Glutathione
Author(s): N. W. Pirie
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Braunstein, A. E., Goryachenkova, E. V. & Pashkina, T. S. 1949 *Biokhimiya*, **14**, 163: *Chem. Abstracts* **43**, 6264d.
Butenandt, A., Weidel, W. & Becker, E. 1940 *Naturwissenschaften*, **28**, 63.
Butenandt, A., Weidel, W. & Schlossberger, H. 1949 *Z. Naturforsch.* **4***b*, 242.
Caspari, E. 1943 *Science*, **98**, 478.
Dalgleish, C. E. 1951 *Quart. Rev. Chem. Soc.* **5**, 227.
Dalgleish, C. E., Knox, W. E. & Neuberger, A. 1951 *Nature, Lond.* **168**, 20.
Dawes, E. A., Dawson, J. & Happold, F. C. 1947 *Biochem. J.* **41**, 436.
Dawes, E. A. & Happold, F. C. 1949 *Biochem. J.* **44**, 349.
Evans, W. C., Handley, W. R. C. & Happold, F. C. 1941 *Biochem. J.* **35**, 207.
Ellinger, A. & Flamand, C. 1907 *Ber. dtsch. Chem. Ges.* **40**, 3029.
Ephrussi, B. 1942 *Cold Spr. Harb. Symp. Quant. Biol.* **10**, 40.
Happold, F. C. & Hoyle, L. 1935 *Biochem. J.* **29**, 1918.
Happold, F. C. & Hoyle, L. 1936 *Brit. J. Exp. Path.* **17**, 136.
Harris, L. J. 1949 *Hopkins & biochemistry*, p. 39. Edited by J. Needham and E. Baldwin. Cambridge University Press.
Haskins, F. A. & Mitchell, M. K. 1949 *Proc. Nat. Acad. Sci., Wash.*, **35**, 500.
Hopkins, F. G. 1906 *Analyst*, **31**, 385.
Hopkins, F. G. 1913 *Rep. Brit. Ass.*, p. 652.
Hopkins, F. G. & Cole, S. W. 1901 *J. Physiol.* **27**, 418.
Hopkins, F. G. & Cole, S. W. 1903 *J. Physiol.* **29**, 451.
Jakoby, W. B. & Bonner, D. M. 1953 *J. Biol. Chem.* **205**, 699.
Knox, W. E. & Mehler, A. H. 1950 *J. Biol. Chem.* **187**, 419.
Knox, W. E. 1958 *Proc. Colloquia 4th Int. Congr. of Biochemistry, Vienna.*
Krehl, W. A., Tepley, L. J., Sarma, P. S. & Elvehjem, C. A. 1945 *Science*, **101**, 489.
Kuhne, W. 1875 *Ber. dtsch. chem. Ges.* **8**, 206.
Kuss, E. 1959 Ph.D. Thesis, University of Munich.
Lester, C. 1961 *J. Bact.* **81**, 964.
Lester, G. & Yanofsky, C. 1961 *J. Bact.* **81**, 81.
Mehler, A. H. & Knox, W. E. 1950 *J. Biol. Chem.* **187**, 431.
Monod, J. & Cohen-Bazire, G. 1953 *C.R. Acad. Sci., Paris*, **236**, 530.
Scott, T. A. 1960 Ph.D. Thesis, University of Leeds.
Wilcock, E. G. & Hopkins, F. G. 1906 *J. Physiol.* **35**, 88.
Wiss, O. 1952 *Z. Naturforsch.* **7***b*, 133.
Wood, W. A., Gunsalus, E. C. & Umbreit, W. W. 1947 *J. Biol. Chem.* **170**, 313.
Woods, D. D. 1935 *Biochem. J.* **29**, 640, 649.
Yanofsky, C. 1960 *Bact. Rev.* **24**, 221.

