FRANCIS JOHN WORSLEY ROUGHTON
1899–1972
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BY Q. H. GIBSON, F.R.S.

LIFE

Francis John Worsley Roughton was born on 6 June 1899 at Kettering, and died suddenly at Cambridge on 29 April 1972. His only sister died in 1933. His father was the fifth consecutive Roughton to practise medicine in Kettering, and his family had numerous connexions among the merchants and farmers of the area. Perhaps the most colourful of his forebears was Thomas Sturgeon, footman to the Duke of Rockingham who, about 1775, eloped with the Duke’s daughter, the Lady Henrietta Wentworth. Using her fortune, he set up a china factory in Rouen, in which he introduced the use of coal for firing his products (the local industry had used wood), but the venture was not a financial success and became insolvent two or three years before the beginning of the French Revolution. Sturgeon, however, after the end of the Napoleonic wars, applied for a large sum in compensation for the loss of the business, and did in fact receive some £6000, a small fraction of his original claim for £40 000, but still a stately sum in those times. He showed great perseverance in pressing the claim, and the papers show that the matter finally engaged the attention of the Duke of Wellington who suggested settlement to be rid of the affair. The connexion with Sturgeon was that a daughter of his was one of Roughton’s great-great-grandmothers. There was an oral family tradition, roughly as described, and about 1955 it occurred to Roughton to pursue the story. The project expanded into a continuing hobby which occupied his leisure hours for some years, and led to expeditions to Rouen to consult the voluminous French archives, as well as to many hours of library work with the English papers, much of the correspondence of Sturgeon and Henrietta with the Duke of Rockingham having survived. He intended to publish the material in book form, but the project was not completed. In an expansion of this interest, he collected data for a family tree of some size which remains among his papers. A methodical man in most things, he did not leave any biographical information on deposit with the Society, so it seems that death took him by surprise. In the circumstances, it has been necessary to draw on the memories of his colleagues and of his wife,
Dr Alice Roughton. It is a pleasure to thank her, Lord Adrian, Professor Malcolm Dixon, Professor H. Hartridge, and Dr J. Needham, who have all contributed to this account of Roughton’s earlier life.

As a young man he suffered from attacks of paroxysmal tachycardia. At the time the outlook was regarded as sombre, and he was advised to treat himself as a cardiac invalid. A first consequence was that he was unable to serve in World War I, and instead, on leaving Winchester, came up to Cambridge in 1917. His plan to pursue a career in medicine was likewise thwarted since it appeared that his physical stamina would not be great enough to support the stresses involved, and he was advised that his life expectancy was also substantially less than normal. The cardiac disability and its associated régime, added to the problems of an only son, made personal relations difficult for him, and he continued in a measure of shyness and awkwardness throughout his life. The reasons for this were not widely known and he was sometimes regarded as remote and uninterested in the people with whom he associated. He was further divorced from common concerns in that, if not wealthy, he had few real financial cares. The need to follow a restrictive régime from a comparatively early age also ingrained some of the attitudes of the invalid, and he was inclined to expect rather exact compliance with his wishes in minor things. In addition, he always disliked parties and socializing as an end in itself. The net result was that he presented an austere image, and many people, perhaps a majority of those who came into contact with him, never penetrated the façade.

His intellectual gifts were early apparent, and, coming up to Trinity as a scholar, he distinguished himself to such a degree that, although he was unable to sit the examinations in Part II because of repeated cardiac attacks, he was treated as if he had done so, and became a graduate student. On completing his thesis he was awarded a research fellowship at Trinity. The years which followed were busy ones in which he laid the foundations of his career in research working with Dr H. Hartridge to make the first rapid kinetic measurement on a solution by the mixing method. His interest at this time was mainly in physiology, and with three other notable young men, he belonged to an informal association they called the Canula Club, which met to discuss topics in physiology and especially Bayliss’s book on General Physiology. All four spent their lives in Cambridge, and three became Fellows of the Society. Besides Roughton they were Malcolm Dixon, Joseph Needham and Hubert Tunnicliffe, noted for his effective teaching of physiology to medical students.

