1/3, 0). This means that two-thirds of the reflections are only weakly phased, by isomorphous replacement or anomalous scattering. It is therefore highly doubtful whether the solvent-flattening procedure used to resolve the phase ambiguity could have produced reliable phases for these reflections. Even in more favorable circumstances, a procedure which relies on a single isomorphous replacement (SIR) map for recognition of the solvent boundaries has its obvious dangers.

The shape of the particle deduced from the octamer crystals may therefore be misleading for the following reason. In electron density maps, the general distribution of density in the unit cell and hence the boundaries of the protein molecules are defined, initially, by the intensities and phases of the low order reflections. The intensities of two-thirds of these reflections would have remained almost unchanged in the single heavy atom derivative because of the closeness of the heavy atom to the special position, so that these reflections would hardly have been phased at all. If this had led to a wrong choice of envelope, it could not have been rectified by flattening the calculated SIR density outside it, although various isolated high resolution features common to the correct and chosen boundaries, such as a piece of right-handed helix, might still show through in weakened form. However, the larger scale distribution of density in the final map would be unreliable. Hence, it is not surprising that an attempt to interpret it has led to a structure which conflicts with all other firm results in the field.

A. KLUG
J. T. FINCH
T. J. RICHMOND
MRC Laboratory of Molecular Biology, Cambridge, CB2 2QH United Kingdom

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15. For a prolate ellipsoid of dimensions 110 × 70 Å, the radius of the equivalent sphere is 40.7 Å, giving $f_0 = 7.74 \times 10^{-8}$ g sec. From standard tables, f/f_0 is 1.03, for zero hydration, whence $f = 7.97 \times 10^{-8}$. The sedimentation constant $s = M(1 - \bar{V}\rho)/Nf$, which gives, using the experimentally measured value of 0.167 for $\delta\rho/\delta c = 1 - \bar{V}\rho$ (14), $s = 2.98 \times 10^{-20}/f$. Hence, for zero hydration, $s = 3.7$; any allowance for hydration will increase f, and hence reduce s.
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We agree with two of the major points in the letter of Klug *et al.* (*1*): the structure of the histone octamer determined by us (*2*) is very different from the structure determined earlier by them; and both structures cannot correctly represent the histone octamer even if a small DNA-dependent increase in its compaction is assumed. We differ on all their remaining points, which represent prevailing views and interpretations rather than "firm data." We can show that the properties of our structure are not inconsistent with the reported data.

1) We never made a value judgment as to whether the "isolated histone octamer is more relevant to the structure of chromatin than that of the octamer within nucleosome core particles." The histone octamer is a physiological entity that exists as such within cells (*3*) before it becomes associated with DNA and not after it "has lost all its DNA." It deserves to be described for its own sake. However, nucleosome core particles are not naturally "present in chromatin," but result from the deliberate enzymatic digestion of chromatin. We questioned not the significance of the removal of H1 or the putative "linker DNA" but instead the consequences of eliminating the torsional information present in the continuum of the native chromatin domains. This is a legitimate and logical question since the work of the Mirzabekov group (*4*) suggests that some internal rearrangements do take place. We believe however, that the structures of both the histone octamer and the nucleosome need to be accurately determined.

2) We did not challenge the crystallization procedures of the MRC group. To do so would have been presumptious on our part since their exact crystallization conditions [references 3 and 4, in (*1*)] such as *p*H, temperature, cofactors, and others, remain unpublished and thus beyond analysis. Likewise, we have not challenged their data handling procedures, such as the heavy atom parameters in each of the approximately 40 (our assumption) data sets and their scaling, the specifics of which have not been published [reference 4 in (*1*)].

Klug *et al.* (*1*) questioned the "curious physical chemical properties" of our structure which were not measured by them, but presumed. They, as we did at the beginning, must have assumed that on first approximation, the volume of our rugby-ball-shaped octamer is equivalent to that of a smooth-surfaced prolate ellipsoid 110 Å long and 70Å diameter. However, our photograph [figures 3 and 6, a and b, in (*2*)] demonstrate that the overall circumference of the model at planes perpendicular to its long axis is definitely not circular but indeed deeply concave at several places. We have now directly measured the volume of our model. The model was placed inside a thin, unsealed, water-tight bag and was submerged in water which caused the bag to conform to the surface topography of the model. Extending the Archimedes principle, we determined that the volume for the octamer is 184,000 Å^3, which is equivalent to a sphere of 35.3 Å radius. Using the formulas and the partial specific volume cited by Klug *et al.*, we calculate that the sedimentation coefficient (*S*) of our octamer is 4.4, not 3.7. If instead, the more accurately determined value of 0.753 (*5*) for partial specific volume is used in the calculation, the *S* value of our octamer is 4.98. It appears that the hydrodynamic properties of our structure are just fine within the scope of the criticism and the treatment of the data outlined by Klug *et al.* However, we have reservations concerning some assumptions employed in this treatment.

