Biochem. J. (1989) 264, 625–632 (Printed in Great Britain)

REVIEW ARTICLE

Sulphane sulphur in biological systems: a possible regulatory role

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INTRODUCTION

The labile sulphane sulphur atom has been shown to have effects in biochemical systems which suggest that it may have natural regulatory functions. This conclusion is supported by evidence that sulphane sulphur is generated by known metabolic pathways, that carrier proteins for stabilizing and transporting it are widely distributed, that it is effective *in vitro* at very low concentration in regulating the activities of many enzymes, and that malignant cells with multiple defects in sulphur metabolism require this form of sulphur for proliferation *in vitro* [1]. This evidence is reviewed here along with a growing body of evidence relating defective sulphur metabolism to cancer, virus infection, and immunodeficiency.

THE NATURE OF SULPHANE SULPHUR

'Sulphane' is a relatively new name for compounds containing sulphur-bonded sulphur [2]. In many sulphane compounds, the sulphur is labile, readily coming out of the structure to react with cyanide ion, to deposit as elemental sulphur, or to be reduced to H_2S by reducing agents. The following labile sulphane compounds have been shown to be involved in biochemical systems: elemental sulphur (S_8), hydrodisulphides frequently called persulphides ($R-S_2H$), polysulphides ($R-S_n-R$) where n must be 3 or greater, polythionates ($^-SO_3-S_n-SO_3^-$), thiosulphate ($S_2O_3^{2-}$), and thiosulphonates ($R-S_2O_2^-$). Another class of compounds, although structurally dissimilar to the above, contains sulphur atoms with properties very similar to the above. These are disulphides with a double-bonded carbon adjacent to the sulphur-bonded carbon. The double-bonded carbon can be part of the following groups: ene, as in alkenyl disulphides ($R-S-S-CH_2-CH=CH-R$); carbonyl, as in disulphides of β-mercaptopyruvate ($R-S-S-CH_2-CO-CO_2H$) or 2-mercaptoacetaldehyde ($R-S-S-CH_2-COH$) (specific examples cited below); or enol, as in disulphides of 3-mercaptopropionaldehyde ($R-S-S-CH_2-CH=CH-OH$).

The most characteristic reaction of sulphane sulphur is its combination with alkaline cyanide ion to form thiocyanate. This reaction is given by all of the classes of compounds named above and is the basis of a quantitative analysis [3]. Thiosulphate and thiosulphonates require heating for reaction with CN^- under standard conditions [3] but heating is not required for thiosulphate if Cu^{2+} ion is present [4]. Under the alkaline conditions of the cyanolysis reaction, the singlet sulphur atoms of polythionates ($^-SO_3-S_n-SO_3^-$) react quantitatively with CN^- when n is 1 or greater [4] but at pH near 7 in biochemical systems, the sulphur is labile only when n is 3 or greater [5]. Disulphides of 2-mercaptoacetaldehyde (RS = 2-mercaptoethanol, 2-mercaptoethylamine, or 1-thioglycerol) [1] or β-mercaptopyruvate (RS = cysteine or β-mercaptopyruvate) contain cyanolysable sulphur, but the sulphur in the reduced mercaptans is not cyanolysable (J. Toohey, unpublished work). Triphenylphosphine ($phenyl_3$-P) behaves like CN^- in abstracting the labile sulphur from sulphane compounds and is frequently used as a test reagent [6–8].

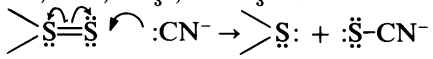
Rapid deposition of elemental sulphur at neutral pH indicates especially labile sulphur and is characteristic of allyl disulphides [7], hydrodisulphides [9], and polysulphides [10]. The latter two structures are more stable in strong acid or alkali (see preparative methods below) or when part of a protein molecule (see carriers below). Polythionates with $n \geqslant 3$ deposit elemental sulphur in a complex reaction catalysed by hydroxyl ion, and thiosulphate decomposes in weakly acidic solution with the outer sulphur atom giving rise to elemental sulphur (reviewed in [11], pp. 20–34).

Sulphur is released as H_2S from all labile sulphane compounds in the presence of excess thiol such as GSH or cysteine [12]. This is the basis of a quantitative analysis which is 10-fold more sensitive than the cyanolysis method [12]. Although the sulphur of reduced β-mercaptopyruvate is not cyanolysable (see above), it is quantitatively released as H_2S by excess thiol [12].

The lability of sulphane sulphur appears to be due to its presence in the thiosulphoxide form, -S(S)-, either by primary structure, as in thiosulphate and thiosulphonates, or by tautomerization. Thiosulphoxide tautomerization and its probable role in labilizing the sulphur atom have been reviewed by Kutney & Turnbull [8]. Tautomerization of allyl disulphides is well documented, $R-S-S-CH_2-CH=CH_2 \rightleftharpoons R-S(S)-CH_2-CH=CH_2$, and the thiosulphoxide tautomer is believed to be an intermediate in many reactions involving the labile sulphur of allyl disulphides [7,13,14]. A similar mechanism undoubtedly occurs in disulphides with carbonyl or enol groups in place of the ene group. These groups are known to labilize adjacent C-S bonds [15,16] and, like the ene group, they probably promote thiosulphoxide tautomerization of disulphides. This process appears to be essential for making the sulphur cyanolysable as evidenced by the acquisition of cyanolysability when β-mercaptopyruvate is converted to the disulphide; only the latter can give the -S(S)- form. This tautomerization can also occur in polysulphides and the lability of the sulphur in polysulphides with $n \geqslant 3$ compared with the stability of dialkyldisulphides ($n = 2$) again reflects the capacity to tautomerize, $R-S-S-S-R \rightleftharpoons R-S(S)-S-R$ [8].

Sulphane sulphur has an apparent oxidation state of zero (6 electrons) in contrast to sulphide sulphur (R-S-R, 8 electrons, 2^-). In the biochemical literature, this unusual property has been represented by the symbol,

S^0, [17–19], e.g. $S^0 + CN^- \rightarrow SCN^-$ [19], and the sulphur has been incorrectly called 'zero valence sulphur' [18,20] (sulphur does not exist as a single atom and, therefore, always has some valence). This property of sulphane sulphur is attributable to the thiosulphoxide isomer which provides a structure from which the sulphur atom is readily removed with 6 electrons by nucleophilic groups such as CN^-, RS^-, SO_3^-, or Ph_3-P:



FORMATION OF SULPHANE SULPHUR

Chemical methods

For demonstrating short-term effects of sulphane sulphur in test systems, hydrodisulphides or polysulphides ($\lambda_{max.} = 335$ nm) can be obtained in low yield and impure form by the following simple procedures: by shaking elemental sulphur in solutions of NaHS [21], thiols [21,22], or proteins [23,24] ($R\text{-}SH + S_8 \rightarrow R\text{-}S\text{-}SH$); by adding Na_2S to alkaline solutions of disulphides such as cystine [25,26] or proteins [27] ($R\text{-}S\text{-}S\text{-}R + S^{2-} \rightarrow R\text{-}S\text{-}S^- + R\text{-}S^-$); by heating G-S-S-G with Fe^{3+} ion [18]; or by heating cystine with Cu^{2+} ion in alkaline solution [28]. Preparative chemical methods for obtaining specific hydrodisulphides, polysulphides, and polythionates in pure form are reviewed in detail by Roy & Trudinger ([11], pp. 43–58) and by Feher [29].