The pattern of full-time research and predominantly scientific interest was broken by marriage in 1925 to Dr Alice Hopkinson, a vigorous and independent individual, who brought Roughton into contact with a new family grouping. Her father had been Professor of Engineering at Cambridge and a Fellow of the Society, and her mother was born Siemens of the German electrical firm. Roughton and his Hopkinson grandmother-in-law took very much to one another, and he was a frequent visitor to her home at 9, Adams Road until her death in 1933. During this period two children were born, Geoffrey and Rosemary, the Roughtons living at first in a house of their own, and after 1933 at 9, Adams Road,
very much according to the conventional pattern of the time. All this was changed after war broke out in 1939. Roughton went to the Harvard Fatigue Laboratory in 1940 to conduct war-related research, chiefly on the effects of carbon monoxide on respiration. Geoffrey and Rosemary were already in the U.S., while Dr Alice Roughton stayed behind in Cambridge at 9, Adams Road, which became the wartime home of the Ballets Jooss. The gardens were in part cultivated, in part used for pasture for cows and the housekeeper was replaced by a Landgirl. After the war, the informality lingered on, and the house was divided into several apartments which were occupied by overseas students or post-doctoral workers. All met at mealtimes in the kitchen, where a savoury stew, fresh bread, cider and a warm welcome awaited the residents and their friends. Stories of the ménage soon spread, and the tale circulated in Colloid Science that a German visitor had appeared there seeking Professor Roughton. On being told that the Professor was working at home he asked: ‘Where lives he?’ ‘9, Adams Road.’ ‘So it is not possible. While in Cambridge I live at 9, Adams Road.’ Some relics of the past remained, however, including a Rolls-Royce convertible which Roughton operated until the middle ’60s, when he finally decided that the time had come to treat it as a vintage car rather than as regular transportation. Until then he travelled on scientific visits carrying an array of boxes and cases with clothes, books, calculator and typewriter which quite filled the interior.

Through all these changes Trinity College was a fixed element in Roughton’s life, and he was associated with it continuously as a Fellow from 1923. When in Cambridge he visited it at least once a day, saying he had to eat the free meal to which he was entitled. In fact, he contributed substantially to its building fund and took a keen interest both in its current affairs and in its history. In his later years he travelled extensively, visiting his collaborators both in Britain and overseas, and entertained many visitors in Cambridge. He was a delightful house-guest who fitted in easily with domestic routine, and most imaginative and successful in his choice of gifts. As a host he was similarly successful, depending to some extent on careful planning of a routine, and in Cambridge, making use of the facilities of Trinity. He had, however, a tendency to abstraction, and on social occasions it was sometimes only too clear that he wished he were somewhere else.

His habit of work was to start moderately early in the morning, say at 10 o’clock and continue to lunch, which in later years was followed by a rest, during which the Daily Telegraph crossword was completed—in the U.S. in the airmail edition. Then a constitutional, and back to work until dinnertime. Half-an-hour with coffee, weak with much milk, and back to work again until 10 or 11 p.m. Little time was spent in recreation as such, though he played golf well, and tennis in carefully measured moderation when younger. He enjoyed taking a part in university and college politics, adopting usually a moderately conservative viewpoint. He also enjoyed acting as a visitor to the British Gelatine and Glue Research Association, and as a director of a private company which owned one or two large Residential Clubs for professional people in London. These duties represented a break in routine, and he performed them thoughtfully.
and conscientiously, bringing his full powers to bear. In special circumstances, such as a threatened take-over of the company, its affairs even displaced scientific matters at the forefront of his mind.

