CHAPTER 5

The Thiosemicarbazones

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A. Introduction

The thiosemicarbazones stand as milestones in the emerging era of the specific treatment of viral diseases. They were the first true antiviral substances to be synthesized; although a number of random events had to take place before their potential was realized. The thiosemicarbazones were the first compounds to be found active in virus-infected animals. They were also the focus of the first systematic studies on the relationship between chemical structure and antiviral activity. Furthermore, these compounds were the first to be effective in humans and placed in clinical medicine. Finally, there are no other compounds known which appear able to block the genetic expression of such a wide variety of viruses.

There is an impressive literature on the antitumor activity of the thiosemicarbazones (SARTORELLI et al. 1977; AGRAWAL and SARTORELLI 1978; PETERING 1980). Even though a well-established link exists between viruses and malignancy, this area will not be included here. Two excellent reviews on the antiviral activity of the thiosemicarbazones are available (BAUER 1972; LEVINSON 1973). I will largely avoid an in-depth coverage of the same material, but will stress literature published in the last 10 years.

B. History

The first publication about an effective antiviral agent virtually escaped attention. In 1950, HAMRE et al. reported that *p*-aminobenzaldehyde-3-thiosemicarbazone (Fig. 1, $R_1 = NH_2$, $R_2 = H$, $R_3 = H$) caused a significant delay in death, as well as survival of a small percentage, of chick embryos and mice infected with vaccinia virus. At that time there was a general atmosphere of pessimism about the discovery of antiviral agents. This mood was enhanced by the unfortunate false start made with the discovery that antibacterial agents were effective against the rickettsia and lymphogranuloma–psittacosis groups, considered for many years to be viruses. However, it was the sensitivity of the tuberculosis bacillus to the thiosemicarbazones that led HAMRE and colleagues to test these compounds against viruses.

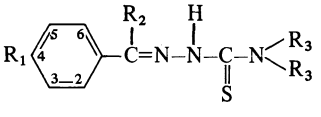


Fig. 1

P. E. Came et al. (eds.), *Chemotherapy of Viral Infections*

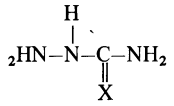


Fig. 2

The antibacterial activity of thiosemicarbazones appears to have been revealed by chance. DOMAGK (the discoverer of the first sulfa drugs), when engaged in structure–activity studies with BEHNISCH, found that sulfathiazoles were potent inhibitors of *Mycobacterium tuberculosis* (DOMAGK et al. 1946). Further structure–activity studies led to the synthesis of sulfathiadiazoles. Essential intermediates in the latter's preparation were 2-aminothiadiazoles which were formed from the corresponding thiosemicarbazones by oxidative ring closure with ferric chloride. One of the thiosemicarbazones synthesized, and tested for biologic activity, was benzaldehyde thiosemicarbazone (Fig. 1, $R_1 = H$, $R_2 = H$, $R_3 = H$). It has been claimed (BOCK 1957) that at the time of this discovery the investigators at I.G. Farben also found that benzaldehyde thiosemicarbazone had no activity against a variety of pathogenic microorganisms. These included, pneumonia virus of mice, influenza virus, lymphocytic choriomeningitis virus, and two members of the poxvirus family – ectromelia and canarypox. Many years later, in the same laboratories, the murine antipox activity of thiosemicarbazones was confirmed (BOCK 1957) and shown to be quite dependent on the strain of virus used.

Benzaldehyde thiosemicarbazone was first synthesized by FREUND and SCHANDER 44 years prior to the discovery of its biologic potential. In that era, semicarbazide (Fig. 2, X = O) was standardly used as a derivatizing agent for aldehydes and ketones. FREUND and SCHANDER (1902) suggested, apparently without historical impact, that thiosemicarbazide (Fig. 2, X = S) should be used instead, because the presence of the sulfur atom could be easily detected in the precipitates (thiosemicarbazones) formed in the presence of aldehydes or ketones in a reaction mixture.

C. Chemistry

This part of the review is centered on studies of the introduction of substituents into various thiosemicarbazones and the subsequent effect on the compounds' antiviral properties. Many of the key structure–activity relationships were established in mice infected with poxviruses, and were subsequently shown to hold true in studies with other virus families. However, as shown in many of the following sections, antiviral agents with in vitro activity have been found or tested much more frequently than those with in vivo effectiveness. The structure–activity relationships found in any one of these systems should not necessarily be taken to hold true for the other, even though the same virus was used. The investigations discussed here will be divided into sections dealing with specific types of thiosemicarbazones. The radical nomenclature is that which is currently accepted (RIGAUDY and KLESNEY 1979).

I. Aryl Thiosemicarbazones

The thiosemicarbazone (TSC) first chosen for antiviral studies by investigators at the Squibb Institute for Medical Reseach was *p*-aminobenzaldehyde-TSC (Fig. 1, $R_1 = NH_2$, $R_2 = H$, $R_3 = H$), because it was the most water-soluble compound in their collection (HAMRE et al. 1950). One of the initial reliable methods for testing antiviral agents had just been developed there (BROWNLEE and HAMRE 1951) with vaccinia as the model virus. The compound was indeed shown to have chemotherapeutic activity in vaccinia-infected eggs and mice. It was most fortunate that this virus was used because it would be another 10 years before other thiosemicarbazones were found that had activity against nonpox viruses (O'SULLIVAN and SADLER 1961; see Sects. C.IV, D.IV). This initial investigation also included one other thiosemicarbazone: *p*-acetamidobenzaldehyde-TSC (Fig. 1, $R_1 = CH_3\text{-}\overset{\overset{O}{\|}}{C}\text{-}NH\text{-}$, $R_2 = H$, $R_3 = H$). It was somewhat less active than the primary compound, but significantly more toxic (the 10% aqueous triethylene glycol necessary for solubilization was innocuous).

A wider series of benzaldehyde thiosemicarbazones was then studied for antiviral activity. HAMRE et al. (1951) found that substitution of glucose or cyclohexane for the benzene ring resulted in almost complete loss of activity. The substitution of oxygen for sulfur in the thiosemicarbazone moiety also caused loss of activity (greatly reduced activity of semicarbazones would be repeatedly shown in future investigations with related compounds). Furthermore, thiosemicarbazide (Fig. 2, X = S) had little, if any, activity (another result that would be confirmed in most later investigations). The effect of substitutions in the benzaldehyde moiety (other than the p-amino and p-acetamido groups) on chemotherapeutic activity was examined next. Nine compounds were substituted in the 4 position (Fig. 1), two were disubstituted in the 3 and 4 positions, and the last was unsubstituted. The disubstituted compounds (4-hydroxy-3-methoxy and 4-hydroxy-3-sulfo) had no activity. All monosubstitutions led to either decreased or unchanged activity compared with the unsubstituted compound or the two 4-substituted compounds tested initially. The groups producing little, or no, change in activity at the 4 position were methoxy (CH_3O), propoxy ($CH_3CH_2\text{-}CH_2O$), and ethylsulfonyl ($C_2H_5SO_2$). In further experiments, butyl groups were substituted for both hydrogen atoms at the end of the thiosemicarbazone side chain [Fig. 1, $R_2 = H$, $R_3 = \text{-}(CH_2)_3\text{-}CH_3$]. All compounds altered in this way (the unsubstituted benzaldehyde thiosemicarbazone and the *p*-amino- and *p*-acetamido derivatives) retained activity against vaccinia virus. This behavior is in marked contrast to such dialkyl substitutions at the terminal amino group of isatin-β-thiosemicarbazone (BAUER and SADLER 1960a; Sect. C.III).

The chemotherapeutic activity of benzaldehyde-TSC was confirmed by THOMPSON et al. (1951) both in minced chick embryo culture (Maitland culture) and in mice infected with vaccinia virus. Unlike HAMRE et al., they found that all substitutions at the 4 position of the benzaldehyde moiety (except for NO_2), or at the terminal amino position in the thiosemicarbazone side chain (Fig. 1) reduced activity in both test systems. This cannot be taken as a true contradiction, even though both groups used essentially the same substitutions. Whereas HAMRE et al. used

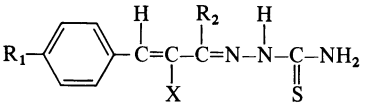


Fig. 3

concentrations close to the toxic dose of each compound, THOMPSON et al. tested all compounds at about the same concentration. Thus, certain mono- and disubstituted compounds were found effective at more than ten times the concentration found to be ineffective by the THOMPSON group. However, the same group (THOMPSON et al. 1953 b) went on to confirm that benzaldehyde semicarbazone and thiosemicarbazide were lacking in activity. They also established that addition of methylene or vinyl groups between the benzaldehyde and thiosemicarbazone portions of the molecule decreased activity.

Integrity of the thiosemicarbazone side chain was found to be critical. Loss of the sulfur atom, as well as substitution of a methyl (CH_3), ethyl (C_2H_5), carbethoxy ($C_2H_5CO_2$), or benzyl ($C_6H_5CH_2$) group at the aldehyde carbon atom (R_2 in Fig. 1) abolished or reduced activity. Except as noted in the following paragraphs, studies on the antiviral properties of benzaldehyde-TSCs virtually ceased in 1953 when attention was focused on the heterocyclic thiosemicarbazones.

That substitution of a methyl group at the aldehyde carbon atom reduced activity against vaccinia virus was confirmed many years later by RUNTI et al. (1968). They carried out more extensive investigations of these acetophenone-TSCs (Fig. 1, $R_2 = CH_3$, $R_3 = H$) particularly with regard to synthesis of lipophilic compounds with substitutions in the benzene ring. Thirteen of these compounds were used in a virus plaque reduction test (the drug is placed in a center well of an agar plaque assay plate; as the drug diffuses the highest concentrations lead to a clearly visible cell toxicity zone, but beyond that there may be a zone of specific inhibition of plaque development) against eight different viruses. The authors concluded that the acetophenone-TSCs had a different antiviral spectrum from the benzaldehyde or isatin (Sect. C.III) series of TSCs. Specifically, *p*-nitro-, *p*-bromo-, and *p*-methoxyacetophenone-TSCs were moderately active against influenza and parainfluenza viruses. Activity could not be demonstrated when the sulfur atom was replaced by oxygen in the TSC side chain, nor was there activity against any of the other viruses, including vaccinia. However, 2,4-dimethoxyacetophenone-TSC was active against vaccinia, but not against any of the other viruses.

Three other reports, all appearing prior to that of RUNTI et al. (1968) claimed that aryl-TSCs were effective against influenza viruses. IWASAKI et al. (1955) tested 26 compounds of benzaldehyde- (Fig. 1, $R_2 = H$, $R_3 = H$), acetophenone- (Fig. 1, $R_2 = CH_3$, $R_3 = H$), cinnamaldehyde- (Fig. 3, $X = H$, R_1,$R_2 = H$), and benzalacetone- (Fig. 3, $X = H$, $R_1 = H$, $R_2 = CH_3$) TSCs against influenza A and B viruses as well as Newcastle disease virus. All four types of unsubstituted TSCs were examined, as well as nitro, dimethylamino [$(CH_3)_2N$], amino, and acetamido para substituents (R_1 in Fig. 3) in each of the four categories. In addition, a bromine substituent (X in Fig. 3) was added to the cinnamaldehyde and benzalacetone series of compounds. From their results the following generalities can be made for the influenza viruses:

a) The unsubstituted TSCs were the most active; these were, in increasing order of effectiveness, benzaldehyde, acetophenone, cinnamaldehyde, and benzalacetone
b) Most of the *para*-substituted compounds were reduced in activity; the order of the greatest to the least reductions was: acetamido, amino, dimethylamino, and nitro substituents
c) In general, the bromo substitutents had little, or no, effect on antiviral activity

With regard to Newcastle disease virus, benzalacetone-TSC was the only active unsubstituted compound. The three other active compounds were the *p*-nitro derivatives of benzalacetone-, cinnamaldehyde-, and benzaldehyde-TSCs. LUM and SMITH (1957), apparently unaware of the paper by IWASAKI et al. (1955), showed that *p*-nitrobenzaldehyde-TSC, *p*-hydroxybenzaldehyde-TSC, anisaldehyde-TSC (Fig. 1, $R_1 = NO_2$, HO, and CH_3O, respectively) and α-pentylcinnamaldehyde-TSC [Fig. 3, X = H, $R_1 = CH_3(CH_2)_4$, R_2 = H] inhibited influenza A and B viruses in chick chorioallantoic Maitland cultures and also spared 50% of the mice inoculated with these viruses. ZAK (1959) noted that isopropylbenzaldehyde-TSC (Fig. 1, $R_1 = isoC_3H_7$) caused a slight reduction in the febrile period of patients with influenza virus infections.

In connection with studying cyanothiophene-TSCs (Sect. C.II) WINKELMANN and ROLLY (1972) synthesized a number of corresponding cyanobenzaldehyde-TSCs. Replacement of the cyano group (N≡C) in both series of compounds by carboxylic acid, carboxamide, ester, or imidate groups produced less active or inactive products when tested in vaccinia-infected mice. In the benzaldehyde series, when the cyano group was introduced at the 2,3, or 4 positions (Fig. 1) of the aromatic ring, the 4-isomer was found to be most active.

II. Quinoline, Pyridine, and Thiophene Thiosemicarbazones

In 1953 a second major advance was made that would eventually lead to clinical application (Sect. H). It was the discovery that heterocyclic TSCs could possess antiviral activity (THOMPSON et al. 1953b). Compounds containing pyridine (Fig. 4), quinoline (see Fig. 5), thiophene (see Fig. 7), and isatin (see Fig. 8) groups protected mice to a degree equal to, or greater than, that obtained with the benzaldehyde-TSCs. The bulk of the structure–activity relationship studies would be carried out with the isatin-TSCs (Sect. C.III), but it should be noted that this initial study with pyridine- and quinoline-TSCs established an important feature of these compounds – the thiosemicarbazone moiety affixed to the heterocyclic ring in a position α to the ring nitrogen atom, as in 2-formylpyridine-TSC (Fig. 4), had no activity in mice infected with vaccinia virus. When the TSC side chain was placed in another position, as in 4-formylpyridine- or 4-formylquinoline-TSC (Fig. 5), the

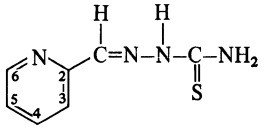


Fig. 4

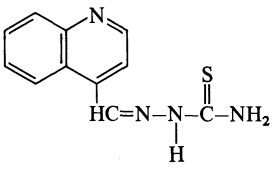


Fig. 5

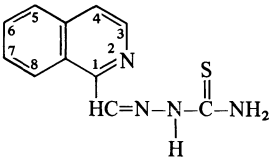


Fig. 6

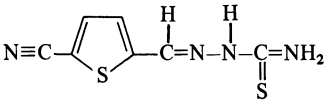


Fig. 7

compound would protect against lethal infection (THOMPSON et al. 1953 b). Further studies indicate that the β position was optimal (Sects. C.III, VI). However, in tissue culture studies using vaccinia as well as other viruses, the opposite appeared to be true. BROCKMAN et al. (1970) found that pyridine- and quinoline-TSCs would prevent herpes-induced cytopathology in tissue culture only if the TSC was α to the ring nitrogen atom. 2-Formylpyridine-TSC was active but the 3- and 4- formyl isomers were not (Fig. 4). Furthermore, 1-formylisoquinoline-TSC (Fig. 6), and its 5-hydroxy derivative, were active (as was 6-formylpurine-TSC), but no protection was noted with isatin-β-TSC or 1-methylisatin-β-TSC (Fig. 8, R_1 = H or CH_3, R_2 = H, R_3 = H_2, X = S). KATZ et al. (1974) found that 5-hydroxy-2-formylpyridine-TSC (Fig. 4) and 1-formylisoquinoline-TSC (Fig. 6) would prevent vaccinia plaque formation in tissue culture. However, the mode of action of these compounds was unique compared with four other active TSCs where the side chain was not α to the ring nitrogen atom (Sect. F.II). More recently LEVINSON et al. (1977 b) found that 1-formylisoquinoline-TSC (Fig. 6) and 2-pyridine-TSC would inhibit the production of Rous sarcoma virus. Further extension of these studies with 2- and 4-formylpyridine-TSC (Fig. 4) showed that the former was much more active than the latter (KASKA et al. 1978). Parenthetically, one notes that the minimum requirement for antitumor activity of pyridine- and isoquinoline-TSCs is that the TSC side chain must be attached α to an unencumbered ring nitrogen atom (AGRAWAL and SARTORELLI 1978).