Cell-compatible generating systems

Preformed sulphane compounds are not suitable for adding to most biological systems because they decompose so rapidly at neutral pH that they do not have time to be effective. The following systems generate sulphane sulphur at very low rates for several days and are suitable for addition to biological systems such as cell cultures [1]. Three of the sulphane-generating systems described below involve disulphides in processes which

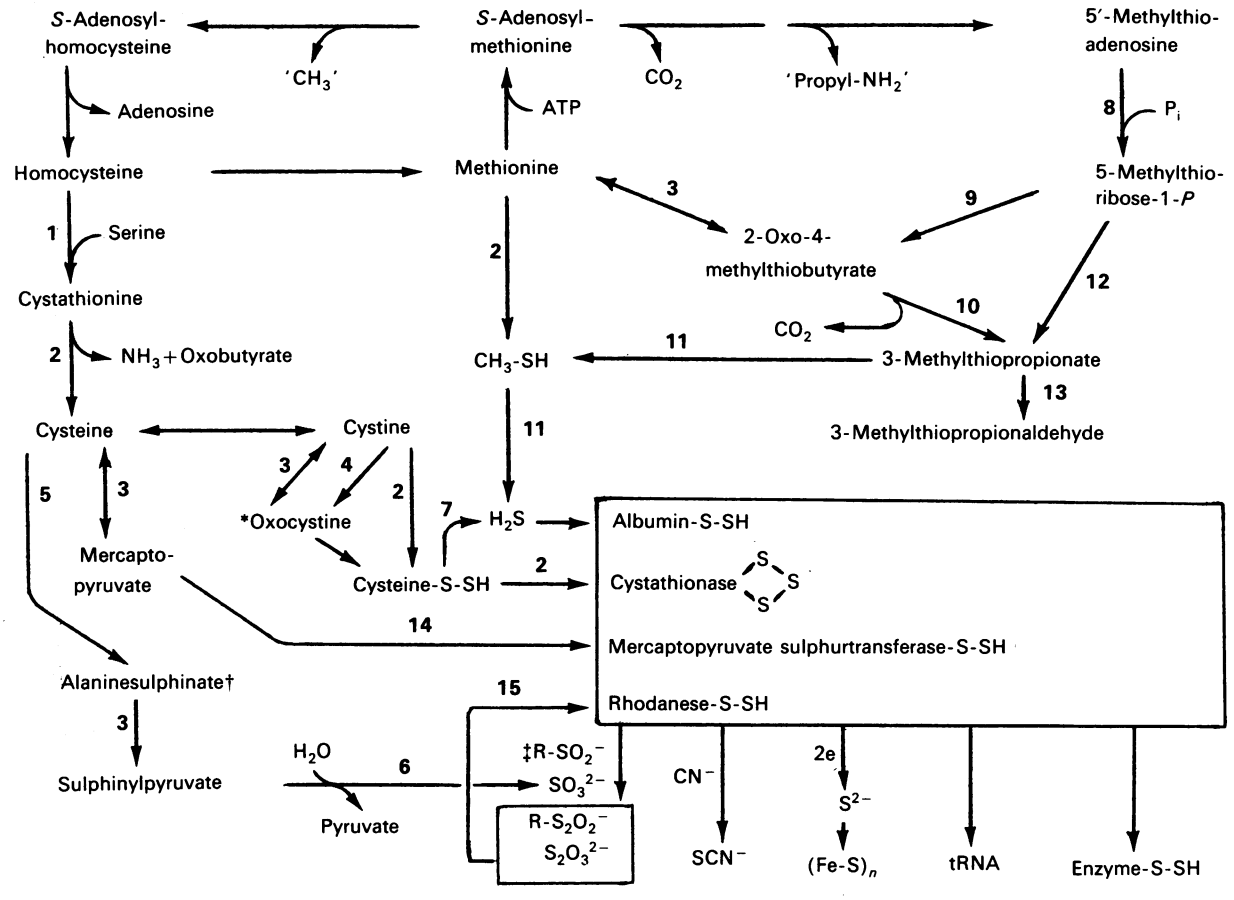


Fig. 1. Sulphur metabolism related to sulphane sulphur

The primary sources of sulphane sulphur in mammals, methionine, cysteine, and cystine, are metabolized as shown to give the immediate sources, β-mercaptopyruvate, cysteine hydrodisulphide, hydrogen sulphide, thiosulphate, or thiosulphonates. The latter react with carrier proteins to give protein-bound sulphane sulphur (large box). Within the protein-bound pool, sulphane sulphur can undergo some interchange between the various carriers (see [63]). The metabolic distribution from the protein-bound pool is shown at lower right. The pathways have been demonstrated in mammalian systems except as noted below. **1**, cystathionine synthetase; **2**, cystathionase; **3**, amino acid:oxo acid aminotransferase; **4**, L-amino acid oxidase; **5**, cysteine dioxygenase; **6**, spontaneous hydrolysis [16]; **7**, reduction at the expense of intracellular cysteine or GSH [49]; **8**, 5'-methylthioadenosine phosphorylase; **9**, multi-step [51,120]; **10**, see [52]; **11**, products identified, details unknown [53,54]; **12**, multi-step in bacterial system [55]; **13**, product identified in yeast system, details unknown [56]; **14**, mercaptopyruvate sulphurtransferase; **15**, rhodanese. Notes: *, Cysteine-mercaptopyruvate disulphide; †, 3-sulphinoalanine, frequently called cysteine sulphinate; ‡, the principal physiological sulphinate is alaninesulphinate.

cause formation of a carbonyl or enol group at the carbon adjacent to the C–S bond. The kinetics of sulphane sulphur formation in each system are easily followed by the cyanolysis or diffusion assays [1].

Pyridoxal catalyses the non-enzymic β-elimination reaction of cystine yielding cysteine hydrodisulphide, pyruvate, and ammonia [30]. The effectiveness of pyridoxal increases from pH 7 with increasing pH; pyridoxal phosphate is also effective with a pH optimum at 7 but pyridoxine and pyridoxamine are ineffective (J. Toohey, unpublished work). A system suitable for generating sulphane sulphur in cell cultures contains 1 mM-cystine and 50 μM-pyridoxal [1].

Diamine oxidase catalyses the monoxidation of cystamine, $(NH_2-CH_2-CH_2-S-)_2$, [31,32] and the resulting aldehyde contains labile sulphane sulphur [1,33]. Diamine oxidase is widely-distributed in tissues [34] and blood [32] and it is not necessary to add additional enzyme to systems containing serum [1]. The optimum concentration of cystamine in cell cultures is 10 μM [1].

