# John Hunter as a Pioneer of Veterinary Science

THE annual oration of the Royal College of Surgeons commemorating the life and work of John Hunter, was delivered at the Mansion House on February 28, by Sir Frederick Hobday, emeritus professor of surgery, and formerly principal and dean at the Royal Veterinary College. This made a departure from the usual tradition that a member of the medical profession delivers the address.

Sir Frederick Hobday in his introduction remarked that he felt it appropriate that a member of the veterinary profession should be honoured by the invitation to deliver the oration, for John Hunter was one of the founders of the Royal Veterinary College in 1791. He is described as being "the life and soul of the undertaking", and with him were associated Sir John Banks, Sir George Baker, the Duke of Northumberland and certain members of the Odiham Agricultural Society, who collected the necessary funds, and they were instrumental in bringing to England Prof. Charles Vial St. Bel, of the Veterinary School at Lyons, to be the first principal of the London Veterinary College. The early and unexpected death of St. Bel caused much anxiety regarding the future of the College, and Hunter helped to tide over the emergency.

Sir Frederick Hobday then proceeded to review John Hunter's contributions to veterinary science. One of the first operations performed by St. Bel in Great Britain was the removal of accessory feet that grew from the fetlocks of the two fore-legs of a horse, and it is stated that Hunter personally assisted (he was surgeon at St. George's Hospital) and gave St. Bel "some useful and friendly hints". John Hunter was a genuine lover of horses, and has left some excellent advice upon their management and on the proper way to shoe colts in order to correct defects in the manner in which they may use their fore-legs. His observations on what is termed 'roaring' are of particular interest, and although he did not correctly recognize its cause (paralysis of the left laryngeal nerve), he clearly distinguished this condition from 'broken wind'. Hunter wrote, too, "upon the effects that medicines have upon horses", and was untiring in his studies of the anatomy and physiology of animals—from the mouse to the elephant—birds, reptiles, insects and fishes. The illustrations in his book entitled "Certain Parts of the Animal Economy" cannot be surpassed for clearness and accuracy even with the aids that are now available. His chapter on the glands between the rectum and bladder, the vesiculæ seminales, describes minutely the comparative anatomy and uses of these glands both in man and in numerous animals, and the alterations that ensue after an animal has been unsexed.

The body temperature of animals was another subject to which John Hunter devoted considerable attention. He performed an experiment in which he froze the edge of the comb of a cockerel and remarks that "when I cut off a little bit it did not bleed nor did the animal show any signs of pain"—pioneer work in the use of a freezing mixture as an anæsthetic.

Hunter's writings on digestion bear witness to his keen powers of observation, for he concluded that something secreted in the coat of the stomach acts upon the food and assimilates this to the nature of the blood. He had learnt that it is not possible to make a horse vomit, and recognized the different actions that medicinal agents have upon different species of animals. In regard to the last-named, Sir Frederick remarked that this could have been only through direct observation. Thus, morphine is a narcotic for man and the dog tribe, but produces disastrous delirium in the horse and cat; strychnine and mercurials are much more toxic for the cow and dog than for the horse.

In the realm of surgery, John Hunter's classical experiment of grafting a cock's spur into its comb is well known—definite pioneer work of the various grafting operations that are performed to-day. He also made a series of observations on the reunion of divided tendons.

In conclusion, Sir Frederick Hobday alluded to the importance to a country of its agriculture, its animals and arable products, and of comparative medicine in the fight against disease, for it is here that the medical man and the veterinary surgeon meet on common ground.

# Crystal Structures of the Proteins

## AN X-RAY STUDY OF PALMER'S LACTOGLOBULIN

By Dr. Dorothy Crowfoot and Dennis Riley, Department of Mineralogy, Oxford

THE crystallization of a lactoglobulin from the albumin fraction of cow's milk by Palmer in 1934[1] was notable both for the size and stability of the protein crystals and for their occurrence in two different crystallographic modifications. The stable tabular form occurs alone when salt solutions of the protein are dialysed at an initial *p*H of 5·8 followed by adjustment to a *p*H of 5·2 with dilute HCl, whilst direct dialysis at a *p*H of 5·2 against distilled water produces needles which may later recrystallize in the tabular form. We have been able to study the inter-relationship of these two varieties through the kindness of Dr. R. A. Kekwick of the Lister Institute, who gave us preparations of them both.

