REVIEW ARTICLE

SELENIUM ANALOGUES OF BIOLOGICALLY ACTIVE SULPHUR COMPOUNDS

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Previous reviews relating to selenium have dealt with the chemistry (Bradt and Crowell, 1932; Painter, 1941) or with the toxic properties (Moxon and Rhian, 1943; Trelease and Beath, 1949; Underwood, 1956; and Moxon, 1958), although a recent review (Schultze, 1960) surveyed the biochemical relationships of selenium-containing Factor 3 and Vitamin E in the animal body. This non-toxic activity of seleniferous material was initially discovered when an essential dietary factor for rats was shown to contain selenium in bound form (Schwarz and Foltz, 1957). Earlier reports had shown the limited essential nature of selenium in plants (Trelease and Trelease, 1938) and in micro-organisms (Pinsent, 1954). While interest in selenium compounds as possible therapeutic agents has been increasing in recent years, this demonstration of the prophylactic-therapeutic properties of Factor 3 can be expected to stimulate further research in this field. In view of the medicinal importance of very many sulphur compounds, it seems appropriate to focus attention on the selenium analogues of some of these and to compare their biological activities.

Isosterism and Bio-isosterism

The earlier work in this field has been well reviewed by Schatz (1960) but a short introductory summary is included. Langmuir (1919) introduced and applied the term "isosteric compounds" or "isosteres" to molecules in which the number and arrangement of electrons was the same. Extensions of this theory led ultimately to the concept that, if compounds "fit the broadest definition for isosteres and have the same type of biological activity", or are directly antagonistic, they are "bio-isosteric" (Friedman, 1951). Nitrous oxide and carbon dioxide, termed isosteric by Langmuir, were later shown to be reversibly anaesthetic to a slime mould, and so fit Friedman's definition of bio-isosteres. Fieser and Richardson (1948) used the term "isolog" in preference to "isostere", although isologous compounds need not always be isosteric. The same terminology was used by Mautner and Clayton (1959) in referring to several series of oxygen, sulphur and selenium compounds, which had previously been described by Mautner (1956) as "almost sterically identical". Isosteres need not be bio-isosteres, but simple isosteric replacements often give compounds of interest and value, and the successful results already obtained through isosteric replacement show that this type of variation is useful in modelling new compounds. Isosterism, however, "will not accomplish for molecules what the periodic table has accomplished for the elements, namely correlation of similar behaviour with similar electronic structure". Molecular size and shape must also be considered in determining biological properties.

Bio-isosterism in the group oxygen, sulphur, selenium and tellurium was mentioned by Schatz (1960), but selenium and tellurium derivatives were considered to be only of minor importance. Friedman (1951) noted that sulphur was surprisingly less bio-isosteric with oxygen than might have been expected, probably due to polarity differences, while Mautner (Mautner and Günther, 1960), in recent extensive work on selenium analogues of biologically active sulphur compounds, has repeatedly emphasised the isosteric similarity between sulphur and selenium. He drew attention to the fact that the radius of doubly bound sulphur (0·94 Å) is close to that of doubly bound selenium (1·07 Å) and that sulphur and selenium analogues crystallised in identical forms which differed from the oxygen analogues (Mautner, 1956; Mautner and Kumler, 1956).

Considering now biological reactions, it was suggested that the mechanism for the excretion of selenium from the animal body resembled that for sulphur (Moxon, Schaefer, Lardy, Dubois and Olson, 1940). Thus dogs excrete sulphur compounds in the presence of bromobenzene as *p*-bromophenylmercapturic acid (I), and similarly, selenium compounds are excreted as "selenomercapturic acid" (McConnell, Kreamer and Roth,

$$Br{\cdot}C_6H_4{\cdot}S{\cdot}CH_2{\cdot}\underset{\displaystyle NH{\cdot}CO{\cdot}Me}{\underset{|}{CH}}{\cdot}CO_2H$$

(I)

1959). In a different context, Mautner and Günther (1960) have shown that selenopantethine (II, X = Se) can replace pantethine (II, X = S)

$$(HOCH_2{\cdot}CMe_2{\cdot}CH(OH){\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}X)_2$$

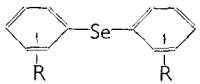
(II)

completely on a mole for mole basis in *Lactobacillus helveticus.* The selenium compound, if supplied to the organism preformed, can replace its sulphur analogue in the biogenesis of coenzyme A, which thereafter performs a biological function.