A leading characteristic was pertinacity, perhaps inherited from Sturgeon. It is hard to recall anything significant which he gave up easily. Not only did he persevere himself, he urged his collaborators to continue in a line which he felt to be correct; once convinced of error, however, he accepted the verdict completely. He believed fully in what he was doing and in its value, and took great pains over every detail of the work and its writing-up. This belief came through clearly on one occasion during a paper-writing session which was not going well. I felt that the work input was out of proportion to the significance of the idea to be expressed, and Roughton, sensitive to this mood, reproved me. ‘Come, Gibson,’ he said, ‘remember, we are writing a classic.’ In this particular instance the verdict of time has come in, on my side, I am sorry to have to admit.

He was a splendid correspondent. His letters were full of thought and reflected his real interest in others. They always contained items of personal news as well as dealing with the scientific matters which prompted them. It is interesting that in a correspondence extending over 20 years and involving hundreds of letters, he and I continued to address each other as ‘Dear Gibson’ and ‘Dear Roughton’, and though formality gradually disappeared from the content of the letters both of us enjoyed the preservation of an unchanging ritual over the years.

A keen and realistic critic of himself and of others, he seldom expressed his opinions, favourable or unfavourable, without due occasion, and rarely gossiped or reminisced.

The appointments which he held were as Lecturer in Biochemistry 1923–27, and then as Lecturer in Physiology 1927–47. He was then invited to become Professor of Colloid Science in succession to Rideal. The decision to accept the Chair was not easily reached for he was doubtful both about the scope of the subject and about his own qualifications for the position. In the event he came to preside over a rather mixed group in Free School Lane whose interests ranged from the physical chemistry of proteins to ore flotation. During his 20-year tenure of the Chair he made little attempt to build up a personal research group, and occupied only a small part of the laboratories on the second floor with Mr Ken Edwards, his long-time technician, and a secretary. The experimental work done there was chiefly concerned with the haemoglobin-oxygen equilibrium, and he had a room wholly devoted to Van Slyke-Neill apparatus and modifications of his own design for special determinations. Relatively little rapid kinetic work was carried on, and that mostly by continuous-flow methods, primarily on reactions of carbon dioxide. In the last 5–10 years of his tenure he attempted to redirect the work of the department towards the study of membranes and biological surface effects, which he saw, correctly, as a key area in biology, but on his retirement the doubts about the existence of a definable subject called Colloid Science were so strongly and widely shared that the title of the department was extinguished in favour of Biophysics. In the last 15 years of his life he extended his oversea contacts and spent an increasing
amount of time working in California and in Milan on the resolution of basic problems of respiratory physiology and of carbon dioxide–haemoglobin interaction, respectively. He continued this pattern after retirement, and had returned to Cambridge from a visit to Milan only a week before his death from a vascular accident. His last letter to me, written from Milan about two weeks earlier, was full of continuing interest and optimism about the resolution of some of our joint problems.