The X-ray measurements show that the two types of lactoglobulin crystals differ not only in crystal structure but also probably in their water content. The tabular crystals are evidently, even on superficial examination, heavily hydrated. They occur commonly either as plates {001} up to 0·3 mm. across and bounded by pyramid faces or as prisms elongated along [010] and showing (100) dominating. They prove to be orthorhombic but are very markedly pseudo-tetragonal, and appear isotropic when viewed along the *c* axis. The birefringence is negative, about 0·004. When these crystals are removed from their

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mother liquor they shrink visibly in the direction of the $c$ axis, and the birefringence falls to less than 0·001. This shrinkage is accurately determined by the X-ray measurements which were carried out both on the air-dried crystals and on crystals mounted in their mother liquor. For the wet crystals $a = b =$ 63·5 A., $c$ = 145 A.; for the dry crystals $a = b =$ 59 A., $c$ = 105 A. The space group in the case of the wet crystals is $P2_12_12_1$, showing, in X-ray intensities as in dimensions, a close approach to the tetragonal space group $P4_22_1$, whence there are probably 8 molecules in the unit cell. There were too few X-ray reflections from the dry crystals to indicate the space group, but it seems most probable that this unit cell also contains eight molecules, but without water of crystallization.

The wet needle crystals occupy a place intermediate between the wet and dry tabular crystals. They appear to be exactly tetragonal, elongated along $c$. The cell dimensions are $a = b$ = 63·5 A., $c$ = 125 A., space group $P4_22_1$. The fact that the needles show positive birefringence suggests that they differ from the tabular form in the orientation of the molecules as well as in water content, and this is confirmed by the drying phenomena. There is little apparent change in shape or birefringence on exposure of the needles to air, but the very weak X-ray diffraction effects obtained from the dried crystals indicate a new unit cell with approximately $a = b$ = 54 A., and $c$ = 125 A.

The unit cell dimensions of the different lactoglobulin crystals suggest that in each case there are eight protein molecules in the cell, associated with different quantities of water of crystallization. Some difficulty was experienced, however, in making measurements of the crystal density to check this, owing to the great solubility of the protein crystals in salt solutions. A value of 1·257 was obtained in sugar solutions for the density of the wet tabular crystals, and this may be regarded as an upper limit for the density (*cf.* Adair and Adair[2]). It is in agreement with the presence of a considerable proportion of water in the crystals. It has so far proved impossible to measure the density of the needle crystals in any solvent, since even in sugar solutions the crystals rapidly imbibe liquid, with resultant swelling and loss of shape. The density of the dry tabular crystals in *o*-dichlorbenzene and toluene was found to be 1·27, which is lower than might be expected, probably owing to the occlusion of air in the irregular structure formed in the drying process. The molecular weight of the protein if calculated from this value and the cell dimensions given above is 35,300, or assuming the more probable density of 1·31 (that of dried insulin) 36,500. These values are in agreement with that obtained by ultracentrifuge measurements—about 39,000[3], within the limits of experimental accuracy.

It is of most interest to compare the crystal structure of these protein crystals with those previously measured, pepsin and insulin, and with hæmoglobin and chymotrypsin (recorded below). The similarity in cell dimensions extends also to the types of photographs obtained from wet and dry crystals of lactoglobulin. The wet tabular crystals give very beautiful X-ray pictures with reflections covering the whole field and extending out to spacings of less than 2·4 A. Those from the needle crystals are less good owing to their much smaller size. But with the dry crystals the photographs are very poor indeed, and this is evidently due to the poor orientation of the protein molecules in the collapsed structure. There are no X-ray reflections with spacings less than 20 A. The sharpness of the limit is reminiscent of that already observed on the much better X-ray photographs obtained from air-dried insulin[4], and suggests that in the insulin crystals also a certain degree of molecular disorientation prevails. As might be expected the crystal structure of dry insulin is of a very compact type, while in the wet lactoglobulin crystals, as in pepsin, the molecular arrangement must be loose and extended.

| | | Pepsin | Hæmoglobin | Chymotrypsin | Lactoglobulin tabular | Lactoglobulin needle | Insulin | Tobacco seed globulin |
|---|---|---|---|---|---|---|---|---|
| Wet | $a$ | 116 | 109 | $\frac{1}{2}\times 99$ | 63·5 | 63·5 | | |
| | $b$ | 67 | 63·2 | 67·8 | 63·5 | 63·5 | | |
| | Layer-spacing | 9×51·2 | 50·2 | 65 | 2×72·5 | 2×62·5 | | |
| | Molecular volume (A.$^3$) | 67,000 | 174,000 | 55,000 | 73,000 | 63,000 | | |
| Dry | $a$ | | 102 | 45 | 59 | 54 | 130 | 2×61 |
| | $b$ | | 56 | 62·5 | 59 | 54 | 74·7 | 2×61 |
| | Layer-spacing | | 35 | 53·5 | 2×52·5 | 2×62·5 | 30·6 | 2×61 |
| | Molecular volume (A.$^3$) | | 101,000 | 38,000 | 46,000 | 46,000 | 50,000 | 455,000 |
| | Shrinkage per cent | | 42 | 31 | 37 | 27 | | |