These two random examples illustrate the fact that selenium can react in biological systems as would sulphur, and can in some cases replace it.

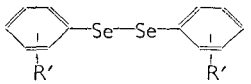
Selenium Analogues of Biologically Active Sulphur Compounds

Interest in organoselenium compounds as possible therapeutic agents dates back only some twenty years. Matti (1940), stimulated by the antistreptococcal properties of the sulphonamides and sulphones, prepared several selenium compounds with a view to their possible use in the treatment of cancer and leprosy. The compounds prepared were derivatives of diphenyl selenide (III) and of diphenyl diselenide (IV). Some of these were tested in the treatment of leprosy and streptococcal infections.



(III)

R = *o*- and *p*-NO_2
o- and *p*-NH_2
o- and *p*-Me·CO·NH



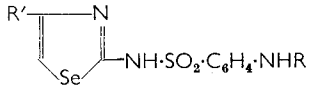
(IV)

R′ = *o*- and *p*-NH_2
o- and *m*-Me·CO·NH
p-OH

No action was noted against streptococci, while in leprosy, only the simplest derivatives showed favourable activity.

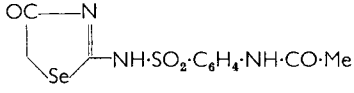
In the same year Painter, Franke and Gortner (1940) investigated some organoselenium compounds with a view to studying selenium compounds in cereals.

Sulphanilamide derivatives containing selenazole (V) and "seleno-hydantoin" (VI) residues were prepared (Roy and Guha, 1945), but no report of their biological properties has been found.



R′ = Me,Ph; R = CO·Me, H

(V)



(VI)

Selenium Amino-acids

Besides these isolated reports, several papers described selenoamino-acid derivatives. A crystalline amino-acid complex containing sulphur and selenium was isolated from *Astragalus pectinatus* (Horn and Jones, 1941) This material analysed as a complex of two parts selenocystathionine (VII, X = Se) and one part cystathionine (VII, X = S). The isolation of such plant products, in a pure form, is extremely difficult since the

$$HO_2C{\cdot}CH(NH_2){\cdot}CH_2{\cdot}X{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$$

(VII)

selenium compounds occur in very small proportions in conjunction with analogous sulphur compounds with very closely related properties. This difficulty was further exemplified by the isolation of another seleno-amino-acid from *A. bisulcatus* by the use of ion-exchange- and filter-paper-column techniques (Trelease, Di Somma and Jacobs, 1960). The indications were that this amino-acid was *Se*-methylselenocysteine (VIII, R = Me; X = Se), still not completely separated from S-methyl-cysteine (VIII, R = Me; X = S).

$$R{\cdot}X{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$$

(VIII)

Stekol (1942), in a short note, described the product obtained from the interaction of cysteine hydrochloride and sodium selenite as selenium tetracysteine, which analysed as $Se(C_3H_6NO_2S)_4$, but no structure was given. Fredga (1937) synthesised selenocystine (IX), while (±)-seleno-cystine (IX) and derivatives of selenocysteine (VIII, X = Se; R = Ph, $Ph.CH_2$) were prepared as part of an investigation of selenium compounds in plants (Painter, 1947a). Painter's approach was to synthesise the

$$HO_2C{\cdot}CH(NH_2){\cdot}CH_2{\cdot}Se{\cdot}Se{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$$

(IX)

selenium analogues of sulphur-containing amino-acids and to compare them pharmacologically and chemically with the isolated plant products. This was reasonable, since it was accepted by then that selenium occurred

as a constituent of amino-acid material (Horn and Jones, 1941). This work was extended by Painter (1947b) with the synthesis of the selenium analogues of (±)-methionine (X) and (±)-homocystine (XI), since cystine

$$Me{\cdot}Se{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$$

(X)

$$HO_2C{\cdot}CH(NH_2){\cdot}CH_2{\cdot}CH_2{\cdot}Se{\cdot}Se{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H$$

(XI)

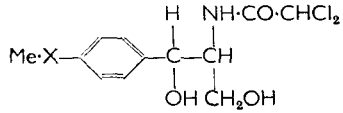
(or cysteine) and methionine carry nearly all the sulphur in cereal proteins. Homocystine, although never identified in plants, was of interest because of its known ability to supply animals with their sulphur-containing amino-acid requirements. In the same year, the synthesis of these two selenium compounds was improved (Klosterman and Painter, 1947). Experiments were later reported by Klug and Petersen (1949) suggesting that selenium tetracysteine (Stekol, 1942) and selenium dicysteine (Painter, 1947a) were really mixtures of cysteine and, probably, selenium dicysteine. This, however, conflicted with other work (Williams and Ravve, 1948), in which the selenium analogue of (±)-cystine was synthesised by two different routes, to give a compound melting at 215°, compared with 215° (Fredga, 1937) and 222° (Painter, 1947a).