3. Glutathione

By N. W. Pirie, F.R.S.
Rothamsted Experimental Station

Just before the First World War, Hopkins was interested in the part that keto-acids might play in muscular contraction and used Rothera's test in a semi-quantitative way. With characteristic candour he mentions (Hopkins 1921) that he did not realize at first that the colour was being given by a mercaptan, though his early training as an analyst in the Government Chemist's laboratory must have made him thoroughly familiar with that application of sodium nitroprusside. Then Arnold (1911) claimed that there was free cysteine in many tissues and this

brought to Hopkins's attention Heffter's (1908) observations on the general distribution in tissues of substances reacting with nitroprusside. A widely distributed reactive substance able to 'play a real part in cell dynamics' was precisely what Hopkins's outlook on biochemistry at that date demanded. He greatly enlarged the list of tissues giving the nitroprusside reaction and methodically set about the isolation of the material responsible. The sequence in which reagents were used varied but the basic principles of the purification procedure depended on precipitation from a boiling water extract with lead, mercury, and copper, and removal of contaminants with uranium and phosphotungstic acid.

Many years earlier, de Rey-Pailhade had published a series of papers on the reduction of elementary sulphur to H_2S by tissue extracts. He gave the name 'philothion' to the hypothetical substance responsible for this phenomenon and attributed to it varied and often improbable properties. When Hopkins called the substance he had isolated 'glutathione' he was influenced by the earlier name, although it was the reaction with sulphur that justified the *-θεῖον* root in the first name and the presence of sulphur in the molecule that justified it in the second. There is no logical justification for the *-one* termination and it would probably not be accepted by a modern editor. Hopkins said he was influenced by the analogy with peptone and with the earlier name (philothion); he may also have been influenced by the fact that he had initially been looking for a ketone. The name was at one time criticized; but some casualness in the application of the conventional rules is not uncommon in biochemistry. Vitamin B_2, for example, continues to be called ribo- instead of ribi-flavine.

Glutathione yielded glutamic acid and cystine (or cysteine) on hydrolysis but no other amino acid was found. It could be argued that Hopkins was insufficiently assiduous in the search and that he should have determined which of the three possible constitutions the supposed dipeptide had. But these were points with which, rightly, he was not greatly concerned. He had a substance and knew enough about its structure and properties to be able to think of it in relation to metabolic processes; for him biochemistry was not the study of the structure of substances but of their activities in tissues. Nearly half of the first paper (Hopkins 1921) is devoted to discussion about, and experiments on, oxidations, reductions and the part that an —SH to —SS— reaction may be playing in them, both *in vivo* and *in vitro*.

Any qualms Hopkins may have had about the constitution were removed by the claim (Stewart & Tunnicliffe 1925) that glutamyl cysteine had been synthesized and was identical with glutathione, so that when Hunter & Eagles (1927) suggested that serine was present as well he (Hopkins 1927) wrote vigorously in defence of what he was later (Hopkins 1929) to call 'My junior Colleagues'—supposed accomplishment'. Hunter & Eagles did not isolate serine and they concluded that GSH contained two free amino groups; nevertheless, as Hopkins (1929) clearly states, their work raised uncertainties in his mind. These uncertainties were fanned by widespread scepticism, or even ribaldry, in the laboratory about the 'synthesis' and by increasing doubts about the validity of van Slyke amino-N determinations on cysteine and glutamic acid and their peptides. The observation that glutathione,

when boiled in water at its own pH, yielded glycine-cysteine diketopiperazine (Hopkins 1929) made the presence of glycine very probable and improvements in the method of preparation gave consistently crystalline products having the analytical composition, equivalent weight, and other properties of a tripeptide containing glutamic acid, cysteine and glycine.