## MOLECULAR WEIGHT OF A TOBACCO SEED GLOBULIN

By Dr. Dorothy Crowfoot, Dept. of Mineralogy, Oxford, and Dr. I. Fankuchen, Crystallographic Laboratory, Cambridge

In order to determine the structure of protein crystals from X-ray diffraction effects it is usually necessary to measure fairly large single crystals. An exception to this rule is the case of crystals belonging to the cubic system, for which powder photographs should provide sufficient data. A preparation of a cubic tobacco seed globulin isolated by H. S. Vickery of the Rockefeller Institute, New York, was given to us by Dr. D. M. Wrinch, and this we have examined by the X-ray method in the form of the dry powder.* The crystals were very small but well-formed isotropic octahedra.

Three powder lines were recorded on the X-ray photographs which correspond in spacing to the first three reflections from a cubic face centred lattice of dimensions $a$ = 123 A. This value permits us to calculate the maximum molecular weight of the asymmetric unit as 363,000, accepting the presence of only the four molecules in the unit cell required by the symmetry, and the density of 1·287 ± 0·001, measured in a sodium phosphate buffer at $p$H 5·0. Since the crystals lose 10·4 per cent of their weight when dried in a vacuum at 100° due to loss of what may be considered water of crystallization, the maximum molecular weight of the dried protein may be calculated as 325,000. This molecular weight is in agreement in order of magnitude with the sedimentation constant of 12·7 × $10^{-13}$ found for the same protein by J. Philpot in the Department of

*W. T. Astbury[5] has previously measured the large angle scattering.

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Biochemistry, Oxford, and is similar to those of other seed globulins, excelsin and edestin given by Svedberg as about 300,000[6]. The X-ray measurements provide some additional evidence to show that the units present in these proteins are of very considerable size, although at this stage we cannot exclude the possibility that the true chemical molecular weight of the tobacco seed globulin is a sub-multiple of 325,000.

## AN X-RAY STUDY OF CHYMOTRYPSIN AND HÆMOGLOBIN

By Prof. J. D. Bernal, F.R.S., Dr. I. Fankuchen and Max Perutz, Crystallographic Laboratory, Cambridge

We have recently been fortunate in obtaining well-developed crystals of two proteins—chymotrypsin and hæmoglobin. The former were prepared for us by Dr. Northrop of Princeton and the latter —methæmoglobin of horse—by Dr. Adair at the Physiological Laboratories, Cambridge. In both cases the crystals were well formed and large ($\frac{1}{2}$ mm.) and well suited for X-ray analysis.

Chymotrypsin crystallizes in thick diamond-shaped plates at first thought to be orthorhombic but afterwards discovered to be monoclinic twins. It was examined both in the native state in its mother liquor[7] and dried. The cell dimensions are $a = 49 \cdot 6$ A, $b = 67 \cdot 8$ A., $c = 66 \cdot 5$ A., $\beta = 102°$ for the wet crystals, and $a = 45$ A., $b = 62 \cdot 5$ A., $c = 57 \cdot 5$ A., $\beta = 112°$ for the dry. The space group in both cases is probably $P2_1$. The cell volumes are 219,000 and 151,000 A.$^3$ respectively. It is evident that very considerable shrinkage takes place on drying, nearly all of which is in the direction of the $c$ axis. The density of the wet crystals could be measured by the methods of Adair and was found to be 1·277. If we assumed four molecules per cell this would give a molecular weight including water of crystallization of 42,300. In the case of the dry crystals it was unfortunately impossible to measure the density on account of the difficulty of removing crystallized salts. Assuming the density of the dried protein to be the same as that of dry insulin, namely, 1·31, the molecular weight is 32,000. This value for the molecular weight of a protein seems rather lower than is usually obtained by the centrifuge method, which, however, has not as yet been applied to chymotrypsin. It is difficult without a deeper analysis to say much about the inner structure of the crystals, but the extreme strength of (001) indicates a layer structure, while the weakness of (*hol*) when $h$ is odd points to a pseudo-glide plane parallel to (010).

