholera Advisory Committee and that commercially available from Schwarz/Mann. In the mouse adrenal cell system, duplicate bioassay of dissolved crystals and separated mother liquor, in parallel with CT which had not been manipulated, showed no significant difference in specific activity. In this assay, the transition to observable cell rounding occurred between 2 and 4 pg per 250 μl per well with each of the above CT samples. Complete dose-response determinations of the activation of rabbit ileal epithelial cell adenylate cyclase by dissolved crystalline material yielded a dissociation constant (K_d) of $5 \times 10^{-10} M$, in comparison with $K_d = 5 \times 10^{-9} M$ (*21*) for preparations from NIH and Schwarz/Mann. Both values are in agreement with values reported by Cuatrecasas (*23*) for the interaction of CT with fat cell ($K_d = 4.6 \times 10^{-10} M$) and liver ($K_d = 1.1 \times 10^{-9} M$) membrane.

In conclusion, (i) the density of the crystal is consistent with five, rather than six, B subunits, and (ii) there is no expression of a molecular rotation axis in the symmetry of the crystal lattice. This, of course, does not rule out rotational symmetry in the arrangement of the B subunits which could exist within the asymmetric unit.

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Infectivity Reacquisition by *Trypanosoma brucei brucei* Cultivated with Tsetse Salivary Glands

Abstract. *Reacquisition of infectivity for mice was observed in cultures of* Trypanosoma brucei brucei *grown in the presence of tsetse fly salivary gland explants in a medium based on the amino acid composition of* Glossina *hemolymph and containing fetal bovine serum. High infection rates were obtained in mice inoculated with about 1.5 × 10⁸ organisms. Infectivity reacquisition was correlated with invasion of the salivary glands by the parasites. Few small trypanosomes with subterminal kinetoplasts (metacyclic-like forms) were found in the infective inoculums. The parasitemias in mice consisted of pleomorphic cultivable trypanosomes. Cultures initiated by these organisms and then placed with the head–salivary gland preparations became infective for mice.*

Trypanosoma brucei brucei, an important cause of disease in cattle, constitutes a prime factor in human malnutrition in Africa. Other subspecies of *T. brucei*, *T. b. rhodesiense* and *T. b. gambiense*, cause human sleeping sickness. Heretofore, infectivity for mammalian hosts was lost, typically irreversibly, by subspecies of *T. brucei* after they were established in culture. The noninfective culture forms resemble in their structure (*1*) and antigenic composition (*2*) the stages of the parasites found in the alimentary tract of their natural vectors, tsetse flies. Further development culminating in the formation of metacyclic trypanosomes infective for mammals occurs in the salivary glands of some of the flies, but induction of this development in cultures could not be controlled. In the present report we describe a method whereby infectivity reacquisition can be controlled in *T. b. brucei* cultures. Our results can be viewed as a first step toward defining the factors that control the completion of the parasite's life cycle to the infective (metacyclic) stage. The system we describe might also lead to development of a vaccine against trypanosomiasis.

Improved methods for the cultivation of arthropod tissues and organs have facilitated studies of the vector stages of various parasites. Records of attempts to employ tsetse organ cultures to produce infective stages of salivarian trypanosomes were published by Trager (*3*) and Nicoli and Vattier (*4*). Using this system with *Trypanosoma vivax*, Trager succeeded in infecting two sheep, but neither he nor Nicoli and Vattier were able to infect rats with *T. b. brucei* (*3*) or *T. b.*