Weisberger, Suhrland and Seifter (1956) reported that selenocystine (IX) was effective in low concentrations in decreasing the incorporation of radioactive L-cystine in leukaemic leucocytes *in vitro*. Diets deficient in the sulphhydryl amino-acid L-cysteine had previously been reported to suppress the growth of malignant tumours in animals. It was not known whether selenocystine also competitively inhibited cystine incorporation in the intact animal, but it was shown (Weisberger and Suhrland, 1956a) that selenocystine decreased the incorporation of L-[^{35}S]cystine by rat murphy lymphosarcoma tumour cells both *in vitro* and *in vivo*, whilst benzylselenocysteine (VIII, X = Se; R = $CH_2{\cdot}Ph$) did not. Selenocystine also decreased tumour growth in the intact arimal. Under clinical conditions (Weisberger and Suhrland, 1956b), selenocystine had a rapid and striking effect on leukocytes in both chronic and acute leukaemia. The effect was greater on immature than on mature leukocytes and the action took place at the source with a reduction in spleen size, as well as by attack of the leukocytes in circulation. It was of interest to note that selenocystine was effective in patients with acute leukaemia resistant to cortisone, aminopterin or 6-mercaptopurine. A decrease in leukocyte count was also reported in chronic myeloid leukaemia resistant to irradiation, Fowler's solution, urethane and busulphan. One case was quoted where the condition which had become resistant to 6-mercaptopurine became responsive again to 6-mercaptopurine after selenocystine treatment. The mechanism of action was unknown, but selenocystine seemed to have a specific toxicity towards immature leukocytes, since no organic changes, attributable to selenium toxicity, were discernible. Nausea and vomiting, which were often very severe, proved a serious disadvantage with selenocystine and difficulty was found in continuing treatment.

Cowie and Cohen (1957) showed that selenomethionine (X) could completely replace methionine for normal growth of a methionine-requiring mutant of *Escherichia coli*, and that radioactive selenium, from selenite, was incorporated into the bacterial proteins. Selenomethionine was identified in protein hydrolysates of *E. coli* (Tuve and Williams, 1957: 1961) and it was suggested that selenocystine may also be produced by *E. coli* grown in the presence of sodium selenite. Thus, since cystine is an intermediate of methionine production in *E. coli* and since selenomethionine has been identified, it is believed, but so far not proven, that selenocystine may also be produced.

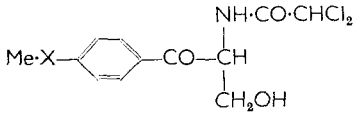
Chloramphenicol Derivatives

The sulphur (XII, X = S) and selenium (XII, X = Se) derivatives of chloramphenicol (XII, X = O) were synthesised (Supniewski, Misztal and Krupinska, 1954) and shown to be strongly antibacterial, with the selenium compound about ten times more active than its sulphur analogue.



(XII)

Gram-positive were more sensitive than Gram-negative bacteria and both the selenium and sulphur analogues were strongly active against acid-fast bacteria. The toxicity in mice of both compounds was comparable with that of chloramphenicol itself. The lethal doses were 500 mg./kg. weight and the toxic symptoms identical with those of chloramphenicol, while the selenium compound, but not the sulphur compound, decreased respiratory movement, lowered arterial pressure and had a diuretic effect on the cat. The same authors also reported that the ketone derivative of chloramphenicol has strong antifungal activity, but this action was not noted in the sulphur and selenium analogues. No structure was given for these compounds but they are presumed to be as shown in structure (XIII, X = O, S, Se).

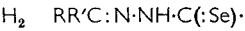


(XIII)

Selenosemicarbazide Derivatives

Selenosemicarbazide (XIV) was isolated in a synthesis of some of its carbonyl derivatives (XV) (Hulls and Renson, 1956a). 4-Phenylselenosemicarbazones (XVI) were also isolated by Huls and Renson (1956b), but no report of their biological activity has been found.