Although the thiophene-TSCs have received little attention (KATZ et al. 1974), 5-cyanothiophene-2-formylthiosemicarbazone (Fig. 7) has been reported (ROLLY and WINKELMANN 1972) to be superior to 1-methylisatin-β-thiosemicarbazone in its in vivo antivaccinia activity in several animal species (Sects. C.III, D.II). Structure–activity studies indicated that substitutions in the side chain or thiophene moiety, as well as lengthening of the side chain, produced compounds of diminished activity (WINKELMANN and ROLLY 1972).

III. Isatin-β-Thiosemicarbazones

Unlike the thiosemicarbazones discussed previously, long-lasting interest in the isatin series of compounds was maintained because of their potency and potential

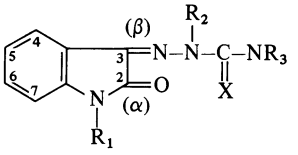


Fig. 8

for clinical application. In 1953 MINTON et al. discovered that isatin-β-thiosemicarbazone (IBT; Fig. 8, R_1, R_2 = H, R_3 = H_2, X = S) was effective when given intraperitoneally in only a single dose. This would protect mice against lethal infection with a poxvirus isolated from a patient presenting the clinical picture of mild smallpox (a finding that would be exploited later by D. J. BAUER at the Wellcome Laboratories and by his colleagues). Initial structural studies with IBT showed that bromo and nitro (MINTON et al. 1953), as well as methyl (THOMPSON et al. 1953 b) substitutions at the 5 position of the aromatic ring (Fig. 8) substantially reduced, or abolished, activity. It was also shown that the semicarbazone had no effect on vaccinia infection in the mouse.

Two years later BAUER (1955) presented results indicating that IBT was even more potent against mouse-adapted neurovaccinia virus than had been previously reported. Using the mouse model, BAUER and SADLER (1960 a) then went on to study the relationship between structure and activity in much greater detail than the initial steps taken by the THOMPSON group.

1. Substitution in the Aromatic Ring

Fourteen derivatives were prepared with single substitutions at the 4, 5, 6, or 7 positions (Fig. 8). Methyl, methoxy, iodo, chloro, or bromo substituents were mostly used. These groups commonly led to reduction or total loss of activity of the compound. Substituents at the 5 position had a particularly marked effect in eliminating biologic activity; except for substituents, such as fluorine, with small atomic or group radii. The interaction between the size of the halogen atom substituted at this position and the degree of antiviral activity was confirmed recently (BORYSIEWICZ and LUCKA-SOBSTEL 1978) using Mannich base derivatives of IBT. BAUER and SADLER (1960 a) found that activity was reduced to a lesser extent in the 4 or 6 positions, and some of the 7-substituted compounds still retained high activity. The importance of the steric effects was further supported by the finding that larger fused ring systems, such as naphthisatins, in which the additional benzene ring is fused to the 4,5, or 5,6, or 6,7 positions, were inactive (BAUER and SADLER 1960 a; SADLER 1965).

2. *N*-Substitution in the Pyrrolidine Ring

Studies were then undertaken on the effect of *N*-substitution in the pyrrolidine ring (BAUER and SADLER 1960 a). Alkylation at this position (R_1 in Fig. 8) produced a conspicuous rise in activity, reaching a maximum with 1-ethylisatin-TSC. This compound had almost three times the activity of the parent compound; although

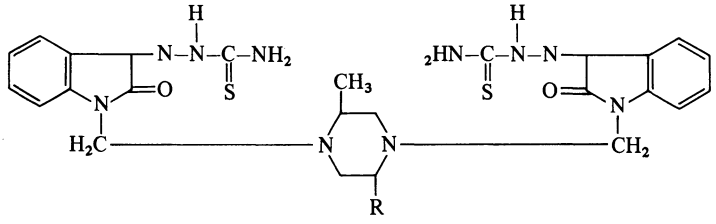


Fig. 9

the *N*-methyl derivative (MIBT) would be used in almost all further studies because of the ease and low cost of production. Activity fell off rapidly with further lengthening of the side chain; the 1-pentyl derivative was practically inactive. More recent attempts to increase activity by substitution at this position have led to the synthesis of *N*-methylthiomorpholine- (CH_2-N⌒S)-IBT (SMEJKAL et al. 1972). It manifested practically the same activity against vaccinia-infected mice as the *N*-methyl derivative. Also examined were a series of 17 mono- and 9 bis-piperazine derivatives of IBT. Studies were carried out in both vaccinia-infected tissue culture systems (BORYSIEWICZ et al. 1973; BORYSIEWICZ and LUCKA-SOBSTEL 1978; ZGORNIAK-NOWOSIELSKA et al. 1973) and mice (ZGORNIAK-NOWOSIELSKA et al. 1976). Both in vivo and in vitro experiments indicated that three bis-piperazine derivatives were the most active (Fig. 9, R = H, CH_3 *cis*, or CH_3 *trans*). In the mouse system, the *N,N'*-bis-(β-thiosemicarbazonemethylisatin)-2-methylpiperazine (Fig. 9, R = H) was more active than the *cis* or *trans* dimethylpiperazine derivatives, but it was slightly less active than MIBT. However, in tissue culture (BORYSIEWICZ and LUCKA-SOBSTEL 1978), MIBT was less active than the Mannich base compounds, with the difference in activity between the three Mannich derivatives being much more pronounced than observed in mice. As stressed in Sect. C, one cannot project in vitro structure–activity relationships to those likely to hold in vivo, or vice versa.

3. Modification of the Pyrrolidine Ring

This showed that the α-carbonyl group (Fig. 8) was essential: 1-acetylindoxyl-TSC (Fig. 10) was inactive, as was isatin-α-TSC (BAUER and SADLER 1960a). Extension of the side chain, as in 1-methyloxindole-3-formyl-TSC (Fig. 11, R_1 = CH_3, R_2 = H, X = =O) resulted in loss of activity. However, indole-3-acetyl-TSC (Fig. 11, R_1, X=H, R_2=CH_3) was found to inhibit vaccinia virus in tissue culture (BUU-HOI et al. 1968) although no animal experiments were reported. Studies on the for-

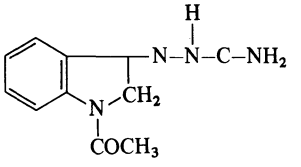


Fig. 10

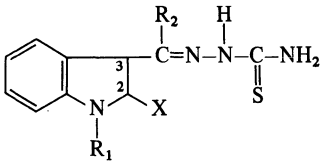


Fig. 11

myl-TSCs were pursued further by ANDREANI et al. (1975, 1978). Their approach was to restore and extend the antiviral activity and spectrum of these compounds by changing the oxygen atom at position 2 (Fig. 11) to another electronegative substituent, and by changing the substituent at position 1 because it was so critical for heightened activity in the isatin series. Of 28 compounds synthesized (ANDREANI et al. 1975), 11 inhibited the growth of vaccinia virus in tissue culture (two of these compounds also inhibited parainfluenza type 3). Under their test conditions, three compounds were even more active against vaccinia than MIBT. These were 1-(*o*, *m*, or *p*-chlorobenzoyl)-2-chloroindole-3-formyl-TSC (Fig. 11, R_1 = CO—  , R_2 = H, X = Cl). Extending these findings with the synthesis of 11 more compounds ANDREANI et al. (1978) concluded that: (a) bromo instead of chloro substitution at position 2 decreased toxicity while retaining antipox activity; and (b) antipox activity was retained with methyl, chloro, and bromobenzoyl substituents at position 1. In this case, as in the initial studies with the chlorobenzoyl substituent, the *meta* positioning of the halogen or methyl group was most active.

4. Modification of the TSC Side Chain

BAUER and SADLER (1960a) showed that modification in any of a number of ways of the TSC side chain led to loss of activity. That methylation at the sulfur atom abolished antipox activity was confirmed in later (TONEW et al. 1974b), more extensive studies on isothiosemicarbazones (see Fig. 13). Further lengthening of this alkyl side chain (Sects. C.V, D.V) led to the emergence of activity in vaccinia-infected mice as well as against the unrelated RNA-containing Mengo virus (VECKENSTEDT and ZGORNIAK-NOWOSIELSKA 1979). After the antiviral spectrum of MIBT was extended to influenza virus (BAUER et al. 1970; Sect. D.III), studies were undertaken by SMEJKAL et al. (1972) to determine if there was potentiation with the well-known anti-influenza drug, amantadine (see Chap. 4). This tricyclic C_{10} primary amine with a very symmetrical structure was combined with MIBT as a single molecule (Fig. 12), but was found to be only moderately more active than amantadine alone. However, MIBT by itself, under the test conditions employed, was even more active against influenza virus.

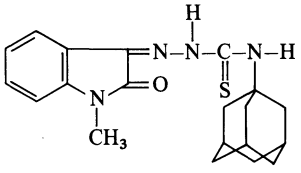


Fig. 12

5. Other Compounds

A number of miscellaneous compounds, including isatin and thiosemicarbazide, were also tested by BAUER and SADLER (1960a) and found to be devoid of activity.

Structural studies on a much smaller scale were also carried out in a poxvirus-infected tissue culture system (SHEFFIELD et al. 1960), as well as in mice infected with various types of poxviruses (BAUER et al. 1962). These confirmed that substitution in the aromatic ring, as well as in the side chain led to loss of activity.

IV. Isatin-β-4′,4′-Dialkylthiosemicarbazones

When BAUER (1955) confirmed the antipox acivity of IBT he showed that the compound was not effective against a number of other murine viruses, including what he believed to be lymphocytic choriomeningitis virus. What was actually tested was pseudolymphocytic choriomeningitis, or ectromelia – a poxvirus (D. J. BAUER personal communication 1975). In 1957 BOCK reported that mice infected with either ectromelia or canarypox were not protected by IBT under conditions which spared neurovaccinia-infected mice. With the realization that ectromelia-infected mice were insensitive to IBT (in tissue culture the compound had very low activity at the limit of detectability; SHEFFIELD et al. 1960), BAUER and SADLER (1960 a) tested this virus along with vaccinia virus against a number of compounds synthesized in their comprehensive studies on structural modification of IBT.

The initial studies showed that monoalkyl substitution at the terminal nitrogen atom of the TSC side chain (R_3 in Fig. 8) resulted in compounds with no activity against either vaccinia or ectromelia viruses. However, further studies on modification of the molecule (BAUER and SADLER 1961) showed that dialkyl substitution (dimethyl, diethyl, dibutyl) at the side chain terminal nitrogen atom (R_3 in Fig. 8) led to high activity against ectromelia and little or no activity against vaccinia. All three derivatives retained activity when methyl or ethyl groups were added to the pyrrolidine nitrogen atom (R_1 in Fig. 8). More detailed studies with the 4′,4′-dialkylthiosemicarbazones (BAUER 1963; O'SULLIVAN et al. 1963) showed that alkylation of the pyrrolidine nitrogen (R_1 in Fig. 8) steadily lowered activity as the carbon chain was lengthened – the exact opposite effect was seen with IBT (Sect. C.III). In a like manner, lengthening of the dialkyl substituents at the terminal nitrogen atom of the thiosemicarbazone moiety decreased activity: the relative effectiveness of the 4′,4′-di-n-butyl compound was six-fold lower than the 4′,4′-dimethyl derivative. Another difference in the effectiveness of the two types of compounds was that with the dialkyl derivatives, mono substitution in the aromatic ring, irrespective of position, produced only small reductions in activity against ectromelia. However, in both series of compounds, disubstitution in the benzene ring abolished activity. These dialkyl compounds were also tested against poliovirus types 1 and 2. Most compounds were inactive against poliovirus type 1, but they were highly effective against poliovirus type 2 (O'SULLIVAN and SADLER 1961). The dibutyl derivative, supposedly because of optimal activity, was chosen for further study (Sects. D.IV, F.IV).

V. Isatin-β-Isothiosemicarbazones

Viral specificity of isatin-β-thiosemicarbazone was markedly changed by dialkyl substitution at the side chain terminal nitrogen atom (R_3 in Fig. 8; see Sect. C.IV). Although initial investigations (BAUER and SADLER 1960 a) showed that methylation at the sulfur atom (a methyl-substituted isothiosemicarbazone, Fig. 13, R_1 = H, R_2 = CH_3, R_3 = H_2) was without activity, more extensive alkylation studies of this type were undertaken with the aim of producing compounds with new virostatic action (TONEW et al. 1974 b; Franke et al. 1975). Confirming previous observations with the benzaldehyde and isatin thiosemicarbazones (Sects. C.I, III),

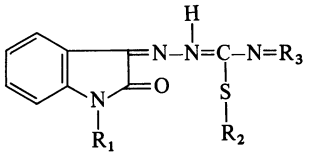
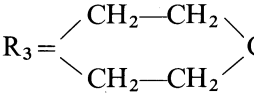


Fig. 13

compounds without sulfur were inactive. Of 24 substituted isothiosemicarbazones synthesized (HEINISCH and KRAMARCZYK 1972) 11 had antiviral activity in tissue culture systems (TONEW et al. 1974b). Of 6 compounds active against vaccinia virus, 5 were also effective against Mengo virus. Derivatives showing this dual activity shared the following structural features:

a. N-Propyl, isopropyl, or n-butyl substituents were present on the sulfur atom (R_2 in Fig. 13)
b. While ethyl substitution at the pyrrolidine nitrogen atom (R_1 in Fig. 13) was more effective than the unsubstituted or methyl-substituted compounds, no activity was found with higher alkylation.

Compounds active only against Mengo virus maintained these structural relationships at the pyrrolidine ring, but methyl, ethyl, or benzyl ($CH_2C_6H_5$) substituents at the sulfur atom also produced active compounds. Furthermore, cyclohexyl substitution at the side chain terminal nitrogen (Fig. 13, $R_1 = C_2H_5$, $R_3 =$  resulted in biologic activity against Mengo virus but not vaccinia virus. Of the 11 compounds active against Mengo virus in tissue culture, 3 (Fig. 13, $R_1 = CH_3$ or C_2H_5, $R_2 = C_2H_5$, $R_3 = H_2$, $R_1 = C_2H_5$, $R_2 = C_4H_9$, $R_3 = H_2$) have been claimed to protect mice against encephalitis induced by Mengo virus (VECKENSTEDT and HORN 1974; VECKENSTEDT and ZGORNIAK-NOWOSIELSKA 1979).

VI. Thiazole Thiosemicarbazones

Antipox activity of thiosemicarbazones was extended to an isothiazolecarbonyl series of compounds (Fig. 14); one of which would be used in clinical trials against smallpox (Sect. H). The most active compound against neurovaccinia-infected mice, of over 40 synthesized (CATON et al. 1965), was 3 methyl-4-bromo-5-formyl-isothiazole-TSC (Fig. 14, $R_1 = CH_3$, $R_2 = Br$, $R_3 = CH = NNHCSNH_2$). Antiviral activity was markedly dependent on the position of the TSC moiety in the isothiazole ring: the 4-formylisothiazole (Fig. 14, $R_1 = CH_3$, $R_2 = CH = NNHCSNH_2$, $R_3 = H$) was marginally active whereas the 3-formyl compound (Fig. 14, $R_1 = CH = NNHCSNH_2$, $R_2 = H$, $R_3 = H$) was totally inactive. In the

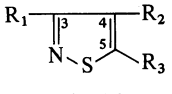


Fig. 14

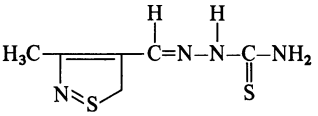


Fig. 15

5-formylisothiazole series, substitution by halogen (Cl, Br, I) in the 4 position increased activity with a decrease in toxicity. The 4-bromo substituent was particularly effective. A 3-methyl substituent decreased toxicity slightly without decreasing activity, and when combined with the 4-bromo substitutent led to the most active compound of the series. Nitro and methyl substituents at the 4 position had little effet on activity, but a carboxy group at this position completely abolished activity. As in the isatin-TSC series, compounds substituted in the side chain were, in general, inactive. Cyclizing the side chain of active formylisothiazoles to mercaptotriazoles or aminothiadiazoles greatly reduced or eliminated activity. A closely related compound (Fig. 15) which was reported to have antipox activity in mice, was 4-methyl-5-formylthiazole-TSC (CAMPAIGNE et al. 1959). This observation has been confirmed in tissue culture using 5-formylthiazole-TSC (KATZ et al. 1974). Little information is available on structure–activity relationships aside from the finding that placing the TSC side chain in the 4 position (α to the ring nitrogen atom; see Sects. C.II, III) resulted in total loss of activity, and that 4-methyl-2-methylthio-5-formylthiazole-TSC was only moderately active (CAMPAIGNE et al. 1959).