The disulphides of 2-mercaptoethanol, 3-mercaptopropanol, and 1-thioglycerol are good substrates for hydroxy dehydrogenases and the resulting aldehydic disulphides contain labile sulphane sulphur [1]. For cell culture systems, the optimum concentration of mercaptoalcohol disulphide is 1 μM [1].

A relatively stable and non-toxic source of sulphane sulphur for biological systems is a sulphide-treated protein [27] such as serum albumin or egg globulin [1]. It is usually necessary to add a thiol, e.g. 0.5 mM-GSH, to make the protein-bound sulphur available. Sulphide-treated proteins can be replaced in this system by fetal serum but not by other commercial sera [1].

Metabolic pathways

Several metabolic pathways are known to generate sulphane sulphur *in vitro* but their relative importance *in vivo* is not known. γ-Cystathionase catalyses the β-elimination reaction of cystine described above [35,36]. Inhibition studies with propargylglycine in intact rat hepatocytes indicate that 50% of cyst(e)ine catabolism occurs via this pathway [37]. In addition, cystine is a highly effective substrate for mammalian transaminase [38] and for L-amino acid oxidase [39,40] and the resulting oxo acid, cysteine-mercaptopyruvate disulphide, contains cyanolysable sulphur (J. Toohey, unpublished work). Cysteine is also a substrate for mammalian transaminases [12,41,42] and the lability of the sulphur in the mercaptopyruvate product was recognized in the 1950's [21]. Homocysteine is a weak substrate for mammalian transaminase [38] and non-mammalian L-amino acid oxidases [43,44] and there is evidence that the sulphur in the imine intermediate [44] or in the oxo product [43] is labile.

Thiosulphate, alanine thiosulphonate, and thiotaurine are formed *in vitro* by enzymic systems, the sulphane sulphur originating in mercaptopyruvate [45], cysteine hydrodisulphide [35], or cystamine [46]. Thiosulphate is a normal constituent of urine [47] and these compounds may simply be excretory products; however, through rhodanese (see below), they may serve as relatively stable sources of sulphane sulphur.

Hydrosulphide ion (HS^-) is converted to sulphane sulphur by reacting with disulphide groups of proteins:

$$\underline{\text{S—S}} + HS^- \rightarrow {}^-\text{S—S-SH} \text{ [27]}$$

Possible sources of hydrosulphide ion in mammals include bacterial metabolism in the digestive tract [48], desulphydration of cyst(e)ine by cystathionase [49], and catabolism of methionine. Formation of H_2S from methionine occurs in mammalian systems by a complex pathway which is not completely characterized. Methionine is first converted to 2-oxo-4-methylthiobutyric acid either directly or by transamination (reviewed in [50], see also [38]) or indirectly through 5′-methylthioadenosine [51]. The oxo acid has been shown to be degraded in a rat liver system in a sequence which involves decarboxylation to 3-methylthiopropionate [52] and cleavage to CH_3-SH and H_2S [53, 54]. In a bacterial system, 3-methylthiopropionate is formed from methylthioadenosine without going through the oxomethylthiobutyrate intermediate [55] and in a yeast system 3-methylthiopropionate appears to be converted to 3-methylthiopropionaldehyde [56]. The latter has an enol group adjacent to a C–S bond and is a likely substrate for the facile release of CH_3-SH. CH_3-SH has been presumed to be the precursor of H_2S in the mammalian system [54] but the details are not known. CH_3-SH can be released directly from methionine by cystathionase [57]. It, or its disulphide, has been identified as a normal constituent of human breath [58] and urine [59,60] and of fetal serum [61].

SULPHANE SULPHUR CARRIER PROTEINS

Related to the metabolic pathways for generating sulphane sulphur, there are specialized carrier proteins which transport the sulphur to various acceptors. A stable protein–sulphur complex has been demonstrated for each of the carriers. Although their functions *in vivo* were obscure at the time of their discovery, they have recently been shown to have functions of possible physiological importance.

Rhodanese

First described in 1933 as thiosulphate:cyanide sulphurtransferase, this enzyme is widely distributed in animal tissues, plants, and bacteria (reviewed in [11], pp. 190–204). It has 293 amino acid residues and carries sulphane sulphur as the hydrodisulphide at Cys-247 [62]. It has many functions in the metabolism of sulphane sulphur: it transfers the outer sulphur atom of thiosulphate and thiosulphonates to CN^-; coupled to cystathionase, it transfers the sulphur of cystine to CN^- [63]; it has thiosulphate synthetase activity, transferring the sulphur from hydrodisulphides and thiosulphonates to sulphite; in the presence of dihydrolipoate, it has thiosulphate reductase activity, $S_2O_3^{2-} \rightarrow H_2S + SO_3^-$ [26]; and it introduces the sulphur into the apoenzymes of many Fe–S cluster enzymes (see below).

Mercaptopyruvate sulphurtransferase

This copper-containing enzyme is widely distributed in animal tissues (reviewed in [11], pp. 204–206). It was originally shown to transfer the sulphur of mercaptopyruvate to itself (giving elemental sulphur) or to cyanide, sulphite, or sulphinates. Recently, it has been shown to transfer this sulphur to the apoenzyme of adrenodoxin [64] and possibly to tRNA (see below).

Cystathionase

Besides its function in generating sulphane sulphur (as

described above), cystathionase also serves as a carrier [65]. The sulphur is thought to be carried as a trisulphide between two cysteine residues [65]. It is transferred to several other enzymes, activating some and inactivating others (see below).

Other proteins

Serum albumin binds elemental sulphur and catalyses its transfer to CN^- [23]. The latter activity was attributed to a distinct enzyme, called rhodanese S, until its identity with albumin was established. Westley's group found that, out of 12 diverse proteins tested, only serum albumin bound elemental sulphur [24]. Szczepkowski showed that the outer sulphur of thiosulphate is bound by serum albumin while the inner sulphur is not [66].

Sulphane sulphur binds to other proteins, such as cytochrome [67] or ferredoxin [17], for which no specific carrier function is known. For example, when ferredoxin or other Fe–S protein is exposed to oxygen, the iron-bound S^{2-} is oxidized to the S^0 state and binds to the protein as a trisulphide [17].

EFFECTS OF SULPHANE SULPHUR IN BIOLOGICAL SYSTEMS

Effects in cell cultures

Several malignant lymphoid cells which are defective in methylthioadenosine phosphorylase [68] and cystathionase [69] require a source of sulphane sulphur for proliferation *in vitro* [1]. The sulphur can be provided by the slow *in situ* alteration of disulphides of cysteine, mercaptoalcohols, or cysteamine (as described above) or by the combination of thiols (unrelated to the above disulphides) with sulphide-treated proteins or fetal calf serum [1,61,70–72]. In the latter system, the thiol (e.g. GSH) must be in the reduced form; its effect is abolished by agents, such as cobalamins, which promote its oxidation [71] and greatly enhanced by metal chelators which retard its oxidation [73]. The defective cells can also obtain the essential factor from feeder cells [61], accounting for their ability to proliferate *in vivo*. Supraoptimal concentrations of the sulphane-generating systems are toxic and cause formation of giant cells (Fig. 2).