Work

In view of the large number of topics which engaged Roughton’s attention it has seemed best to take these in turn rather than deal with the papers in chronological order, though this rule has not been strictly adhered to. Thus, the first and second papers deserve special mention. The first paper was unusual in a number of respects. It was on the respiration and circulation in a case of paroxysmal tachycardia, the junior author serving also as the object of study. The authors included Barcroft, in whose laboratory Roughton was to work for some years, and Dr E. D. Adrian was thanked for making the string galvanometer recordings of the e.c.g. between and during attacks which are reproduced at the end of the paper. As expected, the arterial blood, obtained by radial puncture, was normally saturated, and the arterio-venous difference in oxygen content greatly increased during an attack. Roughton has himself described how his second paper with Barcroft and Shoji came about. His account is given in his tribute to Barcroft presented at the Memorial Symposium in 1948. The paper itself is concerned with a method for determining the oxygen tension in the mixed venous blood. A number of breaths of nitrogen sufficient to lower the alveolar \( p(\text{O}_2) \) to about the required point were taken, and the breath held long enough to allow a reasonable approach to equilibrium between blood and alveolar air. The alveolar \( p(\text{O}_2) \) was then determined and taken as the \( p(\text{O}_2) \) of mixed venous blood. The matter was of great importance in permitting the use of the Fick principle for the determination of cardiac output in the days before the introduction of cardiac catheterization. There followed a series of papers with Hartridge on the rates of reaction of haemoglobin with ligands which not only illuminated their immediate subject, but opened up new prospects in chemistry, biochemistry and physiology. They established a line of work which Roughton was to continue for the remainder of his life, and form a classic contribution to the literature on haemoglobin. Before this work, chemical reactions in solution could be studied only if their half-times were of the order of several minutes, so that samples could be taken for analysis by means of pipettes and ordinary chemical apparatus. Hartridge and Roughton reduced this time to about 1 ms, changing the time scale by perhaps 50 000 times, and so allowing an entirely new range of phenomena to be studied. Their principal method was to mix the reactants intimately together in a time which they showed to be of the order of a tenth of a millisecond, and to allow the mixture to flow down a long observation tube. Knowing the rate of flow and the cross-section of the tube, and assuming mass flow, they could determine the age of the mixture at any distance from the point of mixing. The problem of obtaining its composition remained. For the special...
case of haemoglobin and oxygen this was solved by the use of the reversion spectroscope, invented some years earlier by Hartridge. In this ingenious instrument the observer saw two spectra one above the other, the one having its red end to the left of the field, the other with red to the right. The spectra could be moved relatively to one another so that an emission or absorption line could be made to cross both spectra as an unbroken line, and, with a grating as the dispersing element, its position could be determined to within a few ångströms. This instrument was used to measure the partition of haemoglobin between oxygen and carbon monoxide by means of the difference of about 65 Å in the position of their α-bands, the α-bands of mixtures occupying intermediate positions. In their work on the reactions of oxygen with haemoglobin the weaker spectrum of deoxyhaemoglobin was neglected and the problem of determining the composition of the mixture in the streaming fluid was reduced to that of measuring the proportions of oxy- and carboxyhaemoglobin by means of the difference of about 65 Å in the position of their α-bands, the α-bands of mixtures occupying intermediate positions. In their work on the reactions of oxygen with haemoglobin the weaker spectrum of deoxyhaemoglobin was neglected and the problem of determining the composition of the mixture in the streaming fluid was reduced to that of measuring the proportions of oxy- and carboxyhaemoglobin by means of the difference of about 65 Å in the position of their α-bands, the α-bands of mixtures occupying intermediate positions.

Naturally, these limitations were apparent to Hartridge & Roughton who attempted to replace the reversion spectroscope with a spectrograph. An image of the observation tube was projected on to the entrance slit so as to record the spectrum along a considerable length of the tube in a single photograph, so economizing in reagents, while the photographs could be studied at leisure. The method does not seem to have been applied extensively in practice, and work on it stopped when Hartridge went to London in 1926. A paper in the *Journal of Physiology* in 1928 on the photographic method as applied to static mixtures of oxy- and carboxyhaemoglobins concentrated on determining the exact position of the α-band rather than on spectrophotometry, so that the method remained specific for haemoglobin.

Another more general method of observation was to measure the temperature rise occurring in the mixed fluids as the chemical reaction progressed. A differential thermopile was inserted into the observation tube with one set of junctions near the mixer and the other set sufficiently remote to sense the full temperature rise due to the reaction. Although most chemical reactions are associated with a thermal change, most often a release of heat, the method was insufficiently sensitive to be widely used. A haemoglobin reaction could readily be studied optically with a concentration of, say, 0.1 mM heme in a 1 cm tube, but a reaction with a thermal change of say 10 kcal/mol would provide a temperature rise of only 0.001°C at this concentration, very much at the lower limit of observation. Although Roughton continued to develop the thermal method
of observation after Hartridge had left Cambridge and applied it especially in work with carbon dioxide, it never enjoyed any wide popularity.

In general, it is fair to say that the early work of Hartridge & Roughton, which was entirely original in concept, has received comparatively little recognition. This may have been because of the lack of generality of their procedures and because the method did not undergo evolutionary development. It sprang to life as a complete system from which later work took nothing but the principle of mixing the reagents, and may perhaps be compared with the Daguerre system of photography which shared with later silver bromide photography little but the use of a lens.