VII. Pyrrolidine and Pyrazolone Thiosemicarbazones

Two reports appear to indicate the potential antiviral activity of some pyrrolidine thiosemicarbazones, but the brief nature of the presentations prevents a critical evaluation of the findings. Two TSCs derived from substituted 2,3-dioxypyrrolidines were found to be active against influenza infections in mice (GERZON 1965). These were 1-ethylpyrrolidine-2,3-dione-TSC (Fig. 16, R = C_2H_5) and its β-hydroxyethyl congener (Fig. 16, R = CH_2CH_2OH). The latter was the more effective antiviral agent, a result that was the opposite of the corresponding substitution studies in the antivaccinia isatin series (BAUER and SADLER 1960a). Unlike the isatin compounds, these pyrrolidines had no activity against vaccinia virus.

A series of 13 thiosemicarbazones was synthesized from substituted 3-arylidene-4,5-dioxypyrrolidine nuclei (Fig. 17). Some of these compounds, all of which were tested in tissue culture, were active against vaccinia virus, parainfluenza virus,

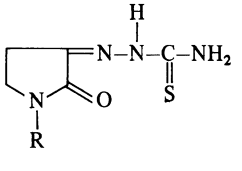


Fig. 16

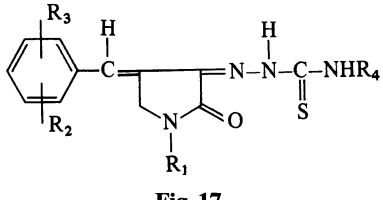


Fig. 17

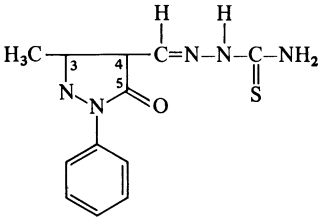


Fig. 18

rhinovirus, coxsackievirus, and influenzavirus (SINGH and SUGDEN 1971). The authors claimed that: none of the compounds was as effective against vaccinia as *N*-methylisatin-β-thiosemicarbazone (Sect. C.III); phenethyl or benzyl substitution at the pyrrolidine nitrogen atom (R_1 in Fig. 17), was necessary for maximal activity while methyl substitution abolished antiviral properties; the nature of the R_2 and R_3 groups (hydroxy, methoxy, halogen) attached to the arylidine ring appeared to have little effect on activity; and substitution of the primary amine group (R_4 in Fig. 17) of the TSC side chain greatly reduced activity. Although no systematic structure–activity studies were carried out on 4-formyl-3-methyl-1-phenyl-5-pyrazolone-TSC (Fig. 18), it was reported to inhibit rhinovirus growth in tissue culture (BUU-HOI et al. 1968).

VIII. Noncyclic Thiosemicarbazones

Cyclic thiosemicarbazones have generally been found essential for antiviral activity. However, there are at least three known exceptions. The first two came to light at the time of intensive investigation of benzaldehyde-TSCs (Sect. C.I). It was found that thiosemicarbazones with an aliphatic oxime nucleus possessed the capacity to protect mice against vaccinia virus (THOMPSON et al. 1953 a). Of six compounds tested, five had antiviral properties. The most effective was butane-2,3-dione-oxime-TSC (Fig. 19, R = H) and the corresponding methoxime (R = CH_3). The structure–activity relationships were not examined in detail but appeared to be similar to the isatin series, in that both oxime and methoxime semicarbazones were inactive. The methoxime was the more active of the two when given intraperitoneally (instead of in the diet). Another observation that the cyclic structure was not essential for antiviral activity was the finding of LEVINSON et al. (1977 a). They showed that kethoxal-bis-(thiosemicarbazone) inhibited the replication of vesicular stomatitis virus in chick embryo culture (Fig. 20). Neither MIBT (Fig. 8) nor 2-

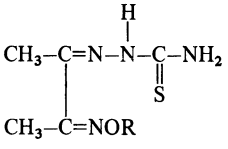


Fig. 19

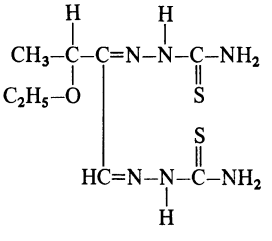


Fig. 20

formylpyridine-TSC (Fig. 4) had any effect on this virus. However, all three compounds were able to inhibit the cell-transforming ability of Rous sarcoma virus (KASKA et al. 1978).

IX. Miscellaneous Thiosemicarbazones

In the isatin series of compounds (Sect. C.III) the carbonyl group at the C-2 position of the pyrrolidine ring was considered essential for biologic activity. However, a series of γ-thiochromanone-4-thiosemicarbazones (Fig. 21) was found to be active against vaccina virus (TSUNODA et al. 1971). Methyl or chloro substitution at the 6 position of the aromatic ring did not alter activity. γ-Thiochromanone-4-thiosemicarbazone appeared to be about as active as 1-methyl-isatin-β-thiosemicarbazone (Sect. C.III) in tissue culture as well as in mice. No additive effect of the two compounds was observed in the animal tests. The thiochromanone-TSC activity in tissue culture has been confirmed (KATZ et al. 1974, 1975).

A single report (BUU-HOI et al. 1968) exists indicating that 3,9-diethyl-6-formylcarbazole-TSC (Fig. 22) inhibits the growth of rhinoviruses in tissue culture, but not influenza or vaccinia viruses.

VARMA and NOBLES (1967) screened indanedione-TSCs for activity in tissue culture against poliovirus type 2, herpes simplex, measles, and influenza viruses. Of six compounds synthesized, two inhibited poliovirus. These were 1,3-indanedione-4′-n-butyl-TSC [Fig. 23, $R_1 = =O$, $R_2 = H$, $R_3 = n\text{-}(CH_2)_3CH_3$] and 1,3-indanedione-4′-methyldithiosemicarbazone (Fig. 23, $R_1 = NNHCSNHCH_3$, $R_2 = H$, $R_3 = CH_3$). The antiviral potential of indanedione dithiosemicarbazones was confirmed by GIANNELLA and GUALTIERI (1970) and by GIANELLA et al. (1973) who synthesized a large number of carbamates, thiosemicarbazones, semicarbazones, and γ-hydroxyiminoketones. Only the dithiosemicarbazone of 2-hydroxyimino-1,3-indanedione (Fig. 23, $R_1 = =NNHCSNH_2$, $R_2 = =NOH$, $R_3 = H$) showed in vitro activity against vaccinia virus. The corresponding semicarbazone was not active. Furthermore, all compounds were ineffective in vitro against herpes simplex and influenza viruses. However, 2-hydroxyimino-1,3-indanedione dithiosemicarbazone was shown to prolong the life of mice lethally infected with influenza A viruses.

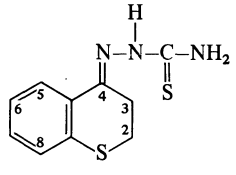


Fig. 21

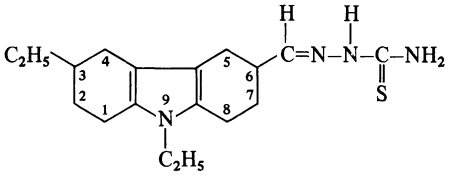


Fig. 22

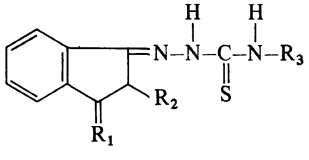


Fig. 23

D. Virus-Inhibitory Spectrum

The ability of thiosemicarbazones to inhibit viruses will be discussed in the order of their general structures as categorized in Sect. C. With few exceptions the effects of specific substituents in a parent compound will not be emphasized. This information can be determined by referring to the appropriate parts of the preceding section.

I. Aryl Thiosemicarbazones

Mice, embryonated chicken eggs, and Maitland cultures derived from them, were found to be protected by specific benzaldehyde-TSCs infected with any of the tested strains of vaccinia virus (Table 1). It was noted (THOMPSON et al. 1953 b) that while benzaldehyde-TSCs having activity in mice usually possessed some capacity to inhibit virus replication in vitro, compounds had been synthesized that were totally inactive in vivo although highly active in vitro. The effect of benzaldehyde-TSCs in rabbits infected intradermally with the IHD strain of vaccinia virus was marginal. THOMPSON et al. (1953 b) reported that, although the number of observations was too small to draw definite conclusions, the impression was gained that treatment did not prevent formation of lesions at the sites of inoculation of virus, although generalization of the infection and development of necrosis at the site of the lesions was prevented.

LUM and SMITH (1957) reported that *p*-nitro-, *p*-hydroxy-, or *p*-methoxybenzaldehyde-TSCs would prevent death in mice infected with influenza A and B viruses. However, HAMRE et al. (1950) could find no murine activity against swine influenza using *p*-aminobenzaldehyde-TSC. Furthermore, BOCK (1957) stated that unpublished data by G. DOMAGK et al. showed that mice infected with an influenza virus were not spared by treatment with an unspecified benzaldehyde-TSC. These discrepancies have remained unresolved and could possibly be explained by chemical, viral, or murine specificities. Mice infected with two members of the Togaviridae family (PORTERFIELD et al. 1978) Semliki Forest (in the genus *Alphavirus*) and

Table 1. Antiviral spectrum of aryl thiosemicarbazones

Compound [a]	Virus	Strain	Host [b]	Primary citation
Bd	Vaccinia	NYBH	Chicken egg, mouse	HAMRE et al. (1951)
		CV II	Chick Mec	THOMPSON et al. (1951)
		IHD	Mouse	THOMPSON et al. (1951)
			Mouse Mec	THOMPSON et al. (1953 b)
		WR	Mouse	BOCK (1957)
Bd, C	Influenca	A, B	Mouse	LUM and SMITH (1957)
A, Bc, Bd, C	Influenza	A, B	Chick Cmc	IWASAKI et al. (1955)
Bc, Bd, C	Newcastle disease	Miyadera	Chick Cmc	IWASAKI et al. (1955)
A	Parainfluenza	1	Tissue culture	RUNTI et al. (1968)

[a] Acetophenone (A), benzalacetone (Bc), benzaldehyde (Bd), cinnamaldehyde (C) thiosemicarbazones

[b] Maitland embryo culture (Mec); chorioallantoic membrane culture (Cmc)

St. Louis encephalitis (in the genus *Flavivirus*) were not protected by treatment with benzaldehyde-TSCs (MINTON et al. 1953). Mice infected with these and a number of other arthropod-transmitted viruses have not responded to treatment with other types of TSCs (Sects. D.II, III).

BOCK (1957) noted that preliminary screening of benzaldehyde-TSCs, done at the time of DOMAGK's discovery of their antibacterial properties, failed to detect activity against two poxviruses; ectromelia and canarypox. Later studies with the isatin series of TSCs would show that ectromelia was insensitive to TSCs that inhibited all other poxvirus murine infections (Sect. D.III). BOCK also reported that in the same studies the disease in mice caused by either lymphocytic choriomeningitis virus or pneumonia virus was not altered by benzaldehyde-TSCs.

II. Quinoline, Pyridine, and Thiophene Thiosemicarbazones

Certain of these heterocyclic compounds were effective against two strains of vaccinia virus and two members of the herpesvirus family (Table 2). Furthermore, isoquinoline- and pyridine-TSCs can inactivate on contact extracellular herpesvirus and Rous sarcoma virus (Table 2; Sect. F.II). While 5-cyanothiophene-2-formyl-TSC (Fig. 7) was unique in its effectiveness against vaccinia virus in rabbits and rats, as well as in mice, it was not active against ectromelia or canarypox viruses (see also Sects. D.I, III for the insensitivity of other TSCs against these viruses). Although active in mice, 4-formylquinoline-TSC (Fig. 5) had limited effectiveness in vaccinia-infected rabbits (THOMPSON et al. 1953b). Poliovirus synthesis in tissue culture has been found to proceed normally (KATZ et al. 1974) in the presence of 1-formylisoquinoline-TSC (Fig. 6) and 5-hydroxy-2-formylpyridine (Fig. 4). In a report dealing with these three types of compounds (MINTON et al. 1953) the general statement was made that thiosemicarbazones were not found to protect mice against St. Louis encephalitis or Semliki Forest virus (Togaviridae, see Sect. D.I).

Table 2. Antiviral spectrum of quinoline, pyridine, and thiophene thiosemicarbazones

Compound[a]	Virus	Strain	Host	Primary citation
P, Q	Cytomegalovirus	NIH	WI-38 cells	BROCKMAN et al. (1970)
P, Q	Herpesvirus	Simplex-HF	HEP-2 cells	BROCKMAN et al. (1970)
I, P		Simplex type 1	[c]	LEVINSON et al. (1974)
I, P	Rous sarcoma	B-77	[c]	LEVINSON et al. (1977b) KASKA et al. (1978)
P, Q, T	Vaccinia	IHD	Mouse	THOMPSON et al. (1953b)
			Mouse mec[b]	
		WR	BSC-1 cells	KATZ et al. (1974)
T	Vaccinia	?	Mouse	ROLLY and WINKELMAN (1972)
			Rabbit	
			Rat	

[a] Isoquinoline (I), pyridine (P), quinoline (Q), thiophene (T) thiosemicarbazones
[b] Maitland embryo culture
[c] Contact inactivation of extracellular virus

III. Isatin-β-Thiosemicarbazones

The most extensive studies on the antiviral spectrum of these compounds have been carried out with isatin-β-thiosemicarbazone and its N_1-methyl derivative. Mice infected with all tested poxviruses, with the exception of canarypox, monkeypox, and ectromelia, will survive when given these drugs (Table 3). As yet, no other in vivo infections with other types of viruses have been convincingly shown to respond strongly to these compounds. Furthermore, as will be shown, many conflicting reports exist concerning the activity of these TSCs against in vitro infection with various viruses.

In 1966 BAUER and APOSTOLOV reported that MIBT at concentrations from 5 to 40 μ*M* could inhibit the replication of adenovirus types 3, 7, 9, 11, 14, 16, 17, 21, and 28 in a HeLa cell tissue culture system. A preliminary study of structure–activity relations of MIBT against these viruses showed that the compound followed the same pattern as that of the poxviruses (Sect. C.III). Replacement of the sulfur with oxygen, substituting two alkyl groups at the terminal amino sequence of the side chain, and changing the methyl group from the N-1 to the C-5 position (Fig. 8) led to loss of activity. Yet, neither HARFORD et al. (1972) nor HERRMANN (1968) could confirm this antiviral activity. Both reports were based on observations in HeLa cells treated with MIBT. In the former case, a 40 μ*M* concentration was used against adenovirus types 2 and 7, while in the latter case, a plaque inhibition test (the drug diffusing from a central disc) was used with adenovirus types 1, 2, 5, and 7. As proposed later (Sect. F), the presence or absence of critical concentrations of divalent cations of first transition series metals should be considered as a possible determining factor in the outcome of these experiments. Isatin-β-thiosemicarbazone has afforded no protection to mice infected with various ar-

Table 3. Response of murine poxvirus infections to isatin-β-thiosemicarbazone and its N_1-methyl derivative

Virus	Strain	Primary citation
Therapeutic		
Variola-vaccinia	Williamsport	MINTON et al. (1953)
Vaccinia	IHD	THOMPSON et al. (1953b)
	WR	BOCK (1957)
Rabbitpox	Utrecht	BAUER and SHEFFIELD (1959)
Cowpox	WCP	BAUER (1961)
	CP	
Alastrim	Schofield	BAUER and SADLER (1960b)
Variola major	Harvey	BAUER et al. (1962)
Noncurative		
Canarypox	Kikuth–Golubschen	BOCK (1957)
Ectromelia	Be	BOCK (1957)
	Sandom	BAUER (1955)[a]
Monkeypox	Copenhagen	CHO et al. (1970)

[a] Ectromelia or pseudolymphocytic choriomeningitis was incorrectly identified in this report as lymphocytic choriomeningitis

Table 4. Studies on herpesvirus sensitivities to isatin or *N*-methylisatin-β-thiosemicarbazones

Virus	Strain	Host [a]	Protection	Primary citation
In vivo				
Cytomegalovirus	Smith	Mouse	No	SCHMIDT-RUPPIN (1971)
Herpesvirus	Simplex	Mouse	No	BAUER and SADLER (1960a)
	Simplex type 1	Rabbit	Marginal [b]	LEVINSON et al. (1974)
Marek's disease	3 K	Chick	Marginal	KUDRIAVTSEV et al. (1977)
In vitro				
Cytomegalovirus	NIH	WI-38	No	BROCKMAN et al. (1970)
Herpesvirus	Simplex	Primary RKF	No	RAPP (1964)
		Primary HTC	Yes	CAUNT (1967)
		HeLa cells	No	HERRMANN (1968)
		HEP-2 cells	No	BROCKMAN et al. (1970)
Infectious bovine rhinotracheitis	Brown	MDBK cells	Yes	Munro and Sabina (1970)
Varicella	Zoster	HeLa cells	No	RAPP (1964)
		Primary HTC	Yes	CAUNT (1967)

[a] RKF = rabbit kidney fibroblast; HTC = human thyroid cells
[b] Thiosemicarbazide used because no suitable vehicle was found for M IBT

thropod-transmitted viruses (BAUER 1955; BAUER and SADLER 1960a). Six viruses from the family Togaviridae (PORTERFIELD et al. 1978) were screened: one from the genus *Alphavirus* (Semliki Forest) and the others from the genus *Flavivirus* (dengue, Ilheus, Nytoya, yellow fever, and Zika). The remaining viruses were in the family Bunyaviridae or were Bunyavirus-like (BISHOP and SHOPE 1979). In the former category were Anopheles A, California, and Wyeomyia; and in the latter category were Anopheles B and Rift Valley fever viruses. The median lethal dose (LD_{50}) of a stock of Rift Valley fever virus was 0.5 log units higher in mice treated with IBT, and this represents the only possible example of slight protection by the drug. BAUER et al. (1970) have reported that the yield of Semliki Forest and Bunyamwera virus in HeLa or monkey kidney tissue culture was reduced by MIBT in a dose-related fashion. The specificity of the antiviral effect was shown by the finding that under similar conditions the yield of another alphavirus (Sindbis) was unchanged in the presence of the drug.