(XIV)



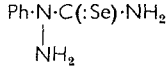
(XV)

R and R′ = Me, Me, Et, H; Me, Et, Pr, H.

(XVI)

R = alkyl, aryl, substituted aryl; R′ = H, Me

2-Phenylselenosemicarbazide (XVII) and a number of its derivatives (XVIII) were also reported (Mautner and Kumler, 1956), but the synthesis of selenosemicarbazide (XIV) by this method, failed. As an extension to the chelating theory of action of thiosemicarbazides in tuberculosis it was suggested by Mautner and Kumler (1956) that the selenium



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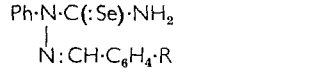
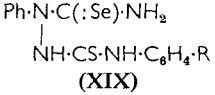


R = NO_2, Cl, Br, I, NMe_2, NH·CO·Me, OMe, OH

(XVIII)

analogues might have greater chelating ability, since selenium is related to sulphur in the same way as sulphur is to oxygen. The sulphur compounds showed antitubercular and antifungal activity, while it was already known that replacement of sulphur by oxygen resulted in complete or partial loss of both types of activity. When the antifungal properties of 2-phenylselenosemicarbazide (XVII), its selenosemicarbazone derivatives (XVIII), phenylselenourea and their sulphur and oxygen analogues were compared by Mautner, Kumler, Okano and Pratt (1956) against plant and animal pathogens and a saprophytic organism, it was found that the selenium compounds were ten to one thousand times more active on a molar basis than the sulphur compounds, while the oxygen analogues showed negligible activity. The possibility of released selenium being the active agent was proved to be of little importance. Finally, it was concluded that selenium compounds were of sufficient activity to warrant further research.

Closely related derivatives of 2-phenylselenosemicarbazide (XVIII, R = NMe_2 and (XIX, R = *p*-Br, *p*-OEt) were synthesised and tested for

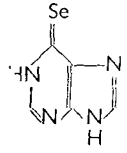


(XIX)

antimicrobial activity (Bednarz, 1957). These compounds were shown to have a weak effect on staphylococci, *Bacillus subtilis* and *E. coli*, a moderate effect on *Mycobacterium phlei*, *M. smegmatis* and the bacillus of Calmette and Guérin and a very strong effect on *M. tuberculosis*.

Selenopurines and Selenopyrimidines

Using similar methods to those for the preparation of the thio-analogues, selenopurine and selenopyrimidine derivatives have been prepared (Mautner, 1956). In this way 6-selenopurine (XX), 6-(methylseleno)purine (XXI), 2-selenouracil (XXII), 2,4-diselenouracil (XXIII) and 2-selenothymine (XXIV) were isolated. It had been noted that the most useful



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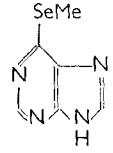
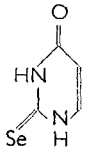


(XXI)



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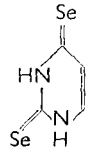
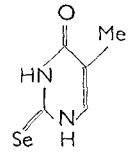


(XXIII)

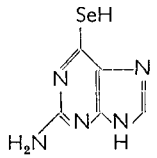


(XXIV)

purine and pyrimidine bases were those in which the size of the new atom or group was closely similar to the atom or group replaced. Since the radius of doubly bound selenium is close to that of doubly bound sulphur, the compounds synthesised were sterically almost identical with the sulphur analogues.

The actions of 6-selenopurine and 6-mercaptopurine were compared against Ehrlich ascites tumour systems and *Lactobacillus casei*, as well as against a wide range of micro-organisms (Mautner, 1958). 6-Selenopurine inhibited a 6-mercaptopurine-resistant strain of *L. casei* as efficiently as it did the wild strain. In contrast, mouse leukaemia L-1210, resistant to 6-mercaptopurine, showed full cross-resistance to the selenium compound (Jaffe and Mautner, 1958a). 6-Selenopurine also inhibited the growth of a fairly wide range of micro-organisms, being more active than the corresponding sulphur compound, while it was shown that the sulphur and selenium compounds appeared to act by similar mechanisms. Another somewhat more detailed report (Jaffe and Mautner, 1958b) showed that with some tumour systems 6-selenopurine had lower antitumour activity and greater host toxicity than equimolar quantities of 6-mercaptopurine and equivalent activity with others. Methylation decreased the activity of both compounds. Since 6-selenopurine was unstable, its effectiveness implied a swift and selective action. The possibility that the decomposition products may be the active species was not supported by the inactivity of the equally unstable 6-(methylseleno)purine (XXI) and 2-selenouracil (XXII).