Although the flow method is remembered nowadays, this was not the method Hartridge had originally hoped to use to follow the haemoglobin–ligand reactions. He knew of the photosensitivity of carbon monoxide haemoglobin and anticipated the method of flash photolysis by some 30 years. In work with Roughton two apparatus were constructed and used. In one, a beam from an arc-lamp illuminated a solution containing oxygen, carbon monoxide, O₂Hb and COHb which was flowing through a tube. After illumination, the solution passed into the observation section of the apparatus where its composition was measured by means of the reversion spectroscope after a period of relaxation towards its dark equilibrium position which was determined by the rate of flow and placement of the spectroscope. In the second apparatus the solution was contained within a cuvette inside an unsilvered Dewar flask. The solution could be examined by the reversion spectroscope during and after illumination with a Pointolite arc lamp. The reversion spectroscope was preset to a suitable wavelength and the time required after illumination until the bands came into coincidence was noted. This apparatus was exactly similar in principle to the apparatus Porter and Norrish used for flash photolysis. The light sources and the methods of observation available were, however, simply not adequate to permit study of haemoglobin–ligand reactions other than the replacement of oxygen by carbon monoxide. Although apparently unpublished, a further approach to the haemoglobin–ligand reaction problem by Hartridge & Roughton may be thought of as anticipating the well-known temperature jump method. They intended to prepare two solutions of oxygen and haemoglobin with the same haem and ligand concentrations but at different temperatures. On mixing, oxygen and haemoglobin would not be at equilibrium at the temperature of the mixture, but would relax towards equilibrium. It was intended to complete the cycle by dividing the effluent from the observation tube and pass it through two coils, one heating and one cooling, in a continuous circulation of the reagents. Given the methods of observation available, it is not likely that good results could have been achieved. In all the foregoing work there was a division of labour: the design and construction of the apparatus was due to Hartridge who built much of it with his own hands. The physicochemical side of things was Roughton’s especial province.

Roughton was concerned with two other types of apparatus development. One was the speeding up of manometric measurement to improve the assay of
carbonic anhydrase and to allow study of the exchange of carbon dioxide between blood and gas. The conventional method of attaching the flask and manometer rigidly together and shaking the whole was abandoned. Instead, the manometer was fixed firmly in place and the manometric flask, which was connected to it by rubber tubing, was shaken vigorously, improving the rate of gas exchange across the gas-liquid interface by a factor of 10 or so as compared with a conventional Warburg manometer. His other interest was in developing methods for extending the capabilities of the Van Slyke–Neill blood gas analysis apparatus. This involved the design and construction of special tonometers for determining points at the extremes of the oxygen and carbon monoxide equilibrium curves. With Pete Scholander he introduced the Scholander–Roughton syringe capillary which permitted accurate (±2%) analysis of small (10 μl) gas bubbles obtained from the Van Slyke apparatus.

The first paper on the oxygen equilibrium curve did not appear until 1931, when Forbes & Roughton published an accurate curve for sheep haemoglobin using the Van Slyke–Neill apparatus. The analysis of their results included the first hint of a two-state model for the haemoglobin–oxygen reaction. . . . since the results in the present paper do not conform to this equation, the Langmuir principle [of independence of sites] cannot apply to all four of the intermediate reactions, but if it be assumed to apply only to the first three intermediate reactions (i.e. \( K_1 : K_2 : K_3 : 4 : \frac{3}{2} : \frac{3}{8} \)), then with only two unknown constants \( K_4 \) and \( K_5 \), good agreement can be obtained between experiment and theory.