The sulphane-dependent cells are malignant lymphoblasts or plasma cells. Many other cells, although not absolutely dependent, are markedly stimulated *in vitro* by sulphane-generating systems. These cells include normal T and B lymphocytes, normal bone marrow stem cells, and malignant human lymphocytes and plasma cells (reviewed in [61]). Some malignant human lymphocytes that are stimulated to proliferate *in vitro* by the sulphur systems [74] have been shown to be deficient in cystathionase [75].

Activation of enzymes

Sulphane sulphur appears to be the physiological source of the sulphur in the iron–sulphur cluster enzymes. Rhodanese, with thiosulphate and a reducing agent, has been shown to introduce sulphur into the apoenzymes of plant ferredoxin [76], bacterial ferredoxin [77], bacterial nitrogenase [78], mammalian succinic dehydrogenase [79], mammalian NADH dehydrogenase [80], and plant

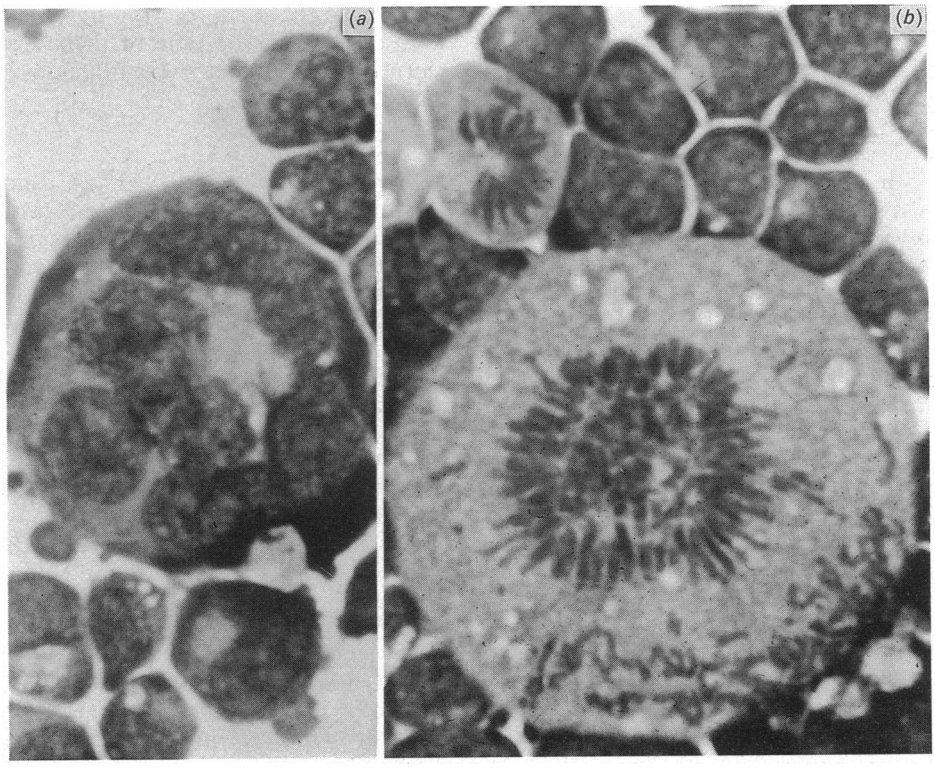


Fig. 2. Giant cells caused by supraoptimal concentration of sulphane sulphur

(*a*) Giant cell and normal-sized murine leukemia cells from culture *in vitro*; (*b*) giant cell and normal cell in mitosis. This effect is produced by slightly excessive concentrations of any of the cell-compatible sulphane-generating systems described in the text. See also [152,153]. Reproduced from [1] with permission.

nitrate reductase [81]. Since the sulphur in the Fe–S clusters is a sulphide ion, the sulphane sulphur must be reduced during incorporation, possibly involving the reductase activity of rhodanese [26]. Mercaptopyruvate sulphur transferase, with mercaptopyruvate, has been shown to introduce sulphur into the apoprotein of adrenodoxin [64].

The molybdenum-containing hydroxylases, xanthine oxidase, xanthine dehydrogenase and aldehyde oxidase, contain cyanolysable sulphur in addition to the acid-labile sulphide of the Fe–S chromophore [82,83]. There are two theories about the nature of the cyanolysable sulphur, one that it is a persulphide of a protein SH group [82,83] and one that it is terminally linked to Mo with a structure resonating between Mo(VI)–S^{2-} and Mo(IV)–S^0 [19,84].

5-Aminolaevulinate synthetase from *Rhodopseudomonas spheroides* is activated by trisulphides (Cy–S–S–S–Cy or G–S–S–S–G) [5], polythionates with $n \geqslant 3$ [5], or cystine + cystathionase [85]. All of these factors were identified in cell extracts, indicating that they may function as natural activators. The mechanism of activation is thought to involve formation of a hydrodisulphide or trisulphide group on the enzyme. Incubation of mammalian malate dehydrogenase with rhodanese and thiosulphate or elemental sulphur resulted in increased activity and incorporation of sulphane sulphur into the enzyme, possibly as a hydrodisulphide group [20]. When adenylate kinase, inactivated by elemental sulphur as described below, was treated with dithiothreitol, the resulting enzyme activity was many-fold greater than that of the untreated enzyme or of the enzyme treated with dithiothreitol alone, suggesting a sulphur-mediated activation process [90].

Inhibition of enzymes

Kato *et al.* first observed that rat liver serine dehydratase was inhibited by cystine + cystathionase or by elemental sulphur [86]. Since then, many enzymes have been shown to be inactivated by sulphane-generating systems or by elemental sulphur, including rat liver homoserine dehydratase [87], bacterial 3-hydroxybutyrate dehydrogenase [87], yeast alcohol dehydrogenase [87], rat liver tyrosine aminotransferase [88], rat liver ornithine decarboxylase [89], and certain isoenzymes of mammalian adenylate kinase [90,91]. The inhibition is reversible by thiols (GSH or dithiothreitol) and is believed to involve formation of a hydrodisulphide group on the enzyme; see the reaction of S_8 with protein-SH groups discussed above [17,20,23,24].