Hopkins got a more spacious and adequately endowed laboratory in 1923 so that more abundant supplies of glutathione could be made. Furthermore, the introduction of cuprous precipitation made the process of isolation simpler and much more reliable. This precipitation has many points of interest. Carius (1862) made cuprous mercaptides from di- and tri-thioglycerine; Klason (1877) made some others and realized that cupric copper was reduced by part of the mercaptan and then precipitated by the remainder. Morner (1904), Friedmann (1903), and others used cuprous copper to precipitate mercaptans but the general value of the reaction was not, and still is not, recognized. Most text books mention the etymologically obvious precipitation with mercury although that metal is notoriously unspecific. Hopkins (1929) recognized that the copper compound made in the earlier (1921) method was cuprous and that its use, though it entailed the loss of some glutathione, was an important step in the purification because the silky sheen of the precipitate shows that it is microcrystalline. He therefore used a suspension of Cu_2O in water as precipitant and so recovered glutathione without loss. Care has to be taken, for cysteine peptides make soluble complexes with any excess of Cu_2O (Pirie 1931) and the precipitate can disappear with disconcerting suddenness, presumably because other components of tissue extracts stabilize some cuprous copper in solution even in the presence of the 0·5 N-H_2SO_4 from which the glutathione is being precipitated. By aeration some of the cuprous glutathione can be recovered and it can all be recovered by complexing the excess Cu with carbon monoxide (Pirie 1931) but, until these tricks were discovered, we often lost valuable quantities of material through failure to hit the end-point precisely. A third factor leading to increased supplies was the introduction during 1929 of a simpler method (Pirie 1930) for making the initial yeast extract; this enabled us to process 20 to 50 kg of pressed yeast each week. In the original method, yeast was boiled in water; this was slow, troublesome, extracted polysaccharides and other materials that often made filtration difficult, and of necessity gave a dilute extract. By killing the yeast with a mixture of alcohol, ether and sulphuric acid the cells themselves act as semipermeable membranes and the extract is so free from other components that it can be precipitated directly with Cu_2O. From being a rarity, glutathione became one of the easier substances of biochemical importance to isolate and, from 1930, its isolation was a routine part of the advanced course in biochemistry. Independently Kendall, McKenzie & Mason (1929) used benzene to liberate glutathione from yeast, but this has not proved so successful and our method is still the one generally used. It has even been patented (Rapkine 1945).

Harington & Mead (1935) established by synthesis that glutathione was γ-glutamylcysteinylglycine; a structure that, more by luck than logic, had been chosen as the most probable by Pirie & Pinhey (1929) from a consideration of the pK values, and by Nicolet (1930) and Kendall, Mason & McKenzie (1930) from the

action of various reagents on glutathione. The constitution was settled before other aspects of biochemistry, and more especially peptide chemistry, were so far advanced as to make this knowledge useful. The initial period of confusion had therefore little lasting effect and the only serious loss was the time that Voss, Guttmann & Klemm (1930) devoted to fifteen attempts to repeat the early 'synthesis'.

Two explanations of the presence of large amounts of glutathione in many tissues came immediately to mind without invoking more teleology than is normally tolerated: it could be a resistant end-product that tissues had difficulty disposing of, or it could be conserved or synthesized as an essential intermediate in metabolism. The ready oxidation to sulphate of glutathione that was fed to or injected into dogs (Hele & Pirie 1931) appears to exclude the first possibility; Hopkins had an intuitive liking for the second and the evidence that has accumulated since justifies it. Modern work on the part that glutathione plays in the action of glyoxalase, formaldehyde dehydrogenase, maleylacetoacetate isomerase and a bizarre enzyme reaction with nitroglycerine need not be discussed here; such actions have been fully discussed in recent symposia (Colowick *et al.* 1954; Crook 1959). It will suffice to recall Hopkins's own work on the subject.

The basic fact was established in Hopkins's paper in 1921; glutathione undergoes oxidation and reduction in biologically significant circumstances. We may now regard methylene blue and washed tissues, or even solvent-extracted and dried tissues, as rather artificial analogues for biochemistry, but it was a bold beginning. With Dixon (Hopkins & Dixon 1922) these studies were extended and this phase of his work finished with an investigation (Hopkins 1925) of the catalytic role of glutathione in the uptake of oxygen by linoleic and linolenic acids. This paper also contains the interesting observation that glutathione will elicit an —SH reaction from some proteins that do not show the reaction after simple heat denaturation.

The next phase was an examination of the substrates that normally maintain glutathione in the reduced state. Hopkins & Elliot (1931) found that the glutathione in an aerated suspension of liver pulp from rabbits and rats began to oxidize after an interval that was diminished if the animal had been starved beforehand. Liver from starved cats, on the other hand, retained the ability to maintain —SH, and even to reduce added oxidised glutathione. This species difference was not pursued—there is in this paper a characteristic personal comment 'we have not cared to work with dogs intentionally deprived of food'.