There are conflicting reports concerning the responses of various herpesvirus infections to IBT and MIBT (Table 4). They are too few in number and superficial to enable one confidently to suggest the possible cause of the discrepancies (see the paragraph on adenoviruses in this section). While murine infections with cytomegalovirus and herpesvirus appear to proceed normally in the presence of these compounds, there was marginal activity in rabbits and chicks infected with herpesvirus and Marek's disease virus, respectively. Because they could not find a pharmacologically acceptable vehicle to dissolve IBT or MIBT, LEVINSON et al. (1974) tested the ability of thiosemicarbazide to ameliorate the course of herpes keratitis in rabbits. While there was a statistically significant decrease in the number and severity of dendritic lesions, the effect was much less than that observed with either iododeoxyuridine or proflavine with exposure to light. With these encouraging results,

Table 5. Studies on orthomyxovirus and paramyxovirus sensitivities to isatin or *N*-methylisatin-β-thiosemicarbazones

Virus	Strain	Host	Protection	Primary citation
Influenza	A/NWS	Mouse	No	BAUER (1955)
	A/Singapore	Mouse	Marginal [a]	SMEJKAL et al. (1972)
	A/Hong Kong	Mouse	No [b]	SMEJKAL et al. (1972)
	A/England	Calf kidney cells	Yes	BAUER et al. (1970)
Parainfluenza	1	Calf kidney cells	Yes	BAUER et al. (1970)
Newcastle disease	?	ERK cells [c]	No	SHEFFIELD (1962)

[a] Survival time increased two-fold
[b] A modest reduction in infectious virus was noted
[c] Derived from embryonic rabbit kidney

it would be of interest to repeat the experiments with isatin-β-thiosemicarbazones. In limited studies we have found that these drugs will dissolve in a nonirritating solution composed of 50% polyethylene glycol 400, 40% polypropylene glycol, and 10% ethanol. Using single, or preferably multiple, injections of IBT or MIBT, KUDRIAVTSEV et al. (1977) found retardation of the growth of Marek's disease virus in chicks infected when 1 day old. The morbidity, as measured by the presence of tumors in internal organs, was over two-fold lower in MIBT-treated chicks, but no difference was seen with IBT. Only MIBT lowered mortality, although no data were presented. Furthermore, it appeared that both drugs delayed, but did not eliminate the virus infection. This may have been partially due to the immunosuppressive properties of these compounds (Sect. G), since virus was no longer detectable in most control chickens 40 days after infection.

Tissue culture studies with the herpesviruses using the isatin-β-thiosemicarbazones were initiated by RAPP (1964). Using 4 μ*M* MIBT in a semisolid overlayer (higher concentrations of the drug precipitated), no reduction in plaque number was noted with either herpes simplex or herpes zoster viruses. This observation was confirmed by HERRMANN (1968), using a plaque reduction method relying on diffusion of either IBT or MIBT from an impregnated antibiotic disc. Both CAUNT (1967) and MUNRO and SABINA (1970), using 20 μ*M* MIBT in liquid tissue culture overlayers, found that the yield of herpes simplex and infectious bovine rhinotracheitis were significantly reduced. Unlike most other studies, IBT appeared to be more effective than MIBT against infectious bovine rhinotracheitis (MUNRO and SABINA 1970).

The number of reports (and their brevity) are too small to evaluate critically the lack of effect of the isatin-β-thiosemicarbazones in influenza-infected mice (Table 5). However, BAUER et al. (1970) found that MIBT was effective against influenzavirus and parainfluenzavirus, as shown by a decrease in the ability of virus-infected tissue cultures to hemadsorb red blood cells. The isatin-β-thiosemicarbazones have shown no activity in mice infected with picornaviruses (Table 6). However, in tissue culture, at about 40 μ*M*, MIBT has reduced the yield of a variety of different picornaviruses in a number of cell lines. Perplexingly, two of the four reports dealing with poliovirus indicate that IBT and its N_1-alkyl derivatives, in concentrations as high as 100 μ*M* were without effect on virus synthesis. Again one

Table 6. Studies on the sensitivity of picornaviruses to isatin or *N*-methylisatin-β-thiosemicarbazones

Virus	Strain	Host	Protection	Primary citation
Encephalo-myocarditis	MM	Mouse	No	BAUER (1955)
Foot-and-mouth disease	A 119	Bovine kidney cells	Yes	POLATNICK (1965)
Poliovirus	MEF 1	Mouse	No	BAUER and SADLER (1960a)
	1, 2	Tissue culture	No	O'SULLIVAN and SADLER (1961)
	1, 2, 3	HeLa cells	No	PEARSON and ZIMMERMAN (1969)
	1, 2, 3	HeLa cells	Yes	BAUER et al. (1970)
	1	KB cells	Yes	LWOFF and LWOFF (1964)
Rhinovirus	1059-H	WI-26 cells	Yes	GLADYCH et al. (1969)
	3242-H	WI-38 cells		
	HGP-M			

could suggest that critical concentrations of divalent cations might have been all-important in the outcome of the experiments.

Returning to the poxviruses, MIBT was not effective when administered (by intubation) to rabbits infected subcutaneously with vaccinia virus (HERRLICH et al. 1965; KACKELL et al. 1966). However, the drug had marginal activity if injected intracutaneously at the site of vaccination (KACKELL et al. 1966). Whereas MIBT did not spare mice infected with ectromelia virus (Table 3), the drug in an aqueous overlayer had borderline activity in suppressing cytopathic effects in HeLa cells infected with the virus (SHEFFIELD et al. 1960). Furthermore, when the drug was in a semisolid overlayer, plaque formation by ectromelia virus was almost completely prevented (BAUER 1972). A similar pattern appeared with monkeypox virus. While MIBT was equally effective inhibiting plaque formation by vaccinia and monkeypox viruses in monkey kidney tissue culture cells, it had marginal or nor activity in chicken eggs, mice, and monkeys (CHO et al. 1970). With the exception of the replication of molluscum contagiosum virus in human amnion (FL) cells (FRANCIS and BRADFORD 1976) the growth of all other poxviruses in tissue culture appears to be inhibited by IBT or MIBT. Virtually all vertebrate viruses can be inactivated on contact with N_1-ethyl- or N_1-methylisatin-β-thiosemicarbazone and divalent cations of first transition series metals (Table 7). Of the first transition series metals (Mn, Fe, Ni, Cu, Zn), copper appears to be the most effective (LEVINSON et al. 1971, 1974; LOGAN et al. 1975). Varying amounts of divalent cations are needed for the reaction to proceed. Even though leukoviruses can be inactivated by addition of IBTs only, interaction with metals appears to be required, since chelating agents abrogate the phenomenon in all systems thus far examined (LEVINSON et al. 1973 b; LOGAN et al. 1975), and deliberate addition of divalent cations results in a strong synergistic effect (LEVINSON et al. 1973 b). While metal "contaminants" are all that are required to interact with IBTs to inactivate leukoviruses, all other viruses require more than trace amounts. Such chelating agents as Tris buffer or histidine (in tissue culture media), when present with IBTs, will prevent inactivation of her-

Table 7. Viruses inactivated on contact with isatin-β-thiosemicarbazones and first transition series metals

Genus	Virus	Reference
Alphavirus	Sindbis	Fox et al. (1977)
	Semliki Forest	Hanson (1977)
Flavivirus	Dengue	Fox et al. (1977)
Arenavirus	LCM	Logan et al. (1975)
	Latino	J. Kubis and C.J. Pfau (unpublished work 1975)
	Parana	
	Pichinde	
	Tacaribe	
Enterovirus	Poliovirus type 1	Fox et al. (1977)
	Poliovirus type 2	
Herpesvirus	Herpes simplex type 1	Levinson et al. (1974)
	Herpes simplex type 2	
	Herpesvirus saimiri	
Leukovirus	Rous sarcoma	Levinson et al. (1971)
	Murine leukemia	Levinson et al. (1973a)
	Murine sarcoma	Levy et al. (1976)
	Visna	Haase and Levinson (1973)
	Maedi	
	Progressive pneumonia	
	Feline sarcoma	Levinson et al. (1973b)
Orthomyxovirus	Influenza	Fox et al. (1977)
Paramyxovirus	Newcastle disease	Fox et al. (1977)
Orthopoxvirus	Vaccinia, rabbitpox	Fox et al. (1977)
Reovirus	Reovirus type 3	Hanson (1977)
Rhabdovirus	Vesicular stomatitis, Rabies	Fox et al. (1977)
Bacteriophage	Lambda	Levinson and Helling (1976)

pesvirus (Levinson et al. 1974). The remaining viruses that have been examined (Logan et al. 1975; Fox et al. 1977) are inactivated by IBTs very slowly unless exogenous $CuSO_4$ is supplied. Invariably, inactivation proceeds faster in phosphate-buffered saline than in tissue culture medium. The reaction is also dependent on pH and temperature (Pfau 1977). Even though in vitro the IBTs in the presence of divalent copper can inactivate all viruses thus far used in murine infections treated with the drug (see Tables 3–6; also Pfau 1975; Levy et al. 1976; Bauer and Sadler 1960a for data on LCM, murine leukemia, and rabies viruses), therapeutic activity has only been demonstrated against the poxviruses. This may be due to their unique content of copper (Sect. F). Enhancement of the murine leukemia infection seen with MIBT treatment may be due to the immunosuppressive potential of the drug (Sect. G).

IV. Isatin-β-4′,4′-Dialkylthiosemicarbazones

None of the compounds in the isatin series with unsubstituted thiosemicarbazone side chains were found to be effective against ectromelia or canarypox viruses (Sect. D.III). However, both isatin-β-4′,4′dimethylthiosemicarbazone and its N_1-

methyl derivative (BAUER and SADLER 1961) protected mice against lethal infection with ectromelia (the only other compound claimed to be effective is 5-cyanofuran-2-formylthiosemicarbazone; WINKELMANN and ROLLY 1972). N_1-methylisatin-β-4′,4′-dibutylthiosemicarbazone (Busatin) has been found to inhibit the replication of poliovirus types 1, 2, and 3 (PEARSON and ZIMMERMAN 1969) and inactivate on contact influenza A viruses (BOPP 1976; M. P. FOX and C. J. PFAU unpublished work 1976).

V. Isatin-β-Isothiosemicarbazones

Various isothiosemicarbazones have been found to be active in tissue culture (TONEW et al. 1974 b) and mice (VECKENSTEDT and ZGORNIAK-NOWOSIELSKA 1979) against vaccinia and Mengo virus infections.

VI. Thiazole Thiosemicarbazones

No studies other than on poxviruses have been reported for these compounds.

VII. Pyrrolidine and Pyrazolone Thiosemicarbazones

Pyrrolidine-TSCs have been reported to protect mice against influenza A infections (GERZON 1965). SINGH and SUGDEN (1971) claimed that certain pyrrolidine-TSCs were active in tissue culture against influenza A virus, parainfluenza (type 1), coxsackievirus, and rhinovirus (types 1 and II). Inhibition of rhinovirus in tissue culture has also been reported for a pyrazolone-TSC (BUU-HOI et al. 1968).

VIII. Noncyclic Thiosemicarbazones

THOMPSON et al. (1953 a) found that oxime- and methoxime-TSCs would spare mice lethally infected with vaccinia virus. Kethoxal-bis-TSC has been shown to inhibit the replication of vesicular stomatitis virus in chick embryo cells (LEVINSON et al. 1977 a), and inactivate on contact Rous sarcoma virus (KASKA et al. 1978).

IX. Miscellaneous Thiosemicarbazones

γ-Thiochromanone-4-TSC has been shown to inhibit mouse tail lesion formation by the IHD strain of vaccinia virus (TSUNODA et al. 1971), and to prevent the cytopathic effects in chick embryo cells infected with that strain of virus as well as in HeLa cells infected with the WR strain of vaccinia (KATZ et al. 1974). 6-Formyl-3,9-diethylcarbazole-TSC was active against the HPG strain of rhinovirus when grown in tissue culture (BUU-HOI et al. 1968). Indanedione-TSCs have been found to be inhibitory in tissue culture to poliovirus type 2 (VARMA and NOBLES 1967) and vaccinia virus (GIANNELLA and GUALTIERI 1970). These compounds have also been shown to prolong the life span of mice infected with either the PR8 strain of influenza A or the Lee strain of influenza B (GIANNELLA et al. 1973).

E. Effects on Normal Cells

In the early 1950s, the beginnings of antiviral chemotherapy as we know it today, relatively little attention was paid to the effect of a drug on normal cells. There were a variety of reasons for this. Some of these were: (a) tissue culture model systems were not readily available; (b) appropriate molecular biology technology was not standardly used or developed; (c) such studies were not considered worthwhile until a drug was found to be effective in primates; and (d) there was less intervention by government regulatory agencies than exists today. Thus, in this context, the amount of literature available depends on when the thiosemicarbazone was discovered, how intensively it was investigated, and during what time period this interest was maintained. The various thiosemicarbazones will be discussed in the order established in Sect. C.

I. Aryl Thiosemicarbazones

No qualitative data is available for the effect these compounds have on normal cells. The only measurements to be found are maximum tolerated doses of drug per egg, mouse, or Maitland culture as measured, respectively, by survival time, weight gain, or ability of cells to divide.

II. Quinoline, Pyridine, and Thiophene Thiosemicarbazones

With the exception of two reports to be cited, the same comments as given in Sect. E.I apply here. BROCKMAN et al. (1970) found a correlation between inhibition of ribonucleotide reductase in uninfected tissue culture cells of epidermoid carcinoma origin and inhibition of herpesvirus growth in these cells. Two inhibitors with this dual function, 1-formylisoquinoline-TSC and 2-formylpyridine-TSC, caused no visible cytotoxicity in control cultures over the time period prior to virus harvest. 1-Formylisoquinoline-TSC has recently been found to induce the synthesis of four new proteins in chick embryo cells (LEVINSON et al. 1979). The details of these studies are given in Sect. E.VIII.

III. Isatin-β-Thiosemicarbazones

As stated in Sects. E.I and II, the early work with eggs and mice was rather uninformative with regard to the specific effect of drugs on normal cells. With the first tissue culture studies on IBT (SHEFFIELD et al. 1960), toxicity was determined by following growth of HeLa cells for 7 days in the presence of drug. The maximum tolerated dose was determined as that concentration that would cause minimal morphological changes in the cells when examined under low power (54×) magnification in the light microscope. That concentration was established as 40 μM, eight-fold more than was necessary to decrease vaccinia virus yields by 90% at 18 h after infection. Even though 5 μM IBT had no effect on cell morphology, SHEFFIELD (1962) investigated the metabolism and growth rate of cells in the presence of the compound. The growth rate was determined by counting cells in a hemocytometer after dispersal with trypsin. Total protein content of the cells was also

determined as well as their rate of utilization of glucose. At 40 μ*M* IBT there was no detectable inhibition of cell growth, protein synthesis, or glucose utilization during the first 24-h period. By extending the duration of the experiment to 5 days, very small differences in growth rates became measurable. BACH and MAGEE (1962) noted that the incorporation of tritiated thymidine into uninfected HeLa cells over the course of 12 h was the same in the presence or absence of 10 μ*M* IBT. MIBT has recently been found to induce the synthesis of four new proteins in chick embryo cells (LEVINSON et al. 1979). The details of these studies are given in Sect. E.VIII.