When intact red blood cells or cell homogenates were exposed to the sulphane-generating system cystine + pyridoxal phosphate, or to mercaptopyruvate, fourteen enzymes were markedly inhibited while eight others were not [92]. Many enzymes have been reported to be inhibited by garlic extracts (reviewed in [93]) and, in an extensive survey, allicin (*S*-oxodiallyl disulphide), the active principle of garlic, was found to inhibit markedly fourteen enzymes while fifteen others were unaffected [93]. When thione compounds (X=S), e.g. carbon disulphide or parathion, are metabolized *in vivo* or *in vitro* by mixed-function oxidase, the sulphur is released from the substrate and becomes attached in a cyanolysable form to cytochrome *P*-450 with concomitant inactivation of the enzyme (reviewed in [94]). This effect is mimicked by benzyl hydrodisulphide [94] and is believed to be due in part to formation of hydrodisulphide groups on the enzyme.

Sulphuration of tRNA

Thiopyrimidines and methylthiopurines are normal constituents of tRNA in all organisms and are thought to have important regulatory functions in translation [95]. The introduction of sulphur is a posttranscriptional modification of tRNA. Enzyme preparations from rat brain [96], liver [97], and hepatoma [97] transfer the sulphur from [^{35}S]mercaptopyruvate to tRNA. The systems require ATP and Mg^{2+} and are specific for preformed tRNA. Tumours with inherently high growth rates may have lower activities of the sulphurtransferase than normal tissue or tumours with low growth rates [97]. A similar enzyme purified 150-fold from *Bacillus subtilis* used cysteine as substrate 350-fold less efficiently than it used mercaptopyruvate [98]. A related enzyme from *Escherichia coli* has been reported to require very small amounts of mercaptopyruvate along with [^{35}S]cysteine as substrate [99]; however, the requirement for pyridoxal phosphate [99,100], the inhibition by amino acids, and the activation by oxo acids other than mercaptopyruvate [100] indicate that the true sulphur donor is mercaptopyruvate formed from the labelled cysteine by transamination. There is some evidence that methylthiolation of adenine in tRNA involves initial introduction of sulphur followed by methylation of the sulphur [101].

Immune enhancement

2-Mercaptoethanol and 1-thioglycerol have been used widely in immune systems *in vitro* because of their marked stimulatory effects (literature cited in [61]). These effects appear to be attributable to the formation *in situ* of sulphane sulphur from the disulphides of these compounds [1]. Diethyldithiocarbamate [$(CH_3-CH_2)_2$–N–C(S)–SH; Imunothiol] has immune-enhancing properties *in vivo* in mice [102] and humans [103]. This compound is metabolized *in vivo* to carbon disulphide (reviewed in [104]) and CS_2 is metabolized to sulphane sulphur (discussed above, see [94]). Therefore, a metabolic pathway exists for generating sulphane sulphur from diethyldithiocarbamate and this is the probable mechanism of the immune-enhancing effects of this drug and its therapeutic effects in AIDS [105].

Other effects of sulphane sulphur

Cystine trisulphide, G–S–S–S–G, or elemental sulphur catalyse the non-enzymic reduction of cytochrome *c* by GSH [18]. Glutathione hydrodisulphide [106] and polythionates [107] are intermediates in the oxidation of elemental sulphur to sulphate by the sulphur and iron-utilizing bacteria, respectively.

Potency of sulphane sulphur

A remarkable feature of sulphane sulphur in biological systems is the very low and narrow concentration range at which it is effective. In cultures of dependent cells, the concentration of sulphane sulphur in the medium at any time, based on kinetic considerations, was less than 1 nM [1]. Adenylate kinase was inhibited by nanomolar concentrations of ethanol-dissolved elemental sulphur [90]. Serine dehydratase and homoserine dehydratase were 100% inhibited by elemental sulphur at concentrations of 1 μM and 0.5 μM, respectively [86]. In the sulphuration

of tRNA with enzymes from rat brain and *B. subtilis*, the K_m for mercaptopyruvate was 7 μM [96,98]; the limited kinetic data published on the *E. coli* enzyme ([99], Table V) show that 0.1 μM-mercaptopyruvate was optimal. Polysulphides and polythionates were effective in activating aminolaevulinate synthetase at 0.5–3 μM [5]. In many of these systems, slightly supraoptimal concentrations of the sulphane compounds were inhibitory [1,5,99]. Because sulphane sulphur is effective at such low and narrow concentration limits, its effects in other systems could easily go undetected.

ALTERED SULPHUR METABOLISM ASSOCIATED WITH CELLULAR PROCESSES

The requirement for sulphane sulphur in enzyme-deficient cells suggests that methylthioadenosine phosphorylase and cystathionase are normally involved in generating sulphane sulphur and that it has an essential physiological role. This is further substantiated by a growing body of evidence that these and other sulphur-related enzymes undergo altered activity physiologically during normal cellular processes and pathologically in disease states. In synchronously-dividing cells, it has been clearly shown that there are two distinct peaks of rapid methylthioadenosine formation [108–110] and phosphorolysis [111] during each cell cycle. Striking biphasic changes in the level of KI_3 or Ag-reactive groups (assumed to be SH groups) occur during mitosis in sea urchin eggs [112,113] and meiosis in plants [114]. The variable component in sea urchin eggs has been shown not to be GSH [113,115]. Its reactivity with cyanide has not been reported.

On the pathological side, there is increasing evidence relating acquired defects in sulphur metabolism to malignancy, virus infection, and immunodeficiency. Methylthioadenosine phosphorylase is absent from some malignant human cells *in vivo* [116–118]. Methylthioadenosine is excreted in the urine of children with severe combined immunodeficiency [119]. An enzyme involved in converting methylthioadenosine to oxomethylthiobutyric acid is absent from a human colon carcinoma cell line [120].

Cystathioninuria occurs in a high percentage of patients with neuroblastoma [121–125] and hepatoma [126,127]. Based on this, Klein *et al.* analysed 27 human neuroblastoma tissue specimens for cystathionase and found that neither enzyme activity nor immunologically-reactive protein was detectable in any of the specimens [128]. This enzyme is deficient in many other malignant cells ([75,128,129], and references cited therein) and the deficiency is usually correlated with a requirement *in vitro* for cystine [129] or a source of sulphane sulphur [74]. Virus-induced malignant transformation of both human and murine cells has been shown to be associated with changes in sulphur metabolism, including deficiency of cystathionase [69], inability to use homocysteine in place of cystine [69], and induction of methionine auxotrophy [130,131].

There are many reports showing that malignant cells cannot use homocysteine in place of methionine *in vitro* whereas normal cells can (reviewed in [132]); however, other work has shown that exceptions to these generalizations occur in both categories of cells [133,134]. *S*-Adenosylmethionine:homocysteine methyltransferase is absent from rat hepatoma cells [135].