The next papers in 1942 and 1944 dealt with the effects of methaemoglobin and carboxyhaemoglobin on the position and form of the oxygen equilibrium curve, which was shown to be shifted to the left and to be less inflected as compared with normal haemoglobin. The equilibrium curve was discussed further in a theoretical paper presented at the Barcroft Memorial Conference. The guidelines were laid down for an attempt to determine uniquely the four constants required by the Adair equation, and in the succeeding years the programme was translated into action in a series of papers culminating in the 1955 paper with Otis and Lyster. These papers were all concerned with sheep haemoglobin, which is technically easy to deal with because of its relatively low affinity for oxygen. Extension to human and horse haemoglobins provided a difficult thesis problem for R. L. J. Lyster, who, though he survived the ordeal, did not work further with haemoglobin. Further extensions were made to dilute solutions of sheep haemoglobin, and human haemoglobin under physiological conditions. In later work with Fatt at Berkeley and Severinghaus at San Francisco, new electrode methods were used to supplement classical gasometry. This body of work probably represents the best that can be done with gasometric methods. Unfortunately, almost all of the work was carried out before the discovery of the effect of 2,3-diphosphoglycerate on the oxygen equilibrium of haemoglobins, and, in addition, the methods were so time-consuming that the stability of the haemoglobin can scarcely be regarded as assured over the period of an experiment. Nevertheless, the work seems unlikely to be superseded in the near future.
Turning to the kinetics of haemoglobin–ligand reactions, the replacement of oxygen by carbon monoxide was studied first, using the photochemical methods already mentioned. The model used for treating the results was not suitable, since it was supposed that the reactions of oxygen with COHb and of CO with Hb were second order. A return to this problem was made in 1934, when a treatment of the replacement reaction was given in terms of the Adair scheme. Experiments were made on a range of concentrations of reagents, and the results were shown to be inconsistent with the Hill equation. A final attack on the replacement reaction was made with Gibson in 1955 when the results were clearly expressed in terms of the velocity constant $k_4$, the rate constant for the dissociation of the first molecule of oxygen from saturated haemoglobin, and the treatment was extended to other ligands in 1957. Except for inclusion of the effects of $\alpha, \beta$ chain differences, the theory then given is current, and the only modification which may perhaps be required is in respect of the assumption that the concentration of species having more than one free gas-binding group is negligible. It may be noted, however, that unexplained anomalies in the replacement reactions of myoglobin have been observed.

The first experiments with the flow method defined the rate of deoxygenation of haemoglobin in the presence of dithionite, and the velocity of combination of deoxyhaemoglobin with oxygen. Both reactions were found to be rapid as compared with the transit time of an erythrocyte through a capillary, thus answering an important physiological question. In this case, Roughton did not re-examine the reactions extensively in later work, though a few measurements were reported by Legge & Roughton in 1950, and considerable effort was invested in joint experiments with Gibson, which, however, remained unpublished because the results could not readily be reconciled with equilibrium experiments on similar solutions.

The carbon monoxide binding reaction was examined early on, but the results were not published until 1934. They included the important finding that, over a 33-fold range of carbon monoxide concentrations, in agreement with the Adair scheme, the rate of CO binding was proportional to its concentration rather than to its 2.5th power as required by Hill’s equation. The reaction was studied further in 1935 by the thermal method, and the first indications appeared that the reaction might speed up as it proceeded, though the point was not noted in print until the data of Bateman & Roughton were re-examined by Roughton in 1948. The carbon monoxide reaction was studied further by the stopped flow method with Gibson, and an attempt was made in 1957 to assign values of the four successive rate constants called for by the Adair scheme. The computational problems were very severe, and it now seems that the values found were not unique minima in the parameter/residual surface. In the light of more recent work it appears likely that the solution given at pH 9.1 is correct, but that for pH 7.1 is probably an alternative set of constants giving a good fit to experiment, but without physical meaning. Roughton, in fact, was always attracted by arithmetical operations and liked to fit experimental data to appropriate schemes. His ostensible object was to extract from the numbers information about the
functional behaviour of haemoglobin, but he was to some extent fascinated by the numbers for their own sake. It was a great misfortune that, born too soon for the computer, his operations were restricted to the capacity of a desk-top rotary calculator. Although he did have expert help and access to modern computers in the U.S. in later years, he never had an opportunity for direct personal work with a computer of his own.