IV. Isatin-β-4′,4′-Dialkylthiosemicarbazones

Although the IBTs had no discernible inhibitory effect on HeLa cells at concentrations well above those necessary to inhibit vaccinia virus (Sect. E.III), this was not the case with the dialkylthiosemicarbazones. At 10 μ*M* MAGEE and BACH (1965) found that N_1-methylisatin-β-4′,4′-dimethylthiosemicarbazone reduced vaccinia virus yield in HeLa cells by 90%, but also reduced tritiated thymidine incorporation into uninfected cells by about the same amount. While studying the inhibitory effect of N_1-methylisatin-β-4′,4′-dibutylthiosemicarbazone on poliovirus synthesis (which does not depend on DNA template function), PEARSON and ZIMMERMAN (1969) found that at 20 μ*M* the compound completely suppressed DNA synthesis within 60 min in HeLa cells. At this time RNA and protein synthesis were little affected, although RNA synthesis became more impaired with time. These inhibitory effects were reversible, for up to 3 h, by washing the cells twice with fresh medium.

V. Isatin-β-Isothiosemicarbazones

TONEW and TONEW (1974) found that tritiated uridine incorporation into the RNA of normal human amnion (FL) cells was reduced by 80% within 6 h after addition of any one of three isothiosemicarbazones (Fig. 13) at concentrations that would almost completely block Mengo virus synthesis in these cells. The isothiosemicarbazones were N_1-ethyl (or methyl)isatin-*S*-ethylisothiosemicarbazone, and N_1-ethylisatin-*S*-*n*-butylisothiosemicarbazone. Since uridine is not a direct precursor of RNA, i.e., it has to enter the cell and then be phosphorylated to uridine-5′-triphosphate, they set out to determine if the effect was due to inhibition of transport or incorporation into RNA. They utilized the previous finding that at low temperatures uridine can be taken up by cells and phosphorylated to the mono-, di-, or triphosphate forms without significant incorporation into RNA. Prelabeling the cells at 16 °C prior to drug addition revealed no diminution, or only a small diminution, of cellular RNA synthesis. This technique was used to show that the compounds had a selective effect on Mengo virus RNA synthesis (Sect. F.V).

VI. Thiazole Thiosemicarbazones

No information is available for the effect of these compounds on normal cells.

VII. Pyrrolidine and Pyrazolone Thiosemicarbazones

No information is available for the effect of these compounds on normal cells.

VIII. Noncyclic Thiosemicarbazones

Kethoxal-bis-thiosemicarbazone (Fig. 20, KTS) has been the only compound in this category to receive attention concerning its effect on normal cells. There is a large literature on the antitumor activity of the compound and in this context its effect on normal cells can be traced through the references cited here. When studying the inhibition of vesicular stomatitis virus (VSV) polypeptides by KTS, LEVINSON et al. (1977a) noted that the drug induced the synthesis of four proteins in uninfected as well as infected chick embryo cells (Sect. F.VIII). Two of these migrated between the L and G viral peptides and two migrated close to the M viral peptide (Sect. F.VIII). Although actinomycin D inhibited their induction, implying the requirement for DNA-dependent RNA synthesis, it blocked neither the synthesis of VSV nor the antiviral effect of KTS. In further studies LEVINSON et al. (1978b) followed the kinetics of synthesis of these proteins and established their molecular weights as 100,000, 70,000, 35,000, and 25,000 daltons. Furthermore, it was clear from their gel analysis that overall protein synthesis was normal, and that under $\times 30$ or $\times 500$ phase contrast microscopy no alteration in the cells could be observed. KTS was also found to induce three or four new proteins of the expected molecular weight classes in human foreskin fibroblasts as well as in mouse and duck embryo cells. Pulse-chase experiments in chick embryo cells revealed neither precursors nor products of these proteins. Since KTS or its copper complex had previously been shown to inhibit nuclear DNA synthesis and also respiration in mitochondria, the authors unsuccessfully tried to induce these proteins, which might have been products to circumvent a blocked metabolic pathway or enzymes with a catabolic function, using cytosine arabinoside, fluorodeoxyuridine, sodium cyanide, or dinitrophenol. The authors next speculated that these four proteins might be drug-detoxifying enzymes (LEVINSON et al. 1978b). Since microsomal mixed-function oxidases are known for their detoxification functions, they tried, again unsuccessfully, to induce the four proteins with sodium phenobarbitol and ethyl alcohol – known inducers of cytochrome microsomal oxidase enzymes. They also considered the possibility that these four proteins were those of endogenous RNA viruses. Although this possibility seemed unlikely since the molecular weights of the induced proteins did not correspond to those of a purified prototype endogenous virus of chicken cells (Rous associated virus) they attempted to determine whether uridine^{3}H-labeled particles with a density of 1.16 were produced in drug-treated cells. No indication of this was found in treated or untreated cells. Another possibility was that the drug–copper complex became bound to DNA and these proteins were induced as a result.This hypothesis was based on their previous observations (MIKELENS et al. 1976) that drug–copper complexes, but not drug alone, were bound to DNA and RNA in vitro. Indeed, LEVINSON et al. (1979) found that a number of chelating agents including 1-formylisoquinoline-TSC (Sects. C.II, D.II), N_1-methylisatin-β-TSC (Sects. C.III, D.III), pyruvaldehyde-TSC, glyoxal-bis-thiosemicarbazone, and a number of other nonthiosemicarbazones would in-

duce these four proteins in chick embryo cells. However, not all chelating agents tested would induce proteins. The determining factor for effectiveness was that the agent be an ionophore for ^{64}Cu. Without coming to any definite conclusions they speculated about the mechanism of induction by copper. LEVINSON et al. (1979) suggested that: (a) copper could interact with sulfhydryl groups in enzymes, and by inactivating them cause their induction (the interaction between copper and thiosemicarbazones, but not semicarbazones, has been described in Sects. D and F; (b) copper could interact with sulfhydryl groups in repressors that become altered sufficiently to allow mRNA transcription for the four proteins; (c) copper could be a cofactor for four metalloenzymes whose synthesis increases when copper concentration increases; and (d) copper could damage DNA sufficiently to induce repair enzymes.

IX. Miscellaneous Thiosemicarbazones

γ-Thiochromanone-1-thiosemicarbazone (Fig. 21; TCT) has been the only compound in this category to receive even superficial attention concerning its effect on normal cells. TSUNODA et al. (1971) noted that 8.4 μM TST showed definte toxicity to chick embryo cells during the first 24 h. No toxicity was observed at 4.2 μM, a concentration that inhibited vaccinia yield by 3 log units 24 h after infection.

F. Mechanism of Action

Most studies have centered on the inhibition of vaccinia virus by isatin-β-thiosemicarbazones. As the molecular strategy of poxvirus infections has been unraveled (MOSS 1974; COOPER et al. 1979), many of the possible modes of action have been eliminated. At this writing we do not know the specific mode of action of any of the thiosemicarbazones. Furthermore, one must keep in mind in the forthcoming discussions that what is found in cultured cells may not reflect what is happening in vivo. This is especially true with the isatin series of compounds which have been repeatedly shown to suppress the immune response (Sect. G). The various thiosemicarbazones will be discussed in the order established in Sect. C.

I. Aryl Thiosemicarbazones

The earliest studies on the mode of antiviral action of the thiosemicarbazones were carried out over 30 years ago with the benzaldehyde series of compounds. Then, only the most basic questions were asked about virus–cell interactions in the presence of these compounds. HAMRE et al. (1950) first suggested that the sparing ability of p-aminobenzaldehyde-TSC (Fig. 1) on vaccinia-infected eggs could not be due to a virucidal effect. They found that the same result was obtained when injection of the drug was delayed until 4 h after infection, compared with the usual 30-min interval. This would also indicate that the compound did not interfere with adsorption of virus to cells. They went on to show (HAMRE et al. 1951) that even when the virus was mixed with 100 times the concentration of drug needed to inhibit vaccinia growth in the chorioallantoic membrane of eggs and held for 2 h at room temperature, there was still no direct inactivating effect. Using benzaldehyde-TSC

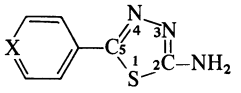


Fig. 24

(Fig. 1) to confirm the sparing ability of this series of compounds in vaccinia-infected mice, THOMPSON et al. (1951) found that there was slightly more inactivation of extracellular virus in the presence than in the absence of the compound. The same result was obtained with the p-acetamido analog, but in both cases the authors appeared to consider the differences insignificant. The same group (MINTON et al. 1953) found that the virus titers in the brains of mice spared by benzaldehyde-TSC were no different from the controls. These results may indicate that the immunopathologic disease pattern was altered (Sect. G), but they suggest strongly that direct inactivation of virus did not occur. Using cinnamaldehyde- or benzalacetone-TSC (Fig. 3), IWASAKI et al. (1955) found that either compound could be added as late as 6 h postinfection to influenza-infected chick embryo Maitland cultures with no decrease in effectiveness. If added at 24 h postinfection (the experiment being terminated after 48 h) the hemagglutination titers were one-half those of the controls. Both cinnamaldehyde- and benzalacetone-TSCs and their p-dimethylamino derivatives had a direct inactivating effect on influenza virus. The virus was suspended in allantoic fluid and the compounds were added at a concentration that would reduce by 80% the hemagglutination titer from infected Maitland cultures. After 24 h at 37 °C the unsubstituted compounds reduced titers by about 2 log units, whereas the dimethylamino derivatives caused about a 1 log unit drop in infectivity. On the other hand LUM and SMITH (1957) found that neither benzaldehyde- nor cinnamaldehyde-TSCs, at maximum nontoxic concentrations, would inactivate influenza viruses. They also observed that the compounds slightly inhibited adsorption of virus to chick chorioallantoic membrane cells, and that there was no noticeable effect on release of newly synthesized virus from cells.

BAUER (1965a) raised the question as to whether the benzaldehyde-TSCs exerted their antiviral activity in the same way as the isatin series of compounds. He focused on the structure of the two groups. The aldehyde-TSCs differ in one marked respect from the TSCs of isatin, which are diketones, in that the side chain contains an extra hydrogen atom. This atom can be cyclized by oxidation with ferric chloride to form a thiadiazole, a reaction that cannot take place with the isatin-β-TSCs. BAUER considered that the cyclization could occur in vivo and that these were the acive compounds. To test this point, benzaldehyde-TSC (Fig. 1) was cyclized to 2-amino-5-phenyl-1,3,4-thiadiazole (Fig. 24, X = C). The compound had antipox activity, but it was somewhat lower than that of benzaldehyde-TSC. The cyclized derivative of 4-formylpyridine, 2-amino-5-(4′pyridyl)-1,3,4-thiadiazole (Fig. 24, X = N), was also active, but again the results did not demonstrate a requirement for cyclization.

II. Quinoline, Pyridine, and Thiophene Thiosemicarbazones

MINTON et al. (1953) found that 5-bromothiophene-2-TSC and 4-formylquinoline-TSC spared mice from lethal infection with vaccinia virus, yet none of the com-

pounds tested was found to reduce the viral content of the brains of these animals consistently. ROLLY and WINKELMANN (1972) confirmed these findings using cyanothiophene-TSCs. These results may indicate that the immunopathologic disease pattern was altered (Sect. G). However, KATZ et al. (1974) found that in HeLa cells there was virtually no replication of vaccinia virus in the presence of 2-formylpyridine-TSC or 1-formylisoquinoline-TSC. The inhibition occurred prior to the synthesis of virus-specific DNA since none could be found in the cells. Vaccinia virus replicates in the cytoplasm (see Sect. F.III for a detailed account of the poxvirus infectious cycle) and thus virus-specific DNA synthesis is relatively easy to follow. This was done by determining the amount of radioactively labeled DNA precursor (thymidine) that was incorporated into cells which had been lysed and centrifuged to remove nuclei. The specificity of the inhibition was shown by the fact that the RNA-containing poliovirus was able to grow normally in cells treated with concentrations of compounds inhibitory to DNA virus replication (KATZ et al. 1974). Furthermore, these findings were well correlated with previously reported observations (BROCKMAN et al. 1970) that these compounds were inhibitors of DNA synthesis.

BROCKMAN et al. (1970), using pyridine- and isoquinoline-TSCs, noted a correlation between antiherpesvirus activity and inhibition of ribonucleotide reductase in the uninfected host cells. Only those compounds examined in which the thiosemicarbazone side chain was affixed α to the heterocyclic ring nitrogen were active in inhibiting both reductase and the herpesviruses. They suggested, and to date there is no support for this, that the activity of ribonucleotide reductase may be the limiting factor in the replication of these viruses. LEVINSON et al. (1974) also studied the effect of these compounds on various herpesviruses. They found that pyridine- and isoquinoline-TSCs could inactivate extracellular herpesvirus on contact as long as the suspending medium was free of agents, such as histidine, capable of chelating divalent cations of the first transition series metals (Sect. F.III). Thus, contact inactivation is an unlikely explanation for the results of BROCKMAN et al. (1970), since these studies were carried out in tissue culture medium containing the blocking amino acids. The same group (HAASE and LEVINSON 1973; LEVINSON et al. 1971, 1973a, b) had found a striking correlation between the ability of various isatin-TSCs to inactivate the infectivity, and reduce virus-particle-associated reverse transcriptase of Rous sarcoma virus and of three slow RNA viruses of sheep. Since MIBT, as well as isoquinoline- and pyridine-TSCs, could inactivate herpesviruses (LEVINSON et al. 1974) a virion-associated RNA or DNA polymerase was sought. Because none was found the site of action was considered to be the nucleic acid template and not the enzyme function since it was shown that MIBT and cupric ions form stable complexes with which nucleic acids associate firmly (MIKELENS et al. 1976). The same binding experiments were carried out with the isoquinoline- and pyridine-TSCs, but no good correlation was found. Although 2-formylpyridine-TSC had the most antiviral activity and complexed well with DNA in the presence of $CuSO_4$, the other TSCs of 5-hydroxy-2-formylpyridine and 1-formylisoquinoline having moderate antiviral activity hardly complexed at all with DNA (LEVINSON et al. 1974; MIKELENS et al. 1976). One could speculate that other metals are involved in the action of these compounds (LEVINSON et al. 1977b). When studying various TSC–metal complexes for their ability to inibit the trans-

forming activity of Rous sarcoma virus and its RNA-dependent DNA polymerase, LEVINSON et al. (1977 b) and KASKA et al. (1978) found that 2-formylpyridine-TSC in the presence of cupric ions was as active as any of the isatin series of compounds that had previously been investigated for these properties. In further studies 2-pyridine-TSC was found to possess these antiviral properties (LEVINSON et al. 1977 b), but its ability to bind to DNA with or without added copper was minimal LEVINSON et al. 1978 a). However, in this regard it was more effective than any of the other thiosemicarbazones when tested in the presence of cobalt (LEVINSON et al. 1978 a). In studying a wide variety of compounds (LEVINSON et al. 1978 a), it became apparent that not all agents active against Rous sarcoma virus bound to nucleic acids and not all ligands that bound to nucleic acids were active against Rous sarcoma virus. Furthermore, some copper-binding ligands were neither active against Rous sarcoma virus, nor were they bound to nucleic acids. At this time there appears to be no simple relationship between the antiviral activity of copper-binding ligands and their nucleic acid-binding ability.

III. Isatin-β-Thiosemicarbazones

Because some of the IBTs spare both mice and humans against smallpox infection, a great deal of attention has been focused on the mode of action of these compounds against poxviruses. All studies have been carried out in tissue culture systems where virus–cell interactions in the presence or absence of drug can be more easily analyzed. The following represents the small amount of data that has led to the generally accepted conclusion that these compounds inhibit virus replication in vivo. MINTON et al. (1953) initially found that while IBT spared mice infected with the Williamsport strain of vaccinia virus, it failed to prevent virus replication as measured in the brain homogenates from these animals. Furthermore, the greatest concentration of virus was present in the brains of treated animals at approximately the same time as those of the untreated mice and the titers were similar. However, in later experiments the same group (THOMPSON et al. 1953 a) showed that serial passage of vaccinia virus in IBT-treated mice led to a gradual decrease in the amount of virus demonstrable in their brains. Specifically, after the fourth serial transfer, virus recovered from untreated mice was comparable in titer to that found in the beginning of the experiment; whereas in treated mice there was a drop of 2.3 log units. In confirming the therapeutic antipox activity of IBT, BAUER (1955) found that under his experimental conditions the drug was much more potent than previously realized. One aspect of this was his finding that on initial exposure to IBT the titers of the IHD strain of vaccinia virus in the brains of treated mice were usually 1 log unit lower than the controls. BAUER and SADLER (1960 a) found that as the chloroform solubility of simple N_1-alkyl derivatives of IBT (see Sect. C.III) increased, decreasing amounts of the compounds were needed to protect vaccinia-infected mice. They speculated that this increased activity could result entirely from their improved lipid solubility, which might enable them to more readily pass the blood–brain barrier and so reach the neurotropic strain of vaccinia (this would also account for the inactivity of compounds bearing strongly polar substituents). Using N_1-ethylisatin-β-thiosemicarbazone, POLLIKOFF et al. (1965) found that vaccinia titers in the brains of treated mice were almost 2 log units lower

than in the controls. The same observation was made by KAPTSOVA and MARENNIKOVA (1967) using N_1-methylisatin-β-thiosemicarbazone and smallpox virus (variola major). Furthermore, histopathologic studies by POLLIKOFF et al. (1965) revealed few recognizable vaccinia virus particles or inclusion bodies (using hematoxylin–eosin or Feulgen reagent staining) in the brains of treated mice.