Two papers followed (Hopkins & Morgan 1936; Crook & Hopkins 1938) on the relations between glutathione and ascorbic acid. In the presence of various plant extracts, containing dehydrascorbic acid reductase and ascorbic acid oxidase, the ascorbic system catalyzes the oxidation of glutathione by O_2 and at the same time helps to protect ascorbic acid from oxidation. During the germination of pea seeds, soluble —SH compounds appear and disappear as growth proceeds. Structural integrity is not necessary for this appearance and disappearance; it can also be demonstrated with pea flour. Hopkins & Morgan (1943) found similar changes in the seeds of a dozen plants and showed, by isolation from germinating peas, that

glutathione was the —SH compound that was appearing. They did not, however, show whether it arose by synthesis or by reduction of the oxidized form. Two years later (Hopkins & Morgan 1945) they isolated glutathione from earthworms, locusts, broad bean and vegetable marrow. They comment here that some insect extracts are troublesome because purines precipitate with the Cu_2O. This trouble is occasionally encountered with yeast, but only when such an excess of Cu_2O has been added that most of the mercaptide has redissolved.

One interpretation of the role of glutathione is that it establishes a generally reducing environment in which enzymes can maintain functional —SH groups; this has been aptly called the 'euphoristic theory'. It was foreshadowed by Hopkins in his early studies on 'fixed —SH group' and explored more fully (Hopkins & Morgan 1938; Hopkins, Morgan & Lutwak-Mann 1938) with succinic dehydrogenase: activity is abolished by oxidized glutathione and restored by reduced glutathione. The role of glutathione as a coenzyme of glyoxalase is well known, though the function of the enzyme remains obscure; Hopkins & Morgan (1945) argued from its very wide distribution that it probably has a function. They found glyoxalase in all but one of sixteen invertebrates, from eight orders, in moulds, red and green algae and many higher plants. It is apparently absent from brown algae and conifers. Hopkins's last paper (Hopkins & Morgan 1948) described a new factor in the glyoxalase reaction and forms the basis of our present picture (Racker 1951) of the three steps in the reaction: the hemimercaptal of methyl glyoxal and glutathione is formed non-enzymically and is then rearranged by glyoxalase I to S-lactoylglutathione which is hydrolyzed by glyoxalase II to lactic acid and glutathione. The new factor was glyoxalase II.

Cysteine, ergothionine, and other mercaptans are the only substances likely to contaminate glutathione when it is isolated by means of its cuprous mercaptide and these potential contaminants are not present in significant quantity in yeast, liver, and the other materials from which it is usually isolated. For many years therefore the peptide was assumed to be unique, but chromatographic techniques show that the assumption is unfounded. Calf lens contains both the thiosulphate corresponding to glutathione (Waley 1959) and tripeptides in which cysteine is replaced by α-aminobutyric acid or by alanine (Waley 1957). Other similar peptides are also present. The concentration of glutathione is 10 to 1000 times greater than that of these other substances; it appears therefore to be the dominant member of a family of γ-glutamyl peptides. There is as yet no definite evidence about their function or whether they arise as stages in protein metabolism, as antagonists in actions involving glutathione, or as aberrations in its metabolism. Several less closely related γ-glutamyl peptides occur in plants (Carnegie 1961).

Hopkins was a biologist who saw that biology had to be interpreted in molecular terms—there is no other basis for a scientific interpretation of the whole of it. He studied glutathione during 30 years because it fitted his outlook perfectly and was an ideal example of the *tertium quid* that he often invoked as an essential part of biochemical processes. He summed up this outlook as the study of 'simple substances undergoing comprehensible reactions'. As time passes and techniques improve, our standards of simplicity alter, but his conception of biochemistry

remains and he would have been at a loss to understand the reason for later attempts to isolate certain aspects of the subject under the label 'molecular biology'. In the nineteen twenties technique was not adequate to make meaningful chemical statements about molecules much larger than tripeptides and he was at first troubled as evidence accumulated that some proteins were 1000 times bigger than glutathione. He feared a return of old vague concepts such as inogen and protoplasm and he was highly sceptical of attempts to deduce protein structure by the principles of pure reason or numerology. Towards the end of his life it became possible to make increasingly concrete statements about the structure of proteins and their behaviour in the cell, so that there is now a substantially unbroken sequence of biochemical experience reaching from glutathione with which he worked for so much of his life to the proteins that he did so much to characterize more than 60 years ago.