Miscellaneous work on ligand-binding kinetics included studies on the reactions of nitric oxide and of isocyanides with haemoglobin and myoglobin.

The kinetics of ligand exchange in erythrocytes is a logical extension of work with haemoglobin solutions, and the matter was taken in hand early in Hartridge & Roughton’s partnership. Their first paper established that the rate of uptake of oxygen by corpuscles was substantially slower than the rate of reaction of haemoglobin with oxygen in solution under comparable conditions, and that this difference in rate could not be accounted for by insufficiently vigorous stirring of the reaction mixture. It was therefore to be attributed to diffusion limitation within the corpuscle, to the effects of the red cell membrane, or to some combination of these. In the next paper (1932) Roughton made calculations on the effect of diffusion, and obtained upper and lower limit solutions which suggested that diffusion within the erythrocyte was scarcely sufficient to account for the slow-down in ligand binding in cells as compared with solution, and that a contribution was probably made by the red cell membrane. Nicolson & Roughton (1951) returned to the problem, giving solutions for the case of carbon monoxide reacting with haemoglobin contained in a slab bounded by membranes of defined permeability, using experimental data obtained by Legge & Roughton with a photoelectric continuous-flow apparatus. The new work suggested a highly significant role for the cell membrane, and also that its permeability might vary considerably with change in physiological conditions, since corpuscles from pregnant ewes showed a large membrane effect, while corpuscles from rams had little, if any. The blood for these experiments was supplied by Sir Joseph Barcroft, and it happened that Barcroft and Roughton spent a part of the morning of the day of Barcroft’s death in discussing the results. Roughton has given an account of this discussion in which arrangements were made for further supplies of blood from the ewes after delivery, in the Barcroft Memorial Symposium, but the planned experiments were never completed.

There followed a theoretical paper on diffusion into spheres and cylinders, and in 1956 with Klug & Kreuzer, a definitive attempt to take account of diffusion and chemical reaction in an artificial system without membranes in which the depth of the solution was such that both diffusion and the chemical reaction had a major effect in determining the course of the observed reaction. The treatment included allowance for the influence of haemoglobin diffusion as well as ligand diffusion. New measurements were also made of the diffusion of gases in haemoglobin solutions of various strengths. The problems of diffusion and reaction continued to occupy Roughton’s attention, and a new photoelectric flow apparatus was set up based on the dual wavelength principle to reduce interference due to scattering of light by the cell suspension. Data were obtained
for oxygen, carbon monoxide, and nitric oxide—the last being especially valuable
in that the rate of chemical reaction is so high as to eliminate chemistry as a
factor in determining the rate of uptake by the erythrocytes. Much of the new
work was undertaken in collaboration with R. E. Forster of the University of
Pennsylvania, and a review was given in 1959 in Progress in Biophysics and Bio-
physical Chemistry. In the most recent work the role of red cell membrane con-
tinues to be in doubt, and the investigations of Roughton and his associates,
extensive and successful as they were, have not wholly closed this chapter of work
with blood.

The last area in which Roughton made major contributions was in relation
to carbon dioxide transport. Here, there was already a substantial body of physico-
chemical information, and a fully developed theory which seemed to account
for the facts in terms of carbon dioxide, carbonic acid and bicarbonate ion. These,
together with the buffering power of haemoglobin, its change in pK on
ligand binding and the chloride shift, appeared to constitute a complete and
internally consistent system. The element of time, however, did not enter into
the classical carbon dioxide transport scheme which was derived entirely from
equilibrium experiments, and the matter does not seem to have been considered
before 1928, although Thiel (1913) and Faurholt (1925) had shown that the
reaction \( \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_2\text{O} + \text{CO}_2 \) does not quickly reach equilibrium. In 1928,
Henriques calculated that about 15% of the bicarbonate in the venous blood
would dissociate to carbon dioxide in the time of passage of a corpuscle through
a capillary (about 1 s), and therefore undertook an investigation of the rate at
which carbon dioxide was set free from blood on shaking violently with a gas
phase. He found that, whereas plasma released carbon dioxide at much the rate
to be expected from Faurholt's experiments, the rate of release from whole
blood was biphasic and much higher. Of the two main explanations, the presence
of a catalyst or the formation of a compound directly with haemoglobin (carb-
aminoohaemoglobin), Henriques, quite reasonably, preferred the second. Other
authors soon showed that a catalyst was present, since an accelerating effect on
the reaction could be observed with blood of haemoglobin solutions diluted
20 000-fold. They attributed the catalytic effect to haemoglobin itself.