When the IBTs were found to prevent cytopathic effects in vaccinia-infected tissue culture, studies were begun on the mode of action of these compounds. The initial investigations were carried out by BAUER and his associates (SHEFFIELD et al. 1960). They first established that IBT had no effect on the virus itself. The inactivation kinetics of neurovaccinia and rabbitpox over a 10-h period at 37 °C in the presence of 40 μM IBT (a ten-fold lower concentration would inhibit poxvirus growth by over 90% in cells derived from embryonic rabbit kidney; SHEFFIELD 1962) were identical. This type of result has been repeatedly confirmed (EASTERBROOK 1962; ZGORNIAK-NOWOSIELSKA et al. 1973; FOX et al. 1977), even though contact inactivation of vaccinia and rabbitpox virus by MIBT occurs in the presence of exogenously added $CuSO_4$ (FOX et al. 1977). The latter is a provocative finding because vaccinia contains copper; at least, it has not been eliminated from virus preparations as purification techniques have improved (HOAGLAND et al. 1941; JOKLIK 1962; ZWARTOUW 1964). Although more will be said later in this section about the possible significance of copper in the intracellular inhibition of poxvirus replication by the IBTs, copper associated with the intact virus may be inaccessible to IBT or held in a chelate of higher stability constant.

I will discuss the remaining mode of action studies, not necessarily in the chronology of their publication, but in the chronology of the sequential stages in the poxvirus–cell interaction. These stages will be divided into five general categories essential for virus replication: (1) adsorption, penetration, and uncoating; (2) transcription of the genome into messenger RNA (mRNA); (3) translation of the messages into virus-specific proteins; (4) replication of the genome; and (5) structural proteins and viral assembly.

1. Adsorption, Penetration, and Uncoating

SHEFFIELD et al. (1960) demonstrated that IBT did not interfere with the adsorption of rabbitpox virus to cells. Their protocol was to infect a number of rabbit kidney cell monolayers with a uniform dilution of virus that would yield a high, but countable, number of plaques after incubation for 40 h at 37 °C. In one-half of the plates the virus inoculum contained 80 μM IBT. The virus was then removed from individual monolayers (with or without drug) at 0.5, 1, 2, and 4 h after infection. The plates were exhaustively washed and incubation continued in the absence of drug. The kinetics of adsorption could be established by following the increasing number of plaques that eventually developed as the time interval was increased between inoculum addition and removal. In more extensive studies of the mode of action of IBT, EASTERBROOK (1962) showed that such cells were able to adsorb a second virus challenge. He found that 22 h after infection of KB (human epidermoid carcinoma) cells with vaccinia virus, initiation of the infectious cycle with rabbitpox virus proceeded equally well as in cells never exposed to the drug. GLADYCH et al. (1969), when investigating the inhibition of rhinoviruses by IBT and

MIBT, also concluded that these compounds did not interfere with virus adsorption to human embryonic lung cells.

Poxviruses enter cells by phagocytosis or by fusion with the cell membrane. Constitutive enzymes of these cells carry out the early stages of the uncoating process which involves release of all the phospholipids in the virion as well as one-half of the viral protein. The resulting essentially noninfectious "core" structures at this stage become a transcription factory for the mRNAs encoding the enzymes (among many others) responsible for degrading the cores and completing the uncoating process. These stages in the poxvirus–cell interaction were shown by EASTERBROOK (1962) to proceed normally in the presence of IBT. He found that the rapid decrease in KB-cell-associated virus during the first 7 h after infection (the eclipse phase) was the same with or without drug. This loss in cell-associated infectivity was also apparent even after a second challenge virus infection. Indirect confirmation of this type of result has come from experiments showing that IBT can be added as late as 3–4 h after infection of ERK (human epitheloid carcinoma) cells without any decrease in antipox activity (APPLEYARD et al. 1965). Furthermore, ZGORNIAK-NOWOSIELSKA et al. (1973), using bis-piperazine derivatives of IBT (Fig. 9), observed that these compounds were as effective in L-132 (human embryonic lung) cells whether added 1 or 4 h after the vaccinia virus adsorption period. Employing electron microscopy, LOVAS and HOLLOS (1969) found that the morphology of vaccinia virus during adsorption, penetration, and uncoating was the same in both MIBT-treated and untreated chick chorionic cells.

2. Transcription

The existence of a DNA-dependent RNA polymerase in poxviruses and the clear separation between host and viral transcriptions sites (these viruses multiply in the cytoplasm) has made the study of vaccinia virus-specific mRNAs particularly illuminating. The overall production of poxvirus mRNAs is clearly biphasic. The initial small burst of mRNA synthesis resulting from transcription of cores is followed by a second wave, representing mostly late mRNA sequences the synthesis of which is dependent on viral DNA replication. About 14% of the viral genome is transcribed from cores and extruded by a mechanism which specifically requires ATP. Early and late mRNA sequences can be distinguished by molecular hybridization competition and by differences in sedimentation rates. The early messenger RNAs appear to have a very long half-life compared with late mRNAs. WOODSON and JOKLIK (1965) established that neither the extent of synthesis of vaccinia-specific mRNA (early as well as late), nor its time course, was affected in HeLa cells exposed to IBT. Their methods depended on the fact that in uninfected HeLa cells all RNAs were synthesized in the nucleus. They found that in the cytoplasm of virus-infected cells, rapidly labeled (with uridine ^{14}C) RNAs were synthesized within 1 h having sedimentation coefficients of 10–14 *S*. The sedimentation coefficients of the rapidly labeled RNA formed 3 h after infection were 16–23 *S*. Both types of RNAs, and not 4 *S* transfer RNAs also found in the cytoplasm, were found to hybridize with DNA from purified vaccinia. KATZ et al. (1978b) have confirmed that IBT did not cause a significant change in the sedimentation profile of the early (1 h) and late (5 h) viral mRNA. WOODSON and JOKLIK (1965) also de-

termined that the mRNAs made prior to genome replication were functional in the sense that they could be incorporated into polyribosomes (packets of ribosomes simultaneously translating an mRNA molecule). Polyribosomes obtained from IBT-treated cells were analyzed by sucrose density gradient centrifugation. For the first 2 h after infection, the distribution of these polyribosomes, as determined by optical density and radioactivity of the mRNAs, was similar to that from the drug-free infected cells.

3. Translation

Primary transcription of approximately 14% of the parental DNA by the virion polymerase leads to the synthesis, not only of early enzymes that function in the biosynthesis of progeny poxvirus genomes, but also of a few of the 30 different polypeptides found in the mature virus particle. The early enzymes induced in vaccinia-infected cells include: thymidine kinase, polynucleotide ligase, DNA polymerase, polyadenylic acid-dependent polymerase, a double-stranded-specific exoDNase, and a single-stranded-specific endoDNase. By combining quantitative immunoprecipitation with autoradiography of gel diffusion plates, it has been established that at least two core polypeptides and one surface polypeptide are made prior to viral DNA synthesis. Temporal studies on viral protein synthesis have been carried out by pulse labeling infected cells with radioactive amino acids, followed by analysis using either immunodiffusion or polyacrylamide gels.

In 1965 MAGEE and BACH found that all of the early enzymatic changes that they could measure in vaccinia-infected cells (thymidine kinase and DNA polymerase) proceeded at normal, or nearly normal, rates in the presence of IBT. MAGEE and BACH (1965), and later BABLANIAN (1968), also noted that IBT had no effect on the early cytopathic effect that had been observed by various investigators in different cell lines within 1 h after infection with vaccinia. All future findings would indicate that this rounding of cells was the result of the synthesis of one or more virus-induced early proteins (BABLANIAN 1968). APPLEYARD et al. (1965) observed antigen–antibody precipitation reactions in gels by the method of Ouchterlony (double diffusion in two directions). They found that IBT had no effect on the incorporation of short pulses of ^{14}C-labeled amino acids into whole cells during the first 2.5–3 h. Using the same approach with additional analysis of solubilized radioactively labeled proteins by polyacrylamide gel electrophoresis, KATZ et al. (1973 b) established that IBT had no effect on the gradual transition from the synthesis of host proteins to early viral proteins. Polyacrylamide gel analysis of early vaccinia proteins formed in IBT-treated cells has repeatedly shown the normally expected pattern (PENNINGTON 1977; COOPER et al. 1979). As will be shown later, IBT profoundly effects viral protein synthesis that occurs after replication of the genome. COOPER et al. (1979) addressed themselves to the possibility that the inhibition of protein synthesis was obtained so late in the infection because IBT took many hours to exert its effect. To rule out this trivial explanation fluorodeoxyuridine (FUDR), an inhibitor of DNA replication, was added to infected cultures to prevent late protein synthesis. In normally infected cells, early protein synthesis is inhibited at the translational level, i.e., a product (possibly a protein) of late mRNA (synthesized from progeny DNA template) is necessary for turnoff of

early protein synthesis. Even after 8 h FUDR treatment IBT did not block early protein synthesis.

4. Replication

The synthesis of viral DNA, which requires continuous protein synthesis, takes place in discrete regions of the cytoplasm not intermingled with the usual cytoplasmic organelles. The number of such foci is proportional to the multiplicity of infection (m.o.i.) and at a low m.o.i. only one focus is evident for each cell. Vaccinia DNA synthesis takes place between 1.5 and 6 h after infection, and maturation of the DNA into virions requires a further period of up to 10 h. A convenient way to determine this DNA synthesis quantitatively is to measure incorporation of thymidine ^3H with time into cytoplasmic acid-insoluble material after separating the infected cells into nuclear and cytoplasmic fractions.

In 1962 EASTERBROOK, as well as BACH and MAGEE, concluded that IBT had no effect on vaccinia DNA synthesis. Using tritiated thymidine and autoradiographic techniques, they counted the number of DNA-synthesizing "factories" in the cytoplasm of infected cells. These findings were confirmed by APPLEYARD et al. (1965) who counted cytoplasmic "factories" by using the nucleic acid-binding dye, acridine orange. BACH and MAGEE also followed the temporal uptake of tritiated thymidine into perchloric acid solubilized DNA from the cytoplasm of infected cells. Unimpeded uptake of thymidine into cytoplasmic DNA of vaccinia-infected cells in the presence of IBT has been repeatedly confirmed (WOODSON and JOKLIK 1965; KATZ et al. 1973 a, b). Several investigators considered the possibility that DNA made in the presence of IBT was defective in some way. MAGEE and BACH (1965) first considered that IBT might be bound to DNA. They observed that IBT gave a 3 °C stabilization of the melting temperature of calf thymus DNA, and a very slight shift to higher wavelengths in the DNA absorption spectrum (in the 260–300 nm range). They concluded that direct interaction between IBT and DNA was not likely to be the primary mode of action of this agent. As shown later in this section, IBT in the presence of divalent copper can change the physical configuration of DNA (MIKELENS et al. 1976). Furthermore, later work would confirm a slight change in the adsorption spectrum of DNA (PILLAI et al. 1977) but a profound change in the adsorption spectrum of MIBT (λ_{max}= 356 nm) in the presence of DNA (or bovine serum albumin) and divalent copper (ROHDE et al. 1979). MAGEE and BACH (1965) next considered if IBT effected the "reading" of DNA. They found that the turnoff of early protein synthesis (specifically thymidine kinase), which depends on translation of late mRNA, occurs normally in the presence of drug. The same investigators (BACH and MAGEE 1962; MAGEE and BACH 1965) also determined that the progeny DNA made in the presence of IBT could be packaged into mature virions. They incubated vaccinia-infected cells in the presence of IBT and tritiated thymidine for the first 4 h after infection. The cells were then washed and normal medium was replaced to allow a partial virus yield. After purification, the specific activity (radioactivity/infectious units) of the virus was determined. This, combined with the appearance of the virus in the electrom microscope, led MAGEE and BACH (1965) to conclude that DNA made in the presence of drug could be incorporated into functional particles. WOODSON and JOKLIK

(1965) also questioned if vaccinia DNA made in the presence of IBT could be coated. They defined coating by the loss of susceptibility to DNase. If IBT was allowed to remain for the entire time of the normal growth cycle no more than 15% of the DNA became insusceptible to enzymatic digestion. KATZ et al. (1973 b) confirmed these findings but also noted, as did MAGEE and BACH (1965), that IBT withdrawal during the early part of the growth cycle allowed DNA to be converted to a DNase-resistant form.

5. Proteins and Viral Assembly

Several observations reported in 1962 indicated that IBT affected a late stage in the poxvirus–cell interaction. BACH and MAGEE found that IBT was effective even when added to cells after cessation of a viral DNA synthesis. EASTERBROOK showed that virus-specific antigens responsible for complement fixation and reaction with fluorescein-labeled antibody were produced in IBT-treated cells. He also followed the development of virus by electron microscopy. The first difference between virus-infected and normal cells as seen in the electron microscope is the appearance of "factory" areas in the cytoplasm 2–3 h after infection. They appear as areas of dense granules and randomly oriented threads devoid of mitochondria and other cytoplasmic organelles. The dense material of the "factory" then aggregates into clumps of compact filaments within and around which membranes form. These membranes appear to develop as arc-like fragments at one edge of the condensate and gradually grow to form a spherical surface which eventually completely surrounds the "immature" particle. Subsequently, small foci appear eccentrically within the "immature" particle. These foci probably contain DNA and are thought to the precursors of the prominent dumbbell-shaped core within each mature virus particle. Final maturation leads to a particle which is smaller or more condensed than the "immature" form, but much more complex structurally, with large lateral bodies and an outer double membrane on either side of the core. EASTERBROOK (1962) was the first to note that, in the presence of IBT, vaccinia-infected cells contained few, if any, mature particles. However, these cells contained a large number of immature forms, most of which appeared to have complete membranes. All subsequent electron microscopic studies showed that IBT or MIBT would both delay virion morphogenesis and block it at the "immature" particle stage (HARFORD et al. 1972; LOVAS and HOLLOS 1969; O'SULLIVAN et al. 1964). It has been stated (ZGNORIAK-NOWOSIELSKA et al. 1978) that a derivative of MIBT [*N*,*N*′-bis(β-thiosemicarbazone-methylisatin) -2-methylpiperazine, Sect. C.III] allows formation of mature vaccinia virus particles, not differing morphologically from virions observed in drug-free infected HeLa cells.

KATZ et al. (1978 a) purified immature particles by sucrose density gradient techniques and compared these high density DNA–protein complexes arising in IBT-treated cells to those found under normal conditions. Using methionine ^{35}S labeled protein they found that both types of immature particles sedimented at approximately the same rate. However, tritiated thymidine labeling (KATZ et al. 1978 a) revealed that the immature particles made in the presence of IBT contained very little DNA. Furthermore, using polyacrylamide gel electrophoresis techniques, these investigators also established that there were profound differences in

the polypeptide composition of both types of immature particles. Up to 30 polypeptides can be resolved from detergent-dissociated mature virus by polyacrylamide gel electrophoresis. The sum of the molecular weights of these peptides (ranging from 10,000 to 200,000 daltons) is 2×10^6 daltons or about 20% of the coding capacity of the genome. Three polypeptides account for 35% of the total protein, 8 peptides for an additional 50%, and 19 for the remaining 15%. At least three major late proteins (4a, 4b, and 10) comprising more than one-third of the protein mass of vaccinia virions are formed by cleavage of higher molecular weight precursors.