Recently, the synthesis and preliminary biological testing of 6-selenoguanine (XXV) was reported by Mautner and Jaffe (1961), following from the antimitotic activity of 6-thioguanine, which involved its incorporation into deoxyribonucleic acid (DNA). Although the mechanism of action of 6-thioguanine was unknown, it was suggested (Mautner and Jaffe, 1961) that charge separation might lead to unusually strong

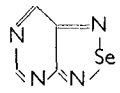


(XXV)

hydrogen bonding with the amino-group of cytosine facing thioguanine in the double helix of DNA and that this could be expected to interfere with its replication. Similar charge separation has been found to be greater in thiocarbamoyl than in carbamoyl compounds, while replacement of sulphur by selenium gives even more marked polarisation (Mautner and Clayton, 1959; Mautner, 1956). Since 6-selenopurine showed antitumour activity in mice despite its instability (Jaffe and Mautner, 1958b; 1960), 6-selenoguanine was similarly tested, having first been shown to be more stable. Effective growth inhibition of *L. casei* was obtained with 6-selenoguanine with one-tenth the required

thioguanine concentration, but the selenium compound was more toxic to mice than the thio-compound in a single dose, although this position was reversed on repeated administration. *In vivo* testing in mice showed that the two compounds had comparable antitumour activity, while the selenium analogue had an appreciably higher therapeutic index. It was shown that if a tumour showed resistance to 6-mercaptopurine, this extended to thioguanine as well as to selenoguanine.

8-Selenopurines (XXVI), related to known purine antimetabolites,

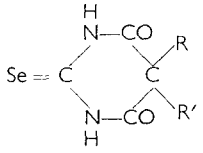


(XXVI)

were synthesised as possible antimetabolites for chemotherapeutic studies in cancer (Carr, Sawicki and Ray, 1958). Carcinostatic activity had resulted when the carbon atom in position 8 of guanine was replaced by a nitrogen atom. In this instance selenium was introduced as a more radical change than the replacement of the nitrogen atom, and while the earlier purine derivatives (Mautner, 1956) had an exocyclic selenium atom, the compounds prepared by Carr, Sawicki and Ray (1958) had a heterocyclic selenium atom. The possible mechanism of action was discussed, but no report of the biological testing of these compounds has been noted.

2-Selenobarbiturates

Several 2-selenobarbituric acid derivatives (XXVII) were prepared (Mautner and Clayton, 1959) as part of an investigation of the relative



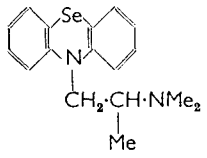
R = H, Ph, CH_2·Ph, C_5H_{11}; R′ = H
R′ = Et; R = Ph

(XXVII)

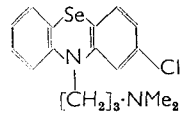
lipid solubilities of oxygen, sulphur and selenium compounds. 6-Selenopurine (XX) and 2-selenouracil (XXII) were similarly investigated. 6-Selenopurine had slightly greater lipid solubility than the thio-analogue, while 2-selenouracil was less soluble at physiological pH than was 2-thiouracil. In the case of the barbiturates tested, the sulphur and selenium analogues had very similar lipid solubilities. It was concluded from these results that, for the types of compound tested, replacement of an oxygen by a sulphur atom is an effective method of increasing lipid solubility, with only minor changes in the steric configuration of the molecule. Further replacement of sulphur by the more metallic selenium did not reduce the lipid solubility, and it was concluded that lack of such solubility should not be a major problem in synthesising selenium analogues of biologically active oxygen and sulphur compounds.

Phenoselenazine Derivatives

The selenium analogues of promethazine (XXVIII) and chlorpromazine (XXIX) were isolated by Müller, Buu-Hoï and Rips (1959), and were



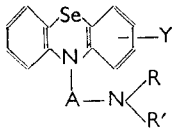
(XXVIII)



(XXIX)

shown to have antihistaminic activity comparable to that of their sulphur analogues.