Several reports demonstrate remarkable anti-cancer effects of sulphane sulphur compounds or progenitors. Treatment with 2-mercapto-3-hydroxypropionaldehyde (a labile sulphur compound) produced greatly prolonged survival and some complete remissions of several transplanted tumours in mice [136]. Apffel *et al.* reported high percentages of complete remission and cure of three transplanted tumours in mice following treatment with cyst(e)amine or derivatives of it [137]. (The thiol, cysteamine, is rapidly oxidized to the disulphide, cystamine, in the presence of oxygen.) 2,3-Dimercaptopropanol [138,139] and cyst(e)amine [140,141] have been shown to inhibit tumour induction by carcinogens. Allyl di- or trisulphides or extracts of plants of the genus *Allium*, which contain allyl or other polysulphides [142,143], have been shown to inhibit the induction of tumours by carcinogens [144–147], the development of spontaneous mammary tumours in C3H mice [148], and the growth of transplanted tumours in mice [149,150]. There is epidemiological evidence that dietary ingestion of *Allium* vegetables (garlic, onions, scallions, chives) is associated with reduced incidence of stomach cancer in humans [151].

The observations on defective sulphur metabolism in cancer cells and the anti-cancer effects *in vivo* of sources of sulphane sulphur suggest that malignant proliferation of cells may be related to a deficiency of sulphane sulphur and the uncontrolled operation of a set of enzymes normally inactivated by sulphane sulphur. On the other hand, circumstantial evidence suggests that overproduction of sulphane sulphur may occur in virus-infected cells. Thus, the multinucleated giant cells produced in cell cultures containing supraoptimal concentrations of sulphane sulphur sources (Fig. 2; [152,153]) are very similar to the giant cells induced by many viruses *in vitro* and *in vivo* [154]. The mechanism by which viruses cause formation of giant cells is not known [154] but the findings reviewed here suggest that it may involve accelerated production of sulphane sulphur induced by the virus, possibly as a mechanism for inactivating a specific set of host cell enzymes.

In summary, sulphane sulphur is generated by known metabolic pathways and has effects in biological systems which suggest that it has a natural regulatory function. Its properties of very high potency and short half-life are consistent with a role as a finely-tuned regulator.

REFERENCES

1. Toohey, J. I. (1986) Biochem. Cell Biol. **64**, 758–765
2. Cahn, R. S. & Dermer, O. C. (1979) in Introduction to Chemical Nomenclature, 5th edn., p. 124, Butterworth, London and Boston
3. Wood, J. L. (1987) Methods Enzymol. **143**, 25–29
4. Sorbo, B. (1957) Biochim. Biophys. Acta **23**, 412–416
5. Sandy, J. D., Davies, R. C. & Neuberger, A. (1975) Biochem. J. **150**, 245–257
6. Challenger, F. & Greenwood, D. (1950) J. Chem. Soc. 26–30
7. Hoffle, G. & Baldwin, J. E. (1971) J. Am. Chem. Soc. **93**, 6307–6308
8. Kutney, G. & Turnbull, K. (1982) Chem. Rev. **82**, 333–357
9. Wood, J. L. (1982) Adv. Exp. Med. Biol. **148**, 327–341
10. Abdobrasulnia, R. & Wood, J. L. (1980) Bioorg. Chem. **9**, 253–260