KATZ et al. (1978a) found that the immature particles formed in the presence of IBT lacked two of the main core polypeptides: 4a and 4b. The KATZ group had previously established by pulse-chase experiments that, while precursors P4a and P4b were cleaved in the presence of IBT to produce the structural polypeptides 4a and 4b (KATZ et al. 1973b), the amounts made were only about 50% of those found in the total cytoplasmic fraction (obtained after boiling with detergent and mercaptoethanol) of cells in the absence of drug (KATZ and FELIX 1977). Furthermore, using quantitative immunoprecipitation and polyacrylamide gel electrophoresis techniques, they found an overall quantitative slowdown in late viral protein synthesis (KATZ and FELIX 1977); a conclusion also reached by PENNINGTON in 1977 (the failure of APPLEYARD et al. 1962, 1965 to observe many late antigens in rabbitpox-infected cells may have been due to the low sensitivity of the immunoprecipitation in agar technique that was used or the inability to detect "insoluble" antigens such as P4a and 4a). Thus, IBT appeared to block the integration of 4a and 4b into the immature particles. This block in core polypeptide integration must be a reflection of the inhibition of either messenger RNA synthesis, the translation process, or interference with processing of precursor. Synthesis of late virus-specific proteins with either enzymatic or structural functions is paralleled by an increase in RNA synthesis and the appearance of mRNA that was not present in the early phase of virus growth. The late mRNA appears to have a very short half-life compared with the early mRNAs. WOODSON and JOKLIK (1965) were the first to examine late mRNAs in vaccinia-infected cells treated with IBT. Under these conditions they found that mRNAs of normal size (16 *S*) and quantity were synthesized and that these mRNAs could be incorporated into polyribosomes. However, these 16 *S* RNAs could only be demonstrated after short labeling periods (3.5 min) with tritiated uridine. Longer labeling periods of 8–13 min produced RNAs with median sedimentation coefficients considerably smaller than 16 *S*, in the neighborhood of 8 *S*. These results fit in with their observations that few polyribosomes were present in cells 4 h after infection in the presence of IBT. WOODSON and JOKLIK questioned whether this was due to lack of formation, or rapid breakdown, of polyribosomes. To explore the latter possibility, cells infected 4 h previsously with vaccinia were exposed to varying concentrations of IBT for 30 and 60 min. Indeed, it was found that polyribosomes were unstable in the presence of the compound (as measured by an increase with time in the number of single ribosomes) in a dose-dependent fashion. KATZ et al. (1978b) confirmed that in the presence of IBT there was a significant decrease of heavy polyribosomes late in infection. Furthermore, using 12-min labeling periods with tritiated uridine they found that late mRNAs synthesized in the presence of IBT appeared smaller or degraded (COOPER et al. 1979).

Utilizing the then recent understanding that eukaryotic mRNAs contain 3′ terminal polyadenylic acid, poly (A), sequences, COOPER et al. (1979) passed the cytoplasmic RNAs through a polyuridylic acid–sepharose column which would retain the mRNAs with poly (A) sequences. In this way it was found that although the total uridine incorporation into the cytoplasmic RNA was unchanged in the presence of IBT (as originally reported by WOODSON and JOKLIK), incorporation into the poly (A) messenger RNA was reduced by about 50%. This mRNA hybridized with vaccinia DNA but some of it appeared smaller or more degraded than that of the control. This group then determined if virus-specific mRNA made in the presence of IBT was methylated. Eukaryotic mRNAs contain 5′ terminal methylated caps and the cap in vaccinia mRNA appears to be required for ribosome binding and efficient translation. Cells 6–8 h after infection were doubly labeled, in the presence or absence of IBT, with uridine ^{14}C and methyl ^{3}H methionine. The RNA was purified by cesium chloride centrifugation and poly (U)–sepharose chromatography, and hybridized to vaccinia DNA. Virus-specific RNA was indeed found to be methylated in the presence of IBT. Since the cells contained considerable amounts of apparently intact virus-specific mRNA, yet late protein synthesis was inhibited by 95% (under their experimental conditions), the authors (COOPER et al. 1979) considered that the mRNA was not translated for some unknown reason. To test this hypothesis a message-dependent, cell-free, protein-synthesizing system was prepared using rabbit reticulocytes. They had previously shown that this system could accurately translate early and late vaccinia virus mRNAs. Using total cytoplasmic RNA obtained from cells at various times late in the infection cycle, they found as much as a 50% reduction in the amount of methionine ^{35}S incorporated into newly synthesized peptides; a figure consistent with the relative amount of intact virus-specific mRNA obtained from the IBT-treated cells. They then determined if the mRNAs isolated from the IBT-treated cells coded for the full spectrum of late virus-specific peptides. Even though total incorporation was reduced by 50%, all polypeptides which could be resolved by the separation methods used were synthesized. However, the synthesis of large polypeptides was reduced more than that of the small polypeptides. They also added IBT directly to the reticulocyte cell-free system containing normal virus-specific mRNA. Even adjusting for the intracellular five-fold increase in concentration of IBT over the extracellular concentration, as found by WOODSON and JOKLIK (1965), translation was not inhibited. As suggested later in this section, copper may possible play a key role in the ability of IBT to inhibit poxvirus synthesis. Thus it would be of interest if these cell-free protein-synthesizing systems could function in the absence of chelating agents so that copper could be deliberately added to the system. COOPER et al. (1979) concluded that further work was necessary to define the mechanism of inhibition of protein synthesis since, in the presence of IBT, approximately one-half of the expected amount of mRNA was capped, properly methylated, polyadenylated, and translatable in a cell-free protein-synthesizing system, yet polypeptide synthesis was inhibited by 95%.

The realization that IBTs could complex with virtually any nucleic acid (MIKELENS et al. 1976) evolved from observations initially made by LEVINSON et al. (1971). While determining if IBTs had any effect on oncogenic viruses they observed that EIBT (the N_1-ethyl derivative) apparently inhibited synthesis of Rous

sarcoma virus in virus-transformed cells. However, sucrose density gradient analysis revealed that particles of Rous sarcoma virus labeled with tritiated uridine were being produced. This paradox was resolved by subsequent experiments in which Rous sarcoma virus was exposed to EIBT prior to infection of cells. These experiments demonstrated that EIBT inactivated the virus on contact. Furthermore, LEVINSON et al. (1973 b) established that either MIBT or $CuS0_4$ could inactivate the virus, and when combined they could inactivate at concentrations where either alone had no activity. In fact, with no deliberate addition of $CuSO_4$ the action of MIBT could be blocked by ethylenediaminetetracetic acid (EDTA), as well as other chelating agents. Thus, LEVINSON et al. (1973 b) considered that MIBT acted as a scavenger (for trace contamination from glassware or media) and vehicle to transport copper to an appropriate site. Parenthetically, $CuS0_4$ alone (or elemental copper in the case of influenza virus) has been found to inactivate on contact herpesvirus (LEVINSON et al. 1974), rabies virus (FOX et al. 1977), Sindbis virus (FOX et al. 1977), and influenza virus (ONG 1958). After inactivation, Rous sarcoma virus was shown still to retain its ability to adsorb to cells (LEVINSON et al. 1973 a).

A striking correlation was found between the ability of various thiosemicarbazones to inactivate the infectivity as well as the virus-particle-associated reverse transcriptase of Rous sarcoma (LEVINSON et al. 1973 b) and visna viruses (HAASE and LEVINSON 1973). For a while it was considered that the contact inactivation of these viruses was a reflection of the thiosemicarbazones and copper to inactivate these enzymes, yet herpesviruses which were lacking this enzyme were still susceptible to the complex (LEVINSON et al. 1974). Further work showed that the Rous sarcoma virus-particle-associated enzymes, lactic dehydrogenase and tRNA nucleotidyl transferase, which do not require template, as well as ribonuclease H which is located on the RNA-dependent DNA polymerase itself, were not sensitive to MIBT (LEVINSON et al. 1973 a; WANG and LEVINSON 1978). This group (MIKELENS et al. 1976; LEVINSON et al. 1977 b) has hypothesized that the mode of inhibition of MIBT on the enzyme activity of the leukoviruses was due to binding of a ligand–metal complex to nucleic acid rather than to enzyme. This was based on their finding that MIBT and $CuSO_4$ formed stable complexes with which any type of nucleic acid of at least 25,000 daltons would firmly associate. This could be quantitatively measured by retention of radioactively labeled nucleic acid on 0.45 μm nitrocellulose filters. Either histidine (at a concentration similar to that in tisue culture medium, see Sect. D.III, or EDTA in a mixture containing MIBT, $CuSO_4$, and nucleic acid would prevent the latter from being retained on these filters. Furthermore, $CuSO_4$ alone (even 1,000 times its effective concentration when combined with MIBT) had no ability to cause trapping of nucleic acid. Other results (FOX et al. 1977) with Sindbis virus and poliovirus also indicated that the possession of virion-associated enzymes was not a prerequisite for contact inactivation and would lend support to the Levinson hypothesis.

When LEVINSON et al. found that nucleic acids bound to MIBT–copper complexes in vitro it was of obvious interest to determine whether the interaction in the test-tube with purified nucleic acids was biologically pertitent. It seemed to be in this sense that MIBT–copper complexes inhibited the transfection of λ phage DNA in *Escherichia coli* (LEVINSON and HELLING 1976). Furthermore, they (LEVIN-

SON et al. 1978a) also attempted to determine whether a nucleic acid–MIBT–copper complex was formed within Rous sarcoma virions when they were exposed under conditions similar to those used to inactivate the biologic activities of the virus. Purified tritiated uridine-labeled Rous sarcoma virus was exposed to $CuSO_4$, MIBT, or a combination of the two. After incubation, the virus was disrupted and excess calf thymus DNA was added to react with any originally unbound MIBT–copper complexes. The samples were then centrifuged in a sucrose density gradient. As in the nitrocellulose filter technique, these experiments demonstrated that MIBT–copper complexes changed the configuration of virus nucleic acid. While $CuSO_4$ had little effect on the sedimentation of Rous sarcoma virus RNA, MIBT treatment resulted in the loss of approximately 50% of the RNA in the normally expected profile. Treatment with both compounds resulted in the complete loss of the RNA. Presumably the RNA was pelleted, rather than degraded, since none of the radioactivity was at the top of the gradient. Furthermore, EDTA added directly to the virus after exposure to MIBT and $CuSO_4$ completely prevented the loss of RNA in the gradient.

Since IBT and its N_1-alkyl derivatives in the presence of divalent copper directly interact with virtually all viruses and nucleic acids, it appears that a good deal of significance should be attached to the ability of the IBTs to be effective in vertebrates against only one family of viruses, the poxviruses. No other viruses but the poxviruses have been reported to contain copper (HOAGLAND et al. 1941; JOKLIK 1962; ZWARTOUW 1964) and this may be what sets them apart in their in vivo sensitivity to the IBTs. THOMPSON et al. (1953a) were aware that thiosemicarbazones were chelating agents and they attempted to determine, not if metals would enhance the antipox activity of these compounds, but rather if they would inhibit their activity in mice. A number of substances, including monovalent cuprous chloride, were found to have no influence on the activity of various thiosemicarbazones. When BAUER (1955) first confirmed the antipox activity of IBT in mice he noted that the maximum degree of protection was obtainable with a quite small dose, and that the effect could not be substantially increased by greatly increasing the dose (or by daily dosage; POLLIKOFF et al. 1965). He felt that the possible limitation of the therapeutic effet was brought about by the presence in the brain (where virus was injected) of a fixed concentration of some substance essential for the activity of IBT. He speculated that a *host* factor, possibly copper or some other metal, might form chelate complexes with IBT and this might be the active compound.

Recognizing that a single report existed (HOAGLAND et al. 1941) concerning the copper content of vaccinia, BAUER (1958) proposed that copper from the virus might somehow play a role in the inhibition of cellular function. He felt that copper salts or compounds, deliberately added to the diet of vaccinia-infected mice might have antiviral activity by competing with the copper from the virus. Indeed he found that copper in the divalent, but not the monovalent, state would prolong the survival time of vaccinia-infected mice by 20%–50%. Divalent copper had no effect on 23 other murine viruses. When BAUER and SADLER (1960a) established that sulfur in the side chain of IBT was necessary for antipox activity, they felt that its ability to chelate metals was a key factor. They suggested that since vaccinia was thought to contain copper it was this circumstance that brought about the selective

therapeutic effect of IBT on neurovaccinia. The present author's hypothesis is that IBTs interfere with late mRNA stability or function because it is only late in the infection when copper is brought into the "factory" or "virosome" areas of the cytoplasm to be incorporated into maturing virions. To support or refute this hypothesis the copper content of mature vaccinia virions could be reestablished using better virus purification and analytical chemistry techniques than were available 15 years ago. The copper content of virus at various stages in the maturation process could also be determined. One wonders whether IBT-resistant mutants of poxviruses (see Sect. H) contain copper. In this regard it is of interst to note that IBT-resistant mutants of both vaccinia and rabbitpox virus lose their resistance to the drug when grown in the presence of the wild-type virus (APPLEYARD and WAY 1966; KATZ et al. 1973c).

IV. Isatin-β-4′,4′-Dialkylthiosemicarbazones

Unlike the monoalkyl or unsubstituted thiosemicarbazones, these compounds are able to protect mice against lethal infection with ectromelia viruses. Isatin-β-4′,4′-dimethylthiosemicarbazone has been clearly shown to block virus replication as measured in the brains of treated mice (O'SULLIVAN et al. 1963). Under the same conditions isatin-β-4′,4′-tetra- or pentamethylenethiosemicarbazone has been found to arrest virus synthesis (BAUER 1963). Although only one published report deals with the mode of action of these dialkylthiosemicarbazones, all interpretations would be clouded because the exquisite poxvirus specificities of the IBTs and dialkylthiosemicarbazones demonstrable in mice (Sect. C.IV) break down in tissue culture (Sect. D.III). MAGEE and BACH (1965) found that, unlike the IBTs, *N*-methylisatin-β-4′,4′-dimethylthiosemicarbazone inhibited both vaccinia and cellular DNA synthesis, the former being more sensitive than the latter.

In tissue culture studies, *N*-methylisatin-β-4′,4′-dibutylthiosemicarbazone was shown by PEARSON and ZIMMERMAN (1969) to be equally effective in inhibiting the replication of poliovirus types 1, 2, and 3. It did not appear to block virus adsorption or penetration, because it could be added to cells 30 min after infection. In fact, the compound could be added at any time during the growth cycle of the virus and would rapidly prevent further virus replication without inactivating previously synthesized virus. Using actinomycin D to unmask virus specific RNA synthesis in HeLa cells, PEARSON and ZIMMERMAN (1969) found viral RNA to be completely blocked within 1 h after addition of the compound, even when viral RNA was being synthesized at a maximal rate. This inhibition occurred before viral polyribosomes were degraded and protein synthesis slowed. Using an in vitro cell-free poliovirus polymerase reaction, they showed that the compund could inhibit the incorporation of uridine ^{3}H 5′triphosphate into acid-precipitable material.

N-methylisatin-β-4′-4′-dibutylthiosemicarbazone has been found to inactivate on contact influenza A (strains WSN and PR8) and B (strain Lee) viruses (BOPP 1976; M.P. FOX and C.J. PFAU, unpublished work 1976). These viruses fully retained their hemagglutination activity. Neuraminidase activity, tested only for the A viruses, was also found to be unchanged (PFAU 1977). In view of the data presented in Sect. F.III (and the poliovirus results) this may indicate that these dialkylthiosemicarbazones also effect nucleic acid function.

V. Isatin-β-Isothiosemicarbazones

A series of studies by E. and M. TONEW and co-workers centered around the mode of action of these compounds against Mengo virus replication in human amnion (FL) cells. The initial studies (TONEW et al. 1974 b) using N_1-ethylisatin-*S*-ethylisothiosemicarbazone (Fig. 13) showed that the compound had no virucidal effect on this RNA-containing virus. Furthermore, it did not intefere with the adsorption or penetration of the virus. The former was measured by the amount of virus that could be washed from the cells after the virus adsorption period. The latter was measured by the loss of ability of virus-specific antibody to neutralize cell-adsorbed virus. Extending these studies to include N_1-methyl-isatin-*S*-ethylisothiosemicarbazone and N_1-ethylisatin-*S*-*n*-butylisothiosemicarbazone TONEW and TONEW (1974) found that these compounds inhibited uridine transport in uninfected FL cells (Sect. E.V). If a nucleotide pool was established in FL cells by allowing uptake of tritiated uridine to occur at 16 °C for 1 h prior to drug addition, incorporation of radioactivity into RNA proceeded normally for a period long enough to follow viral synthesis. Using this technique with Mengo virus-infected cells, along with actinomycin D treatment to unmask virus-specific RNA, they found that the isothiosemicarbazones prevented viral RNA synthesis. These investigators then examined Mengo virus-specific RNA-dependent RNA polymerase activity in FL cells as well as in a cell-free system. No polymerase activity was detected in infected cells treated with these compounds. In the in vitro cell-free polymerase assay (TONEW et al. 1974 a), working concentrations of the drugs inhibited uridine ^{14}C triphosphate incorporation into acid-precipitable material by 50%. Furthermore, UV absorption maxima of these compounds at 360 nm were unaffected by addition of nucleic acids, but showed enhanced optical densities and slight shifts toward red in the presence of protein. TONEW et al. (1974 a) felt that these results excluded the possibility that the compounds were inhibitory because they bound to nucleic acids, but rather that complexes of enzyme components and isothiosemicarbazones were involved in the inhibition of RNA polymerase.