Klug *et al.* cite solution x-ray studies [references 5, 6, and 7 in (*1*)], which incidently are neutron diffraction studies, as providing firm data against our structure. However, Braddock *et al.* found that the pitch of the DNA superhelix in the nucleosome was 37Å. Furthermore they measured the radius of gyration and found it to be "substantially greater than that expected" [reference 7 in (*1*)] for a "flat disc or wedge" shaped model. To resolve this discrepancy, they assigned 25 percent of the histone mass to flexible "tails" and the remaining 75 percent of the octamer mass to the volume of an equivalent cylinder of dimensions 40 by 70 Å, that is, 153,000 Å^3. If we assume standard protein density for the remaining mass [reference 16 in (*1*)], the volume of the whole octamer would be 188,000 Å^3. Although this number compares well with ours, we do not rely on it as support for our structure, in line with earlier arguments of Finch *et al.* that "scattering in solution which yields spherical averages of intensities, can never prove a model" [reference 3 in (*1*)]. Nevertheless, we predict that when

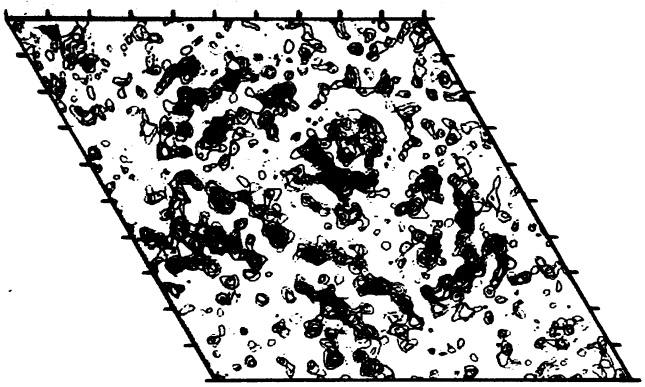

Fig. 1. The unfiltered map. This electron density map shows the range from −0.25 to +0.75 in x, from −0.25 to +0.75 in y, and from −0.048 to +0.048 in z axes. It was calculated from the phases determined by isomorphous and anomalous scattering differences only, with the use of programs from a crystallographic package of G. A. Petsko (Massachusetts Institute of Technology). No filtration of any kind was applied to it. The crystallographic statistics in Table 1 were derived from the same data used to make this map. Comparing this map to that in [figure 2a in (*2*)] it is evident that the filtration process did not alter the overall features of the protein, but eliminated noise in the solvent region, and consequently reduced the well-known artifactual electron density at the heavy atom site while sharpening the density of the protein.

the features of our octamer structure are used to calculate neutron-scatter functions, the resulting curves will correlate more closely with the observed scatter curves than any heretofore calculated.

The statement of Klug *et al.*, "These large discrepancies suggest that the structure proposed by Burlingame *et al.* is wrong; we attribute this to deficiencies in their x-ray analysis," is undocumented and does not serve to resolve the issue. Klug *et al.* have neither analyzed our diffraction data nor have they seen our electron density map in its entirety. The specific criticisms of Klug *et al.* are summarized in the following four points. (i) "[O]nly a single heavy atom (derivative) was used." (ii) ". . . [T]wo-thirds of the reflections are only weakly phased." (iii) Given the above, solvent flattening introduced artifacts. (iv) "Burlingame *et al.* have misinterpreted their map."

1) In 1970, the structure of rubredoxin (*6*), and in 1972 the structure of flavodoxin (*7*) were determined by means of single isomorphous replacement (SIR) and anomalous scattering (AS). More recently, the structures of dihydrofolate reductase (*8*) and troponin C (*9*) were solved with SIR and AS, while the Eco RI–DNA complex (*10*) and two Bence Jones proteins (*11, 12*) have been determined from a single derivative without AS data. Thus we believe that the validity of this technique has been established.

2) We, too, were concerned that the heavy atom might have insufficient phasing power for a significant fraction of the reflections. However, we have justified (*2*, p. 547) why "its position caused no significant problems." As documented in Table 1, the heavy atom contributes with high statistical significance to *all* the reflections, a testament to the superb precision of modern data collection methods.