The hæmoglobin crystals were found to be monoclinic and usually twinned, corresponding very closely to the description given by Reichert and Brown[8]. The dimensions are $a = 109$ A., $b = 63 \cdot 2$ A., $c = 54 \cdot 2$ A., $\beta = 112°$ for the wet crystals and $a = 102$ A., $b = 56$ A., $c = 49$ A., $\beta = 134°$ for the dry. The space group in both cases is $C2$ with a face-centred pseudo-hexagonal cell. The cell volumes are 348,000 and 202,000 respectively. Here the shrinkage takes place apparently more by the increase of the $\beta$ angle rather than by the shortening of cell edges. The density of the wet crystals is 1·242, which gives a molecular weight of protein and water on the assumption of two molecules per cell of 131,000. The molecular weight of the dry crystals has not been determined on account of the salt content, but taking the value

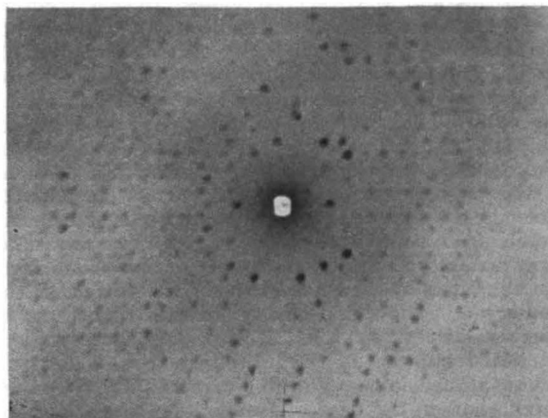

Fig. 1.

WET HÆMOGLOBIN CRYSTAL; 5° OSCILLATION ABOUT $b$-AXIS SHOWING (*hko*)-ZONE AT CENTRE OF PHOTOGRAPH. NOTE THE PSEUDO-HEXAGONAL CHARACTER OF THE PATTERN AND THE INTENSITY OF THE REFLECTIONS AT HIGH ANGLES.

of 1·26 based on that measured by Chick and Martin[9] for the closely similar serum albumin, the molecular weight is 77,000. The air-dried crystals still contain water. Taking the amount estimated by Haurowitz[10] as 9·6 per cent, the molecular weight of the dry protein becomes 69,000, which agrees excellently with the 67,000 found by chemical methods.

The molecular arrangement appears to be based on a layer lattice with a puckered pseudo-hexagonal network. In the dry crystals it is possible to arrive at a structure which accounts qualitatively for the

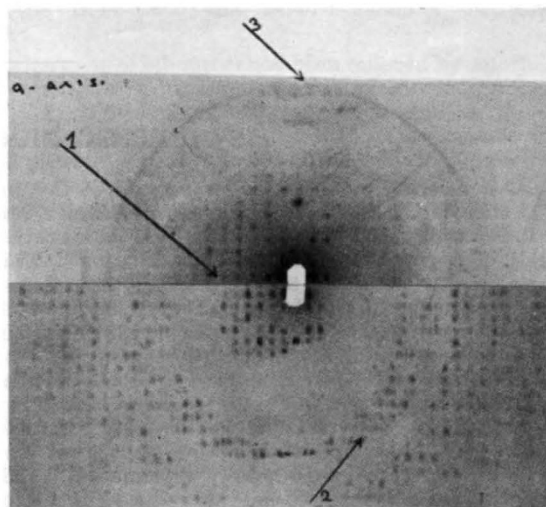


Fig. 2.

COMPARISON PHOTOGRAPH OF CHYMOTRYPSIN: WET BELOW AND DRY ABOVE; 5° OSCILLATION ABOUT $b$-AXIS SHOWING AT CENTRE OF PHOTOGRAPH THE (*okl*)-ZONE. NOTE LARGE DIFFERENCE OF SPACING OF (00*l*)-REFLECTIONS ($\uparrow_1$) AND COMPARATIVELY SMALL DIFFERENCE OF LAYER-LINE SEPARATION. THE WET CRYSTAL WAS A TWIN; THE (1*kl*)-REFLECTIONS SHOW DOUBLING OF SPOTS ($\uparrow_2$). THE RAPID FALLING OFF OF INTENSITY IN THE DRY CRYSTAL PHOTOGRAPHS SHOULD BE NOTED. THERE IS ALSO THE APPEARANCE OF WEAK SPOTS AT A LARGER ANGLE, CORRESPONDING TO A SPACING OF 5 A. ($\uparrow_3$)