A number of stable, non-toxic phenoselenazine derivatives (XXX),



(XXX)

Y = H, halogen, CF_3, lower alkyl or lower alkoxyl group.

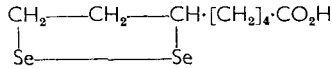
A = Divalent, straight or branched alkylene chain of 2–6 carbon atoms.

R,R′ = H, Ph·CH_2, lower alkyl or, with the nitrogen, part of a monocyclic 5- or 6-membered heterocyclic ring.

unsubstituted or substituted in position 10, have been patented (Smith, Kline and French Laboratories, 1959). These compounds were reported to be particularly useful as tranquillisers or mild sedatives. They also showed fungicidal, antibacterial, antihistaminic and anti-emetic properties. Those substituted in position 10, useful mainly as intermediates, also displayed significant antifungal, vermifugal and antibacterial activity.

6-Selenoctic Acid

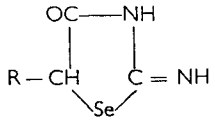
When Schwarz and Foltz (1957) showed that selenium was an integral part of the biologically active Factor 3, they did not report Factor 3 as a pure chemical entity but described some of its properties, as did Patterson, Milstrey and Stokstad (1957). The suggestion by Bergson (1957) that 6-selenoctic acid (XXXI) might be related to, or even identical with Factor 3, was quickly shown to be incorrect (Schwarz, Foltz and Bergson, 1958).



(XXXI)

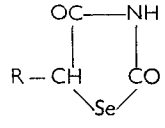
2-Imino-4-selenazolidones and Selenazolid-2,4-diones

A number of 2-imino-4-selenazolidones (XXXII) and their acid-hydrolysis products, selenazolid-2,4-diones (XXXIII), have been prepared and



(XXXII)

R = H, alkyl, Ph.



(XXXIII)

screened for biological and antimicrobial activity. These products were screened for a wide range of activity in view of the diverse activity shown by similar oxygen (Clarke-Lewis, 1958) and sulphur analogues (Brown, 1961). However, no promising activity was shown by the selenium compounds (Comrie, Dingwall and Stenlake, unpublished).

Proposed Metabolic Studies

Pichat, Herbert and Thiers (1961) reported the synthesis of selenocystamine (XXXIV), selenohypotaurine (XXXV) and selenotaurine (XXXVI) in a proposed comparison of the metabolism of organic sulphur and selenium compounds.

$(NH_2 \cdot CH_2 \cdot CH_2 \cdot Se)_2 2HCl$ (XXXIV)

$NH_2 \cdot CH_2 \cdot CH_2 \cdot SeO_2H$ (XXXV)

$NH_2 \cdot CH_2 \cdot CH_2 \cdot SeO_3H$ (XXXVI)

A different approach to the same problem has been taken by Fredga and Lindgren (1961), who have recently reported the commencement of a study of long-chain fatty acids (XXXVII) incorporating a selenium atom in various positions of the chain.

$Me \cdot [CH_2]_n \cdot Se \cdot [CH_2]_m \cdot CO_2H$, where $n + m = 12$ or 13.
(XXXVII)

Conclusion

Whether organoselenium compounds will ever command a place in medicine is rather doubtful. However, the biological reports to date have shown that in certain spheres of activity, selenium compounds may be of some value. The indications are that this seems most likely in the antifungal, antibacterial and carcinostatic fields. It is encouraging to note that very recently at least two groups of workers have embarked on a comparative study of the metabolism of sulphur and selenium compounds. At present, the knowledge of the fate of selenium compounds in the body is limited and such studies can be expected to lead to better understanding of the biological role of selenium. Incorporation of selenium into animal proteins has already been demonstrated (McConnell and Wabnitz, 1957; McConnell, Roth and Dallam, 1959), while urinary excretion apparently follows the same route as for sulphur (McConnell, Kreamer and Roth, 1959).

One of the discouraging features of such studies, however, is the relative instability of selenium compounds. Thus, formulation can be expected to be extremely difficult. The fairly good lipid solubility, if a general characteristic, is likely to be an asset since the presence of water often accelerates decomposition, leading to breakdown products with powerfully unpleasant odours. Perhaps in this field more than in any other, the medicinal use of an active compound will be limited, not only by its toxic properties, but also by its physical, and hence sensory, stability.

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