of this altered distribution must be considered in terms of the normal steady state existence of the plasma corticosterone. In control animals, the plasma concentration of the steroid remains constant, due to an equilibrium between rate of secretion and rate of metabolism. However, when a compound such as phenylbutazone increases the apparent volume of distribution without changing the rate of metabolism, it is obvious that more corticosterone must be produced by the adrenals and the total body content of the steroid must be increased. The action of phenylbutazone may then have two distinct facets: *first,* an increased level of corticosterone in tissues, and *secondly,* an increased secretion of the steroid in the adrenals. The latter action has been observed by Bernauer and Schmitt (13) as an increase in the corticosterone content of adrenal venous blood after administration of phenylbutazone. Studies currently in progress indicate that phenylbutazone interferes with the binding of corticosterone to plasma proteins, resulting in a displacement of steroid molecules and increasing the fraction of corticosterone present in the free (nonbound) form (14).

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The Binding of Xenon to Sperm Whale Deoxymyoglobin

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(Received July 12, 1966)

SUMMARY

Xenon binds to deoxymyoglobin at one specific site within the protein molecule. A major portion of the binding energy is provided by London interactions.

The existence of anesthetic properties among members of the "rare" gases (1) shows that specific structural groupings are not necessary for anesthetic action. Anesthetic ability seems to depend mainly upon the polarizability of the agent molecules.

Solubility studies (2) have shown that anesthetic agents such as cyclopropane, nitrous oxide, and xenon bind to a number

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of proteins, including myoglobin and hemoglobin. The detailed structural analysis of sperm whale metmyoglobin (J. C. Kendrew, H. C. Watson and R. Diamond, to be published) has made it possible to examine the nature of the forces involved in rare gas-protein association (3). In metmyoglobin (4) the heme group lies enfolded by the globin chain, with the central iron atom bound on one side of the heme to nitrogen of the imidazole ring of a histidine residue (F8), and on the other side to a water molecule. The crystallographic investigation of the xenon adduct of metmyoglobin (5) showed that a single xenon atom is bound at a site in the interior of the protein molecule, approximately equidistant from one of the pyrrole rings of the heme group and the ring of the heme linked histidine. It appears that the energy of binding originates mainly in London interactions.

The recent structural analysis of deoxymyoglobin (6) showed that in this form of the protein the iron atom is five-coordinated: there is no water molecule or other group at the sixth coordination position (opposite the heme-linked histidine) as in metmyoglobin. Inspection of the structure shows that this water cavity would be large enough to accommodate a xenon atom, but that in such a case only a few London contacts could be made, these yielding a possible energy of the order of 2 kcal/mole. It was suggested, however, that the increased charge on the heme group might produce a large enough charge-induced dipole to facilitate binding. The present study was designed to answer this question.

Deoxymyoglobin crystals, prepared by the method of Nobbs *et al.* (6) were mounted in quartz capillaries and equilibrated with xenon at a pressure of two atmospheres. X-ray intensities of the hk0 and h0l reflexions were collected to 2.8 Å resolution with multiple-film precession photographs. From these data electron den-