3) Contrary to the assumption of Klug *et al.*, in the iterated single isomorphous replacement (ISIR) procedure (*13*), neither high nor low order reflections dominate the process. In a test (*14*) of the relative influence of high and low order reflections on the boundary of Bence Jones protein Rhe (*15*), the molecular envelope calculated with the 5 to 3 Å reflections is nearly identical to the envelope calculated with the infinity to 3 Å reflections. The ISIR procedure objectively locates the molecular envelope and properly, on the basis of probability, combines the phases of the back transform of the solvent flattened map with the experimentally determined phases.

Figure 1 shows a 3.3 Å resolution map calculated with phases derived solely

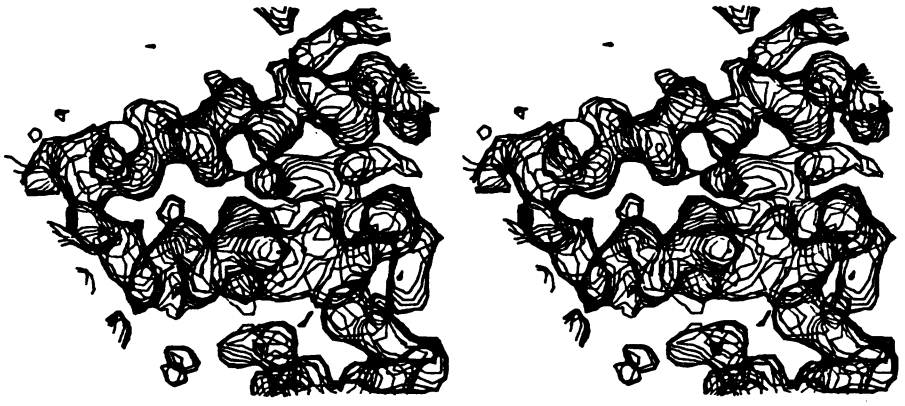

Fig. 2. Helices with visible side chains. Two helices from H2A are contoured at a lower level than shown in [figure 1 in (*2*)] and a thicker slab of electron density is shown. Density above and below the helix can be seen, including side chains. This map is contoured at only the lowest contour level, for clarity.

Table 1. Statistics for the SIR and AS data, without any filtering. For all 12,942 reflections, the phasing power is 1.77 and the figure of merit is 0.57. The phasing power represents the root-mean-square calculated heavy atom contribution to the structure factor divided by the rms lack of closure error. The figure of merits were calculated by the method of Blow and Crick (*17*), and used in obtaining Fig. 1. The maps in (*2*) were generated with phases refined by the ISIR procedure of B. C. Wang (*13*), which calculates the figure of merit by the method of Hendrickson and Lattman (*18*), and yields different numbers. Before noise filtering, the figure of merit calculated by the method of Hendrickson and Lattman was lower than that reported here, while after the ISIR procedure, it was higher. The ISIR procedure substantially improved the phases of the higher order reflections. FOM, figure of merit.

Resolution range	$-h + k + l \neq 3n$			$-h + k + l = 3n$		
	Reflections (No.)	Phasing power	FOM	Reflections (No.)	Phasing power	FOM
120.00–6.30	1290	1.71	0.73	655	4.69	0.97
6.30–5.00	1251	1.89	0.70	631	3.91	0.90
5.00–4.37	1232	1.52	0.58	620	2.13	0.77
4.37–3.97	1217	1.54	0.48	620	1.94	0.55
3.97–3.68	1238	1.32	0.45	615	1.56	0.48
3.68–3.47	1177	1.28	0.43	570	1.04	0.42
3.47–3.29	1209	1.16	0.40	617	0.80	0.34
120.00–3.29*	8614	1.49	0.54	4328	2.34	0.64

*Entire range.

from SIR and AS information and this should be compared with figure 2a in (*2*).These maps are "before" and "after" solvent flattening respectively, and clearly demonstrate that our noise filtering procedure did not introduce artifacts into the map.

4) Klug *et al.*, seem to have overlooked the information presented (*2*, p. 547) and concluded that we have incorrectly chosen the protein boundaries. On the contrary, 95 percent of the boundaries of the octamer are unambiguously delineated by large solvent regions between molecules [Fig. 1 and figure 2 in (*2*)] and the constraints imposed by the crystallographic symmetry elements (*2*, p. 547). Five helices, not two, were mentioned in our paper (*2*), and these plus several smaller helices make up about 50 percent of the protein mass, consistent with circular dichroism and Raman spectroscopy (*16*). Furthermore, we have reported that the chains of H2A and H2B have been traced nearly from end to end, and sufficient segments of characteristic amino acid sequences have been identified in the map to allow the assignment of the polypeptides. The quality of the map itself proves the validity of our procedures. The accompanying stereo pair (Fig. 2) is a replot of the alpha-helical region we showed before [figure 1 in (*2*)], contoured at a lower level and thus illustrating some side chains.