11. Roy, A. B. & Trudinger, P. A. (1970) The Biochemistry of Inorganic Compounds of Sulphur, Cambridge University Press, Cambridge
12. Toohey, J. I. (1983) Arch. Biochem. Biophys. **223**, 533–542
13. Baechler, R. D., Daley, S. K., Daly, B. & McGlynn, K. (1978) Tetrahedron Lett. **2**, 105–108
14. Baechler, R. D., Hummel, J. P. & Mislow, K. (1973) J. Am. Chem. Soc. **95**, 4442–4444
15. Nicolet, B. H. (1931) J. Am. Chem. Soc. **53**, 3066–3072
16. Kearney, E. B. & Singer, T. P. (1953) Biochim. Biophys. Acta **11**, 276–289
17. Petering, D., Fee, J. A. & Palmer, G. (1971) J. Biol. Chem. **246**, 643–653
18. Massey, V., Williams, C. H. & Palmer, G. (1971) Biochem. Biophys. Res. Commun. **42**, 730–738
19. Wahl, R. C. & Rajagopalan, K. V. (1982) J. Biol. Chem. **257**, 1354–1359
20. Agro, A. F., Mavelli, I., Cannella, C. & Federici, G. (1976) Biochem. Biophys. Res. Commun. **68**, 553–558
21. Hylin, J. W. & Wood, J. L. (1959) J. Biol. Chem. **234**, 2141–2144
22. Fletcher, J. C. & Robson, A. (1963) Biochem. J. **87**, 553–559
23. Sorbo, B. (1955) Acta Chem. Scand. **9**, 1656–1660
24. Vennesland, B. V., Castric, P. A., Conn, E. E., Solomonson, L. P., Volini, M. & Westley, J. (1982) Fed. Proc. Fed. Am. Soc. Exp. Biol. **41**, 2639–2648
25. Rao, G. S. & Gorrin, G. (1959) J. Org. Chem. **24**, 749–752
26. Villarejo, M. & Westley, J. (1963) J. Biol. Chem. **238**, 4016–4020
27. Cavallini, D., Federici, G. & Barboni, E. (1970) Eur. J. Biochem. **14**, 169–174
28. de Marco, C., Coletta, M. & Cavallini, D. (1963) Arch. Biochem. Biophys. **100**, 51–55
29. Feher, F. (1975) in Handbook of Preparative Inorganic Chemistry (Brauer, G., ed.) pp. 356–441, Ferdinand Enke Verlag, Stuttgart
30. Cavallini, D., de Marco, C. & Mondovi, B. (1960) Arch. Biochem. Biophys. **87**, 281–288
31. Cavallini, D., de Marco, C. & Mondovi, B. (1956) Experientia **12**, 377–379
32. Bergeret, B. & Blaschko, H. (1957) Br. J. Pharmacol. **12**, 513–516
33. de Marco, C., Bombardieri, F., Riva, F., Dupre, S. & Cavallini, D. (1965) Biochim. Biophys. Acta **100**, 89–97
34. Zeller, E. A. (1963) Enzymes **8**, 313–316
35. Cavallini, D., de Marco, C., Mondovi, B. & Mori, B. G. (1960) Enzymologia **22**, 161–173
36. Flavin, M. (1962) J. Biol. Chem. **237**, 768–777
37. Drake, M. R., de la Rose, J. & Stipanuk, M. H. (1987) Biochem. J. **244**, 279–286
38. Ricci, G., Nardini, M., Federici, G. & Cavallini, D. (1986) Eur. J. Biochem. **157**, 57–63
39. Ricci, G., Federici, G., Achilli, M., Matarese, R. M. & Cavallini, D. (1981) Physiol. Chem. Phys. **13**, 341–346
40. Ubuka, T., Ishimoto, Y. & Kasahara, K. (1975) Anal. Biochem. **67**, 66–73
41. Cammarata, P. S. & Cohen, P. P. (1950) J. Biol. Chem. **187**, 439–451
42. Ubuka, T., Ishimoto, Y. & Akagi, R. (1981) Inherited Metab. Dis. **4**, 65–66
43. Chen, S. S., Walgate, J. H. & Duerre, J. A. (1971) Arch. Biochem. Biophys. **146**, 54–63
44. Cooper, A. J. L. (1983) Annu. Rev. Biochem. **52**, 210–211
45. Sorbo, B. (1957) Biochim. Biophys. Acta **24**, 324–329
46. Cavallini, D., de Marco, C. & Mondovi, B. (1961) Enzymologia **23**, 101–110
47. Gast, J. H., Arai, K. & Aldrich, F. L. (1952) J. Biol. Chem. **196**, 875–884
48. Kadota, H. & Ishida, Y. (1972) Annu. Rev. Microbiol. **26**, 127–137
49. Cavallini, D., Mondovi, B., de Marco, C. & Sciosa-Santoro, A. (1962) Enzymologia **24**, 253–266
50. Benevenga, N. J. (1984) Adv. Nutr. Res. **6**, 1–18
51. Backlund, P. S. & Smith, R. A. (1981) J. Biol. Chem. **256**, 1533–1535
52. Steele, R. D. & Benevenga, N. J. (1978) J. Biol. Chem. **253**, 7844–7850
53. Blom, H. J., van den Elsen, J. P., Yap, S. H. & Tangerman, A. (1988) Biochim. Biophys. Acta **972**, 131–136
54. Steele, R. D. & Benevenga, N. J. (1979) J. Biol. Chem. **254**, 8885–8890
55. Furfine, E. S. & Abeles, R. H. (1988) J. Biol. Chem. **263**, 9598–9606
56. Marchitto, K. S. & Ferro, A. J. (1985) J. Gen. Microbiol. **131**, 2153–2164
57. Binkley, R. (1951) J. Biol. Chem. **192**, 209–211
58. Chen, S., Zieve, L. & Mahadevan, V. (1970) J. Lab. Clin. Med. **75**, 628–635
59. Zlatkis, A. & Liebich, H. M. (1971) Clin. Med. **17**, 592–594
60. Matsumoto, K. E., Partridge, D. H., Robinson,. A. B., Pauling, L., Flath, R. A., Mon, T. R. & Teranishi, R. (1973) J. Chromatogr. **85**, 31–34
61. Toohey, J. I. (1981) J. Supramol. Struct. Cell. Biochem. **17**, 11–25
62. Ploegman, J. H., Drent, G., Kalk, K. H., Hol, W. G. J., Neinrikson, R. L., Keim, P., Weng, L. & Russell, J. (1978) Nature (London) **273**, 124–129
63. Szczepkowksi, T. W. & Wood, J. L. (1967) Biochim. Biophys. Acta **139**, 469–478
64. Taniguchi, T. & Kimura, T. (1974) Biochim. Biophys. Acta **364**, 284–295
65. Yamanishi, T. & Tuboi, S. (1981) J. Biochem. (Tokyo) **89**, 1913–1921
66. Szczepkowski, T. W. (1963) Acta Chem. Scand. **17** Suppl., 180–183
67. Catignani, G. L. & Neal, R. A. (1975) Biochem. Biophys. Res. Commun. **65**, 629–636
68. Toohey, J. I. (1978) Biochem. Biophys. Res. Commun. **83**, 27–35
69. Livingston, D. M., Ferguson, C., Gollogly, R. & Lazarus, H. (1976) Cell **7**, 41–47
70. Broome, J. D. & Jeng, M. W. (1973) J. Exp. Med. **138**, 574–592
71. Toohey, J. I. (1975) Proc. Natl. Acad. Sci. U.S.A. **72**, 73–77
72. Toohey, J. I. & Cline, M. J. (1976) Biochem. Biophys. Res. Commun. **70**, 1275–1282
73. Mohindru, A., Fisher, J. M. & Rabinovitz, M. (1985) Experientia **41**, 1064–1066
74. Epstein, A. L. & Kaplan, H. S. (1979) Cancer Res. **39**, 1748–1759
75. Glode, L. M., Epstein, A. & Smith, C. G. (1981) Cancer Res. **41**, 2249–2254
76. Pagani, S., Bonomi, F. & Cerletti, P. (1984) Eur. J. Biochem. **142**, 361–366
77. Bonomi, R., Pagani, S. & Kurtz, D. M. (1985) Eur. J. Biochem. **148**, 67–73
78. Pagani, S., Eldridge, M. & Eady, R. R. (1987) Biochem. J. **244**, 485–488
79. Bonomi, F., Pagani, S., Cerletti, P. & Cannella, C. (1977) Eur. J. Biochem. **72**, 17–24
80. Pagani, S. & Galante, Y. M. (1983) Biochim. Biophys. Acta **742**, 278–284
81. Tomati, U., Sermanni, G., Dupre, S. & Cannella, C. (1976) Phytochemistry **15**, 597–598
82. Branzoli, U. & Massey, V. (1974) J. Biol. Chem. **249**, 4346–4349