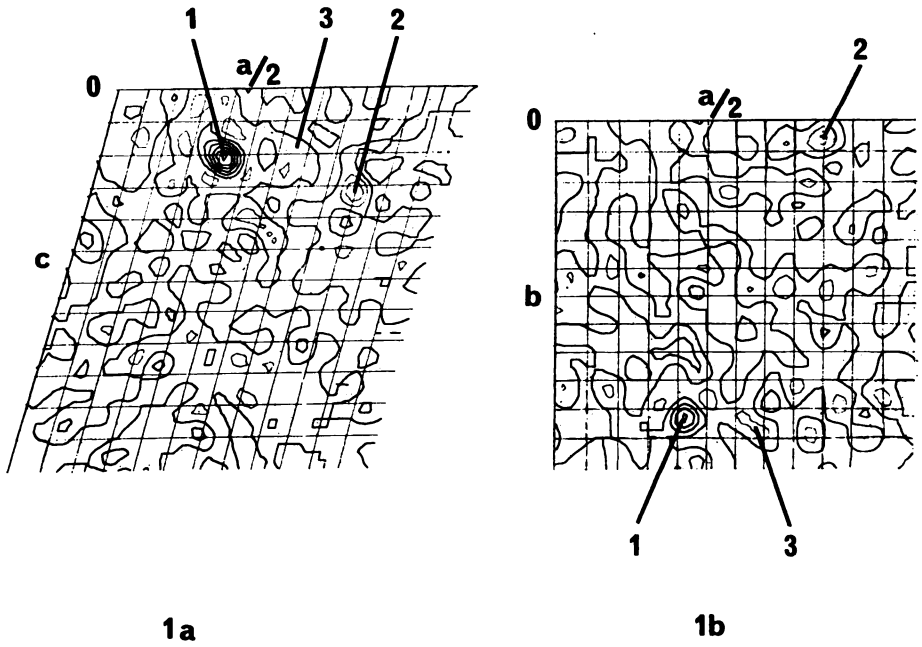

FIG. 1. *Difference Fourier syntheses between deoxymyoglobin-xenon and metmyoglobin*

Calculated for the projection of the structure onto the planes perpendicular to the directions [010] (1a) and [001] (1b). Contours are at an arbitrary interval—positive and zero contours full line, negative contours dotted. The heights of the peaks in Fig. 1a are twice those in Fig. 1b because the former projection is centric. Site *1*, xenon site; site *2*, negative group absent in deoxymyoglobin; site *3*, cavity (water site in metmyoglobin).

TABLE 1[a]
Xenon-deoxymyoglobin interaction distances (<5.5 Å)

Group	Atom	Distance (Å)	Group	Atom	Distance (Å)
Leucine F4	Cγ	3.05	Isoleucine H18	Cδ1	3.40
	Cβ	4.36		Cγ1	4.72
	Cα	5.11		Cγ2	5.30
	C	4.85			
	O	4.75	Heme	NVR	4.35
Alanine F5	N	5.28		C1VR	4.02
Histidine F8	Cδ2	4.20		CD	4.53
	Nε2	4.13		C4VL	5.33
	Cε1	3.73		CR	5.11
	Nδ1	3.47		C4VR	4.32
	Cγ	3.79		C3VR	4.07
	Cβ	4.36		C2VR	3.86
Leucine G5	Cγ	4.66		CAVR	4.27
Phenylalanine H14	Cε2	4.50		CMVR	4.69
	C	3.88		CBVR	5.35
	Cε1	4.68		FE	5.37

[a] Since the studies of xenon-metmyoglobin interaction were made, the atom coordinates of the protein have been further refined by least squares analysis improving the mean coordinate standard deviation to 0.15 Å (J. C. Kendrew, H. C. Watson and R. Diamond, to be published). The myoglobin atom nomenclature is described in references 7 (peptide chain) and 5 (heme group).

sity difference maps between deoxymyoglobin xenon and metmyoglobin were calculated using the phase angles of the native metmyoglobin.

Each of these difference maps (Fig. 1) shows two peaks. The positive peak *1* ($x = 0.177$, $y = 0.864$, $z = 0.168$) represents the position of xenon addition to the molecule, and corresponds to the xenon position as found in metmyoglobin. The negative peak *2* ($x = 0.38$, $y = 0.06$, $z = 0.27$) corresponds to the loss of the negatively charged group which is bound at the surface of metmyoglobin but which is absent in deoxymyoglobin. The remaining areas of the maps are relatively featureless, indicating that no other xenon atoms are present in the protein at subsidiary sites to any appreciable degree (less than 17% occupancy) and that no gross structural change has occurred in the protein itself. No peak is found in the indicated position *3* in Fig. 1 where the previously described water cavity of deoxymyoglobin lies. The accuracy of these projection maps is not sufficient to show the loss of this water molecule.

Thus xenon binds to deoxymyoglobin in a specific site on the histidine-linked side of the heme group; the potential binding characteristics of the water cavity on the distal side seem insufficient to overcome the thermal energies.

The xenon-protein approach distances are listed in Table 1. It is apparent that the side chain of leucine (F4) must move slightly to accommodate the xenon atom (van der Waals radius of 2.2 Å), but it would not be expected that such a movement would be seen on these projected Fourier maps.

The dispersion interaction calculated from the London contacts (Table 1) yields an energy in the region of 10 kcal/mole. The additional binding energies due to the dipole-induced dipole and to the net charge-induced interactions cannot be accurately determined since it is not yet known how the charge on the heme group is distributed; it can be estimated however that these other interactions will not exceed 2 kcal/mole.

This work was supported in part by U.S. Public Health Service Grant NB03625 to R. M. Featherstone, U. C. Medical Center, San Francisco. One of us (C.L.N.) is a Royal Society Stothert Research Fellow.

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