We believe that the resolution of the causes for the differences between the two structures will come about through further experimentation rather than rhetoric and argumentation. The results of our ongoing efforts in fitting the amino acids to the electron density map should shed some light on the resolution of the differences between the two structures.

E. N. MOUDRIANAKIS
Department of Biology,
Johns Hopkins University,
Baltimore, Maryland 21218

W. E. LOVE
Department of Biophysics,
Johns Hopkins University

B. C. WANG
Biocrystallography Laboratory,
V.A. Medical Center,
Pittsburgh, Pennsylvania 15240,
and *Department of Crystallography,*
University of Pittsburgh,
Pittsburgh, Pennsylvania 15260

N. G. XUONG
Department of Physics,
University of California at San Diego,
La Jolla 92093

R. W. BURLINGAME
Department of Biology,
Johns Hopkins University

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We have solved the crystallographic structure of the nucleosome core particle (histone octamer with 146-bp DNA) (*1*). We are having considerable difficulty in rationalizing the interpretation of the histone octamer of Burlingame *et al.* (*2*) with the nucleosome structure we have solved (*1*), with those of Richmond *et al.* (*3*) and Bentley *et al.* (*4*), and with many other biophysical studies. We see no problem with the electron density of the octamer presented by Burlingame *et al.* It appears to be of very high quality. It is the interpretation of the structure that we think should be reexamined. Burlingame *et al.* seem predisposed to assume that the DNA will lie in the grooves of the histone octamer. Our own nucleosome structure, along with those of Richmond *et al.* and Bentley *et al.*, indicated that in many locations rather than lying in the grooves of the histone octamer, the DNA actually rides on the ridges or "high points" of the proteins. The "helical ramp" is therefore somewhat discontinuous and complex. It appears to us, from statements in their article, that Burlingame *et al.* have ignored all but the most obvious (to them) possibilities for placing the model DNA on the octamer.

It seems quite possible to us to place superhelical DNA onto their histone core in a way that is consistent with our own and the other nucleosome structures, and which has a superhelical radius (~43 Å), superhelical pitch (~28 Å), and number of superhelical turns (~1.85 for 146 bp DNA) to be consistent with the bulk of previous experimental evidence about the nucleosome (Fig. 1).

Also, in this orientation, the octamer seems to have a size and extent, and occupy a volume which is not much different from what is seen in the crystallographic studies of nucleosome core particles. Some of our early modeling studies, which tested model nucleosomes with ellipsoidal histone octamers against diffraction data, showed us that the best ellipsoidal representation for the octamer has its long axis *not on the superhelical axis*, but about 45° from it and points in the direction of the DNA ends (Fig. 1). If at 0.15*M* ionic strength, in the presence of the DNA, the H2A region in the octamer of Burlingame *et al.*, (that is, the protein region shaded dark) moves in ~20 Å to fill the apparent solvent channel, the resulting structure (with the DNA bound as we propose) bears a very good resemblance to the crystallographic structures of the whole nucleosome core particle. The maximum thickness of the octamer in the superheli-

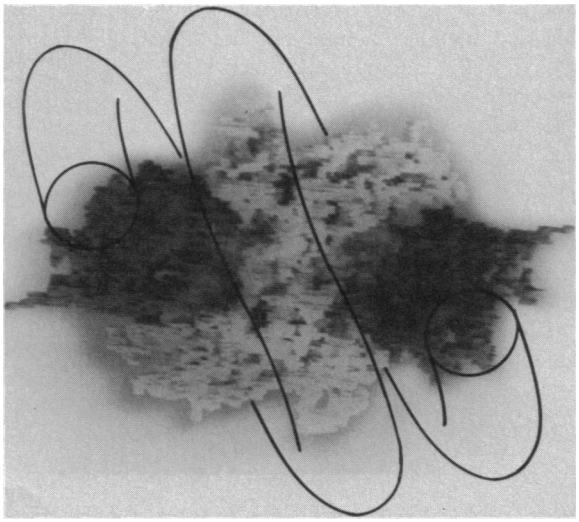

Fig. 1. Diagram of the nucleosome core particle showing proposed placement of superhelical DNA on the histone octamer model of Burlingame *et al.*, which is consistent with the nucleosome crystal structures and other biophysical studies.

Crystallographic Structure of the Octamer Histone Core of the Nucleosome

E. N. MOUDRIANAKIS, W. E. LOVE, B. C. WANG, N. G. XUONG and R. W. BURLINGAME

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