83. Massey, V. & Edmonson, D. (1970) J. Biol. Chem. **245**, 6595–6598
84. Gutteridge, S., Tanner, S. J. & Bray, R. C. (1978) Biochem. J. **175**, 887–897
85. Yamanishi, T., Kubota, I. & Tuboi, S. (1983) J. Biochem. (Tokyo) **94**, 181–188
86. Kato, A., Ogura, M. & Suda, M. (1966) J. Biochem. (Tokyo) **59**, 40–48
87. Pestana, A. & Sols, A. (1970) Biochem. Biophys. Res. Commun. **39**, 522–529
88. Hargrove, J. L. & Wichman, R. D. (1987) J. Biol. Chem. **262**, 7351–7357
89. Murakami, Y., Kameji, T. & Hayashi, S. (1984) Biochem. J. **217**, 573–580
90. Conner, J. & Russell, P. J. (1983) Biochem. Biophys. Res. Commun. **113**, 348–352
91. Russell, P. J., Conner, J. & Sisson, S. (1984) Clin. Chem. **30**, 1555–1557
92. Valentine, W. N., Toohey, J. I., Paglia, D. E., Nakatani, M. & Brockway, R. A. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 1394–1398
93. Wills, E. D. (1956) Biochem. J. **63**, 514–519
94. Sawahata, T. & Neal, R. A. (1982) Mol. Pharmacol. **21**, 464–467
95. Ajitkumar, P. & Cherayil, J. D. (1988) Microbiol. Rev. **52**, 103–113
96. Wong, T., Harris, M. A. & Jankowicz, C. A. (1974) Biochemistry **13**, 2805–2812
97. Wong, T., Harris, M. A. & Morris, H. P. (1975) Biochem. Biophys. Res. Commun. **65**, 1137–1144
98. Wong, T., Weiss, S. B., Eliceiri, G. L. & Bryant, J. (1970) Biochemistry **9**, 2376–2386
99. Lipsett, M. N., Norton, J. S. & Peterkofsky, A. (1967) Biochemistry **6**, 855–860
100. Lipsett, M. N. (1972) J. Biol. Chem. **247**, 1458–1461
101. Agris, P. F., Armstrong, D. J., Schafer, K. P. & Soll, D. (1975) Nucleic Acids Res. **2**, 691–698
102. Renoux, G. & Renoux, M. (1977) J. Exp. Med. **145**, 466–471
103. Pompidou, A., Delsaux, M. C., Telvi, L., Mace, B., Coutance, F., Falkenrodt, A. & Lang, J. M. (1985) Cancer Res. **45**, 4671s–4673s
104. Masuda, Y., Yasoshima, M. & Shibata, K. (1988) Res. Commun. Chem. Pathol. Pharmacol. **61**, 65–75
105. Lang, J. M. *et al.* (1988) Lancet **ii**, 702–705
106. Suzuki, I. (1965) Biochim. Biophys. Acta **104**, 359–371
107. Silver, M. & Lundgren, D. G. (1968) Can. J. Biochem. **45**, 1215–1220
108. Kusunoki, S. & Yasumasu, I. (1976) Biochem. Biophys. Res. Commun. **68**, 881–885
109. Heby, O., Gray, J. W., Lindl, P. A., Marton, L. J. & Wilson, C. B. (1976) Biochem. Biophys. Res. Commun. **71**, 99–105
110. McCann, P. P., Tardif, C., Mamont, P. S. & Schuber, F. (1975) Biochem. Biophys. Res. Commun. **64**, 336–341
111. Sunkara, P. S., Chang, C. C. & Lachman, P. J. (1985) Biochem. Biophys. Res. Commun. **127**, 546–551
112. Rapkine, L. (1931) Ann. Physiol. Physicochim. Biol. **7**, 382–418
113. Sakai, H. & Dan, K. (1959) Exp. Cell Res. **16**, 24–41
114. Stern, H. (1958) J. Biophys. Biochem. Cytol. **4**, 157–161
115. Fahey, R. C., Mikolajczky, S. D., Meier, G. P., Epel, D. & Carroll, E. J. (1976) Biochim. Biophys. Acta **437**, 445–453
116. Kamatani, N., Yu, A. L. & Carson, D. A. (1982) Blood **60**, 1387–1391
117. Fitchen, J. H., Riscoe, M. K., Dana, B. W., Lawrence, H. J. & Ferro, A. J. (1986) Cancer Res. **46**, 5409–5412
118. Chilcote, R. R., Brown, E. & Rowley, J. D. (1985) New Engl. J. Med. **313**, 286–291
119. Mills, G. C. & Mills, J. S. (1985) Clin. Chim. Acta **147**, 15–23
120. Ghoda, L. Y., Savarese, T. M., Dexter, D. L., Parks, R. E., Trackman, P. C. & Abeles, R. H. (1984) J. Biol. Chem. **259**, 6715–6719
121. Gjessing, L. R. (1963) Scand. J. Clin. Lab. Invest. **15**, 474–478
122. Geiser, C. F. & Efron, M. L. (1968) Cancer **22**, 856–860
123. von Studnitz, W. (1970) Acta Paediatr. Scand. **59**, 80–82
124. Helson, L., Fleisher, M., Bethune, V., Murphy, M. L. & Shwartz, M. K. (1972) Clin. Chem. **18**, 613–615
125. Rajnherc, J. R., Gennip, A. H., Abeling, N. G., vander Zee, J. M. & Voute, P. A. (1984) Med. Pediatr. Oncol. **12**, 81–84
126. Voute, P. A. & Wadman, S. K. (1968) Clin. Chim. Acta **22**, 373–378
127. Geiser, C. F. & Shih, V. E. (1980) J. Pediatr. **96**, 72–75
128. Klein, C. E., Roberts, B., Holcenberg, J. & Glode, L. M. (1987) Cancer **62**, 291–298
129. Glode, L. M., Kriegler, M. P. & Livingston, D. M. (1981) Biochemistry **20**, 1306–1311
130. Hoffman, R. M. & Erbe, R. W. (1976) Proc. Natl. Acad. Sci. U.S.A. **73**, 1523–1527
131. Kamely, D., Weissbach, H. & Kerwar, S. S. (1977) Arch. Biochem. Biophys. **179**, 43–45
132. Hoffman, R. M. (1982) In Vitro **18**, 421–428
133. Kreis, W. & Goodenow, M. (1978) Cancer Res. **38**, 2259–2262
134. Kano, Y., Sakamoto, S., Kasahara, T., Kusumoto, K., Hida, K., Suda, K., Ozawa, K., Miura, Y. & Takaka, F. (1982) Cancer Res. **42**, 3090–3092
135. Kuchino, Y., Yasuda, T. & Nishimura, S. (1977) Cancer Res. **37**, 206–208
136. Apple, M. A. & Greenberg, D. M. (1969) Cancer Chemother. Rep. **53**, 195–198
137. Appfel, C. A., Walker, J. E. & Issarescu, S. (1975) Cancer Res. **35**, 429–437
138. Lusky, L. M., Braun, H. A. & Woodard, G. (1947) Cancer Res. **7**, 667–668
139. Crabtree, H. G. (1948) Br. J. Cancer **2**, 281
140. Tatsuta, M., Iishi, H., Yamamura, H., Baba, M., Mikuni, Y. & Taniguchi, H. (1988) Int. J. Cancer **41**, 423–426
141. Marquardt, H., Sapozink, M. D. & Zedeck, M. S. (1974) Cancer Res. **34**, 3387–3390
142. Block, E. (1985) Sci. Am. **252(3)**, 114–119
143. Whitaker, J. R. (1976) Adv. Food Res. **22**, 73–133
144. Sparnins, V. L., Barany, G. & Wattenberg, L. W. (1988) Carcinogenesis (London) **9**, 131–134
145. Belman, S. (1983) Carcinogenesis (London) **4**, 1063–1065
146. Wargovich, M. J. (1987) Carcinogenesis (London) **8**, 487–489
147. Niukian, K., Schwartz, J. & Shklar, G. (1987) Nutr. Cancer **9**, 171–176
148. Kroning, F. (1964) Acta Unio Int. Cancrum **20**, 855–856
149. Weisberger, A. S. & Pensky, J. (1957) Cancer Res. **18**, 1301–1308
150. Lau, B. H., Woolley, J. L., March, C. L., Barker, G. R., Koobs, D. H. & Torrey, R. R. (1986) J. Urol. **136**, 701–705
151. You, W., Blot, W. J., Chang, Y., Ershow, A., Yang, Z. T., An, Q., Henderson, B. E., Fraumeni, J. F. & Wang, T. (1989) J. Natl. Cancer Inst. **81**, 162–164
152. Jackson, J. F. & Lindahl-Kiessling, K. (1964) Exp. Cell Res. **34**, 515–524
153. Jackson, J. F. & Hill, F. S. (1967) Nature (London) **214**, 1155–1156
154. Poste, G. (1972) Int. Rev. Cytol. **33**, 157–252