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intensities of all the observed planes. In this structure the asymmetric unit is a half molecule of approximate diameter 35 A., linked with three others. The molecule observed in solution may be formed of two of these. The optical properties of the wet crystal point to the approximate parallelism of the hæmatin groups which lie approximately parallel to the (100) planes. As yet their position cannot be inferred, but owing to the two-fold axis of symmetry which the double molecule possesses, only two different kinds of hæmatin globin arrangements are possible. In the meantime, it is possible to draw some useful conclusions from the character of the photographs. In both cases the wet crystals showed perfectly definite reflections at spacings as low as 2 A. This proves the complete internal regularity of the protein molecules down to atomic dimensions. The dry crystals, on the other hand, show in the case of hæmoglobin no reflections of spacing less than 20 A, and for chymotrypsin, except for one or two weak reflections at 5 A., none below 8 A. Dr. Crowfoot had already noticed this phenomenon for insulin and more recently for lactoglobulin. The explanation is probably somewhat as follows. The intensity of a reflection from a protein crystal may be considered a function of three factors: the structure factor due to the position of the molecules, that due to the positions of the atoms inside the molecule, and a third factor depending on the regularity of arrangement. It is apparent that there is a general enhancement of spots in the regions of 9 A. and 4·5 A. corresponding to the two main reflections maxima of denatured proteins. In the dry protein, however, the irregularity is such that only the first of these is retained, the spots corresponding to the outer rim being generally too weak to register. If this analysis is correct, it follows that the change involved on denaturation does not require any considerable movement of atoms or amino-acid residues, but only relatively minor rearrangement together with almost complete loss of regularity, the dry protein representing an intermediate stage.

As can be seen from Fig. 2 the dried crystals of chymotrypsin show not only alterations of spacing but also of relative intensities of reflection. If we assume that drying takes place by the removal of water from between protein molecules, studies of these changes provide an opportunity of separating the effects of inter- and intra-molecular scattering. This may make possible the direct Fourier analysis of the molecular structure once complete sets of reflections are available in different states of hydration.

Already sufficient protein crystals have been examined by X-rays to be able to point to significant regularities. The table of lattice dimensions referred to three axes at right angles shows that the proteins fall into two groups: pseudo-hexagonal and pseudo-cubic, in both cases with a side of 60–75 A. This is of the order of twice the diameter of a protein molecule.

The work already done shows the wealth of new data that can be obtained by X-ray methods from reasonably well-crystallized proteins. The full advantage of the possibilities can, however, only be realized by work on a far larger number of protein types under different physical conditions, together with a more detailed application of crystal analysis.

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# Asymmetric-Sideband Broadcasting

AT a meeting of the Wireless Section of the Institution of Electrical Engineers on March 2, Mr. P. P. Eckersley read a paper entitled "A Quantitative Study of Asymmetric-Sideband Broadcasting". This paper directed attention to the fact that the progress of radio broadcasting is being hampered by the lack of a sufficient number of channels in which to contain the increasing number of transmissions. In the present state of the art, few, except local, stations can be received without interference from other broadcasting stations on adjacent frequency channels, unless the upper modulation frequencies of the wanted programme are severely attenuated. This inter-station interference can be reduced if all or part of one sideband of the transmitted spectrum is cut away; and it is the object of the asymmetric-sideband system of transmission to cut away part of one sideband without loss of the higher modulation frequencies and without introducing audible harmonic distortion.

Mr. Eckersley then gave a quantitative analysis of the phase and amplitudes of the sideband components and the effect of these factors in producing distortion. Curves have been derived, which show the required attenuation-constant of filters which cut away part of one sideband but produce no more than a constant, small distortion. A description was also given of practical tests in which the quality of reproduction obtainable from an asymmetric transmitter was compared with that given by orthodox modulation. It is claimed that while a highly trained ear may occasionally be able to detect some slight differences between the two types of reproduction when they are directly compared, no difference is detectable with modern broadcast receiving apparatus.

The last part of the paper showed that the asymmetric system has three possible applications to broadcasting as it is practised to-day. First, it could be used to allow carrier frequency differences of 11 or 12 kc./sec. without introducing any sideband overlap interference. Secondly, it could be applied to existing conditions in which the carrier frequencies differ by about 9 kc./sec., and it would then reduce inter-station interference to about one tenth of its present value. Thirdly, if the existing interferences are considered to be tolerable, then the differences between the carrier-frequencies of stations can be reduced to 6 kc./sec., without increasing the interference; this implies that one and a half times the number of stations working in Europe to-day on exclusive wave-length channels could be accommodated in the existing wave-band.

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