It was at this point that Roughton entered the picture, showing first that the
ratio of catalytic activity to haemoglobin concentration varied from one species
to another, and with Meldrum, separating the catalytic activity from haemo-
globin by alcohol : chloroform precipitation of haemoglobin. The name adopted
for the new enzyme, carbonic anhydrase, was suggested by Phillip Eggleton.
The way was open for new kinetic experiments similar to those of Henriques
in which the enzyme activity, which had been shown to be sensitive to cyanide,
could be suppressed at will. It was now found that in the absence of inhibitor
the release of carbon dioxide proceeded in a uniform manner and at a high
speed, and that, on adding cyanide, the reaction became biphasic as had
originally been observed by Henriques. It thus seemed that Henriques must
have had an inhibitor in his solutions or had succeeded in some way in removing
carbonic anhydrase from them.
The size of the rapid phase was considerably greater than could be accounted for by carbon dioxide in physical solution, and, following Henriques, was attributed to carbaminohaemoglobin. Roughton followed through by showing that Henriques calculations were incorrect and that the uncatalysed reaction would probably account for only about some 2% of carbon dioxide transport, so underlining the importance of carbonic anhydrase.

The significance of carbaminohaemoglobin continued to be a matter of controversy for many years, in part because of the lack of convincingly specific methods for its determination and in part because non-specific experiments using such procedures as osmotic pressure determination yielded equivocal results. Roughton, however, persevered with the problem and returned to it in earnest in the last 15 years of his life when, with Luigi Rossi-Bernardi of Milan, he examined many aspects of the carbaminohaemoglobin problem. Their work finally established not only the existence of carbaminohaemoglobin, but the nature of the groups involved in CO₂ binding, the relative affinity of the α and β chains, and the interrelation between carbon dioxide and 2,3-diphosphoglycerate binding.

There remain for mention the miscellaneous papers deriving chiefly from the wartime research period at the Harvard Fatigue Laboratory. These were concerned with immediately practical matters of carbon monoxide toxicity such as the rate of uptake and elimination of the gas and its determination in small amounts in blood and in air.

To prepare this memoir, it was necessary to read or reread a number of Roughton’s papers. Although many of them may be regarded as belonging to the field of physical chemistry, and have been treated as such, it seems that Roughton was really a physiologist at heart. His papers were not planned because the work was technically feasible, but were intended to answer definite questions with physiological meaning, and to introduce quantitative rigour to the subject where this was possible.

Several papers were apparently intended to be the first in a series, since they were labelled as such in the title but no later members ever appeared. It is not now possible to say why this should have been so, but it is tempting to speculate. It may have been associated with Roughton’s view of himself and of his relation to science. He was perhaps among the later members of the line of Cambridge men to whom science was not a profession but a hobby. His position in society and his self-regard were not bound up with success in science, since these had been established by his being a Wykehamist, a Fellow of Trinity, and financially independent. In science, then, he was an amateur, working to produce carefully written papers for their own sake. Thus he felt little compulsion to complete or to publish (save perhaps when he was up for election to the Royal Society), and was not even importantly dependent on the whims of grant-giving bodies. He belonged indeed to an earlier and happier age of science. He was aware of this, and remarked on several occasions on his good fortune in living largely before the age of Grant Q. Swinger (of the Institute for the Absorption of Federal Funds) and of professionalized science. It followed that he made little attempt to
publicize his work, and though often engaged in controversy, he was content to publish his findings, avoiding the rancour and acrimony so common in scientific debate today.

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