References (Pirie)

Arnold, V. 1911 *Z. physiol. Chem.* **70**, 300.
Carius, L. 1862 *Liebigs Ann.* **124**, 238.
Carnegie, P. R. 1961 *Biochem. J.* **78**, 697.
Colowick, S., Lazarow, A., Racker, E., Schwarz, D. R., Stadtman, E. & Waelsch, H. 1954 (Eds.) *Glutathione*. New York: Academic Press.
Crook, E. M. 1959 (Ed.) '*Glutathione*' *Biochem. Soc. Symp.*, p. 17.
Crook, E. M. & Hopkins, F. G. 1938 *Biochem. J.* **32**, 1356.
Friedmann, E. 1903 *Hofmeisters Beiträge*, **3**, 1.
Harington, C. R. & Mead, T. H. 1935 *Biochem. J.* **29**, 1602.
Heffter, A. 1908 *Med. naturwiss. Arch.* **1**, 82.
Hele, T. S. & Pirie, N. W. 1931 *Biochem. J.* **25**, 1095.
Hopkins, F. G. 1921 *Biochem. J.* **15**, 286.
Hopkins, F. G. 1925 *Biochem. J.* **19**, 787.
Hopkins, F. G. 1927 *J. Biol. Chem.* **72**, 185.
Hopkins, F. G. 1929 *J. Biol. Chem.* **84**, 269.
Hopkins, F. G. & Dixon, M. 1922 *J. Biol. Chem.* **54**, 527.
Hopkins, F. G. & Elliott, K. A. C. 1931 *Proc. Roy. Soc.* B, **109**, 58.
Hopkins, F. G. & Morgan, E. J. 1936 *Biochem. J.* **30**, 1446.
Hopkins, F. G. & Morgan, E. J. 1938 *Biochem. J.* **32**, 611.
Hopkins, F. G. & Morgan, E. J. 1943 *Nature, Lond.* **152**, 288.
Hopkins, F. G. & Morgan, E. J. 1945 *Biochem. J.* **39**, 320.
Hopkins, F. G. & Morgan, E. J. 1948 *Biochem. J.* **42**, 23.
Hopkins, F. G., Morgan, E. J. & Lutwak-Mann, C. 1938 *Biochem. J.* **32**, 1829.
Hunter, G. & Eagles, B. H. 1927 *J. Biol. Chem.* **72**, 147.
Kendall, E. C., McKenzie, B. F. & Mason, H. L. 1929 *J. Biol. Chem.* **84**, 657.
Kendall, E. C., Mason, H. L. & McKenzie, B. F. 1930 *J. Biol. Chem.* **88**, 409.
Klason, P. 1877 *Liebigs Ann.* **187**, 120.
Morner, K. A. H. 1904 *Z. physiol. Chem.* **42**, 351.
Nicolet, B. H. 1930 *J. Biol. Chem.* **88**, 389.
Pirie, N. W. 1930 *Biochem. J.* **24**, 51.
Pirie, N. W. 1931 *Biochem. J.* **25**, 614.
Pirie, N. W. & Pinhey, K. G. 1929 *J. Biol. Chem.* **84**, 321.
Racker, E. 1951 *J. Biol. Chem.* **190**, 685.
Rapkine, L. 1945 U.S. Patent 2,376,186.
Stewart, C. P. & Tunnicliffe, H. E. 1925 *Biochem. J.* **19**, 207.
Voss, W., Guttman, R. & Klemm, L. 1930 *Biochem. Z.* **220**, 327.
Waley, S. G. 1957 *Biochem. J.* **67**, 172.
Waley, S. G. 1959 *Biochem. J.* **71**, 132.