VI. Thiazole Thiosemicarbazones

All mode of action studies have been carried out with 3-methyl-4-bromo-5-formylisothiazole-TSC, one of two thiosemicarbazones to reach clinical trials (Sect. H). Like IBT (Sect. F.III), it protected mice against lethal infection with vaccinia virus, even though fully infectious virus could be recovered from the brains of treated mice – the difference in titers between control and treated mice being over 2 log units (RAO et al. 1965). SQUIRES and MCFADZEAN (1966) summarized evidence (without presenting experimental data) that led them to suggest the possible involvement of an interfering substance induced by the drug–virus combination. This hypothesis was based on four observations. (1) Mouse brain suspensions were prepared from three groups: one treated with drug alone, one infected with vaccinia alone, and one receiving both virus and drug. The suspensions were clarified by centrifugation sufficient to pellet virus, and the supernatants were injected intradermally into mice. Dermal vaccinia was then scarified over the injection site. The virus "took" over the sites injected with material from the first two prepara-

tions, but not over the site from the third (combination) preparation. (2, 3) SQUIRES and MCFADZEAN (1966) assumed that the interfering substance in their brain homogenates might be interferon. Since it had been reported that either steroids or high oxygen tension were generally considered to reduce interferon synthesis SQUIRES and MCFADZEAN determined what effect they would have on the efficacy of isothiazole-TSC. Both cortisone treatment and increased partial pressure of oxygen virtually eliminated the protective activity of the drug. Concurrent research by LIEBERMAN et al. (1966) showed that cortisone had no effect on the antipox activity of EIBT. (4) They had shown previously that the isothiazole-TSC was completely inactive in mice infected with the RNA-containing encephalomyocarditis (EMC) virus. Yet isothiazole-TSC-treated mice which survived an otherwise lethal vaccinia virus infection were more resistant to challenge with EMC virus than the controls. Critical experiments to prove or disprove the interferon hypothesis were not performed. Since the hallmark of interferon production in virus-infected animals is a marked reduction or elimination of virus, a 1 log unit reduction in brain virus (if the isothiazoles act like the IBTs, see Sect. H.II) does not support the hypothesis. Parenthetically, interferon could not be detected in the brains of vaccinia-infected mice treated with the N_1-ethyl derivative of IBT, but was present in the absence of drug (POLLIKOFF et al. 1965).

Using tissue culture APPLEYARD et al. (1965) investigated the mode of action of 3-methyl-4-bromo-5-formylisothiazole-TSC at the same time they investigated IBT. Using techniques described in detail in Sect. F.III, they showed its action to be similar to that of IBT in all respects that were tested. The compound prevented formation of the same viral antigens as did IBT; it did not interfere with viral DNA synthesis; and it inhibited virus growth to the same extent as IBT in several cell lines. Another indication that the mode of action of both types of compounds might be similar was the finding that IBT-resistant mutants of vaccinia virus were almost as refractory to the isothiazole-TSC (APPLEYARD and WAY 1966).

VII. Pyrrolidine and Pyrazolone Thiosemicarbazones

No mode of action studies have been reported for these compounds.

VIII. Noncyclic Thiosemicarbazones

LEVINSON et al. (1977 a) found that kethoxal-bis-thiosemicarbazone (KTS, Fig. 20) inhibited the replication of vesicular stomatitis virus (VSV). VSV is a negative-stranded RNA virus, that is its genome has none of the hallmarks of an mRNA [3′-poly (A) sequences and a capped 5′ end, see Sect. F.III]. Five capped, poly (A)-containing RNA species which anneal to virion RNA can be resolved in infected cells. In a cell-free system, these mRNAs can direct the synthesis of each of the five proteins (L, G, N, NS, and M) which make up the intact virus particle. The enzyme responsible for transcribing the mRNAs is contained within the virion. This transcriptase is easily demonstrated in vitro. Unlike the results with IBTs and Rous sarcoma virus (Sect. F.III), KTS had no effect on the VSV polymerase. However, using labeling and polyacrylamide gel electrophoretic conditions that would differentiate three of the VSV mRNAs, LEVINSON et al. (1977 a) found that KTS would

completely inhibit their synthesis. The synthesis of all classes of protein was also inhibited. Pretreatment with the drug had no inactivating effect on the virus itself but rather enhanced subsequent virus replication. Since the chelating agent EDTA would produce the same effect it was felt that KTS was removing a toxic heavy metal cation. KTS has also been shown to inhibit the transforming activity and reverse transcriptase activity of Rous sarcoma virus (KASKA et al. 1978). On the basis of studies with Rous sarcoma virus and other thiosemicarbazones (Sect. F.III), it may be that KTS inhibits template rather than enzyme function.

IX. Miscellaneous Thiosemicarbazones

γ-Thiochromanone-1-thiosemicarbazone (TCT, Fig. 21) was shown to inhibit vaccinia virus replication beyond the adsorption–penetration–uncoating stage because it could be effectively added to chick embryo cell cultures up to 8 h after infection (TSUNODA et al. 1971). KATZ et al. (1975) using techniques developed in studies on the mode of action of IBT against vaccinia virus in HeLa cells (Sect. F.III) found that TCT did not inhibit the replication of vaccinia DNA. However, as shown by prolonged sensitivity to deoxyribonuclease, it was not incorporated into maturing virions. Although both early and late viral proteins were produced in cells treated with TCT, the cleavage of a later precursor peptide (P4a) into the structural peptide 4a (Sect. F.III) was largely inhibited. In biochemical terms, not only were the modes of action of TCT and IBT similar, but there was also good cross-resistance between mutants that had been isolated which were capable of growing in the presence of one drug or the other.

G. Animal Studies

The distribution and metabolism of thiosemicarbazones with antitumor activity have been extensively investigated (AGRAWAL and SARTORELLI 1978). However, as they relate to antiviral studies, the thiosemicarbazones have received little or no attention with regard to metabolic transformations of the compounds, adsorption from various sites of administration, pharmokinetics, or pharmodynamics. The main emphasis here will be to review the evidence that indicates the potent immunosuppressive nature of thiosemicarbazones. This is stressed since it is now known that the intensive meningitis produced in mice after intracerebral inoculation of vaccinia or ectromelia virus is mediated by T-cells. Thus, the thiosemicarbazones may act (at least in mice) as a two-edged sword, inhibiting both target cells by suppressing virus synthesis and effector cells by blocking lymphocyte proliferation.

I. Aryl Thiosemicarbazones

In the early work on benzaldehyde thiosemicarbazones (Fig. 1), it was noted that the concentrations of this compound in the diet, necessary to spare mice from otherwise lethal intracerebral injection with vaccinia virus, would prevent their normal increase in weight (THOMPSON et al. 1953b). If we assume that toxic substances are especially effective against rapidly dividing cells (as is the case in cancer chemotherapy), then the thiosemicarbazone could have altered lymphocytes that

were proliferating in response to stimulation by the virus. Since the virus titers in the brains of these spared mice were not consistently lower than those in untreated mice (MINTON et al. 1953), and since the inflammatory response in the meninges is T-cell-mediated (see Sect. G.III), it is conceivable that sparing was due to immunosuppressive and not antiviral activity of the compound.

II. Quinoline, Pyridine, and Thiophene Thiosemicarbazones

MINTON et al. (1953) found that, when 5-bromothiophene-2-formylthiosemicarbazone was introduced into their diet, mice could be spared from otherwise lethal intracerebral injection with vaccinia virus. Yet the virus titers in the brains of these mice were not consistently lower than in those of the untreated group. The same observations were also noted with 4-formylquinoline-TSC (Fig. 5). These workers (THOMPSON et al. 1953 b) also noted that each compound in a series of 5- or 3-substituted thiophene-2-formylthiosemicarbazones was quite toxic when given parenterally. As discussed in Sect. G.I and in the next paragraph, these mice may have survived the virus infection, not so much by the antiviral properties of the compounds, but by their ability to compromise the immunologic reaction to the infection.

Using 5-cyanothiophene-2-formylthiosemicarbazone (Fig. 7) at twice the dose (76 mg/kg) necessary to protect 100% of mice receiving 10^3 LD_{50} vaccinia virus (BORYSIEWICZ and TADEUSIEWICZ 1976), ZGORNIAK-NOWOSIELSKA et al. (1978) investigated the compound's ability to suppress both humoral and cell-mediated responses. The humoral response was measured by injecting compound subcutaneously into three inbred strains of mice in a schedule identical to that used in antiviral studies: twice a day for 4 days beginning immediately after antigen injection – in this case sheep red blood cells (SRBC). On day 5, lymphoid cells were removed and the number producing IgM immunoglobulins against SRBCs was determined by the in vitro "direct" Jerne hemolytic plaque assay. Although minor differences were found, there was about a 50% reduction in plaque numbers in relation to the control groups in all three strains of mice receiving the compound. This decreased primary immune response was also reflected by the numbers of IgG-producing cells (most immunogens elicit detectable antibodies of the IgM class before those of the IgG class) as measured by the "indirect" Jerne SRBC–hemolytic plaque assay. The effect of the compound on the secondary immune response was determined by reinjecting SRBCs into previously drug-treated mice on day 9 and removing lymphocytes on day 13. Again the number of IgM-producing cells detected by the Jerne assay was about 55% below that of the control group. The cell-mediated immune response in mice was assessed by allergic contact dermatitis to 2-phenyl-4-ethoxymethyleneoxazolone (oxazolone). The schedule of administration of the thiosemicarbazone to mice was the same as that used in the humoral response studies. The thickness of the ear was measured before and 24 h after painting with oxazolone (on day 7). Up to a 70% decrease in swelling was noted in mice treated with the thiosemicarbazone. In earlier studies BORYSIEWICZ and TADEUSIEWICZ (1976) noted that the relative activity of 5-cyanothiophene-2-thiosemicarbazone, as measured by survival time of infected mice, decreased above 20 mg/kg body weight.

III. Isatin-β-Thiosemicarbazones

The initial quantitative studies on the ability of thiosemicarbazones to inhibit the immune response in mice were carried out by McNEILL (1972, 1973) and McNEILL et al. (1972). The humoral response to sheep red blood cells (see Sect. G.II for details of the assay procedure) was measured 5–7 days after a single injection of cells and daily injection of 1 mg/kg body weight of MIBT (Fig. 8). McNEILL et al. (1972) found that the number of IgM-producing lymphocytes was reduced almost 5-fold compared with the number found in drug-free mice. There was almost a 20-fold difference when the number of IgG-producing cells was determined. In line with these observations, the amount of circulating antibody against SRBCs 5 days after beginning the drug treatment was 15-fold lower than in the control group of mice. Since antibody-secreting cells are derived from the bone marrow, McNEILL et al. (1972) next determined that the total number of cells which could be flushed from femurs was the same whether or not mice had received six daily injections of MIBT. They also established the number of cells in these preparations capable of forming colonies in a semisolid suspension cell assay. Again, very little numerical difference was seen in these cells which are generally regarded as specific progenitors of granulocytes and macrophages. However, using colony forming cells from the spleen, a marked decrease was noted in the drug-treated group. NcNEILL (1972) went on to show that MIBT added directly to the semisolid suspension cell assay would inhibit development of colony formation. Although MIBT reduced the number of colonies, it did not affect the size of the colonies that did develop. He felt that this indicated a selective mode of action of the drug, although there was no change in the relative frequency of granulocyte and macrophage colonies. McNEILL (1973) extended these studies on colony inhibition, using 25 derivatives of IBT (see Sect. G.IV). He found that EIBT was as effective as MIBT but that azo and iodo substituents on the aromatic ring substantially reduced activity (a finding parallel to the compounds ability to spare mice after vaccinia infection, Sect. C.III).

NcNEILL et al. (1972) questioned if the ability of MIBT to compromise the immune system might play a role in its antipoxvirus activity. The poxvirus chosen for study was ectromelia because the bone marrow (and later the spleen) can become heavily infected with virus; and MIBT does not spare mice from the infection (BAUER and SADLER 1961). They assumed that the latter result meant that the drug had no effect on virus replication and would make explanation of the results less complicated. However, interpretation of the experiment was not clear since low concentrations of drug (0.5 mg/kg) had no effect on the death rate in ectromelia-infected mice (33%) while a higher dose (2 mg/kg) increased mortality somewhat (50%). Using experimental protocols and obtaining results almost identical to those found when studying 5-cyanothiophene-2-formylthiosemicarbazone (Sect. G.II), it was established that MIBT inhibited both the B-cell and T-cell response (BORYSIEWICZ et al. 1977; ZGORNIAK-NOWOSIELSKA et al. 1978). The B-cell, or humoral response, was measured in the same way as reported by McNEILL et al. (1972) except that mice received 20 mg kg^{-1} day^{-1} MIBT after a single injection of SRBCs. The T-cell, or cell-mediated response was measured by the degree of allergic contact dermatitis provoked by oxazolone, using the same regimen of drug

administration as previously described. Both ectromelia and vaccinia virus multiply in the meninges and ependyma of mice after intracerebral inoculation. Meningitis is characterized by perivascular cuffing and marked infiltration of mononuclear cells (the category of cells including the B- and T-lymphocytes, monocytes, and macrophages). HAPEL and GARDNER (1974) demonstrated that the severity of meningitis in mice infected with ectromelia coincided with the number of cells in the cerebrospinal fluid (CSF) capable of destroying ectromelia-infected tissue culture cells labeled with ^{51}Cr. These CSF cells with cytolytic activity were shown to be T-lymphocytes because they possessed a surface antigen (θ) not found on B-cells or macrophages. The critical role of these T-cells in disease was further substantiated by treatment of ectromelia-infected mice with antithymocyte serum (the thymus being the site of T-cell differentiation). There was a marked reduction of T-cells in the CSF after treatment, and the normal 35% mortality was reduced to zero. Essentially the same conclusions were reached by MORISHIMA and HAYASHI (1978) while studying vaccinia meningitis. Their study was even more persuasive than HAPEL and GARDNER's since cytotoxic T-cells (measured in a ^{51}Cr release assay using vaccinia-infected tissue culture cells as targets) were obtained directly from the meninges.

HERRLICH et al. (1965) noted that, like benzaldehyde-TSC and 4-formylquinoline-TSC (THOMPSON et al. 1953 b), MIBT did not prevent lesion formation in rabbits after intradermal inoculation of vaccinia virus. Drug was given twice a day, by intubation, for 10 days at concentrations up to 300 mg kg^{-1} day^{-1} (the acute oral LD_{50} of MIBT in rabbits is in excess of 2,000 mg/kg; BAUER 1977). They noted that above 100 mg/kg the weight of the rabbits after 10 days was 20%–30% lower than the control animals. Also, above 100 mg/kg the hemagglutination antibody titer against the virus was as much as 15-fold lower than in drug-free animals. KACKELL et al. (1966) confirmed the lack of activity of MIBT against intradermal vaccinia virus except if the drug was injected at the site of vaccination. Giving MIBT by intubation for 6 days at 100 mg kg^{-1} day^{-1}, they measured the antibody response to the virus at the end of the second week of infection (during an initial 5-day observation period the rabbits were viremic). They found that the vaccinia hemagglutination antibody titer was reduced 20-fold while the neutralizing antibody titer was lowered 8-fold.

There are other examples, beside the response to poxviruses, where MIBT may influence the disease process by altering the immune response to the infection. The natural history of the F1 hybrid of New Zealand Black and New Zealand White mice is characterized by the appearance of serum antinuclear antibodies, and the development of proteinurea and fatal glomerulonephritis. This renal disease is believed to be caused by the deposition of antibody–antigen–complement complexes in the glomeruli. GABRIEL (1971) found that such mice, if given MIBT (100 mg/kg by weekly injections, starting within 24 h after birth and continuing for the rest of their lives), would develop this autoimmune disease much more slowly than the control group. For example, at 10 months 75% of the control group had died while only 25% of the drug-treated mice had expired. While studying the ability of MIBT to inactivate on contact both the transforming ability and RNA-dependent DNA polymerase of murine leukemia virus (Sects. D.III, F.III), LEVY et al. (1976) found that MIBT enhanced rather than inhibited development of leukemia in the mouse.

They found that, 17 days after injection with Friend (leukemia) virus, the spleens of MIBT-treated mice weighed almost twice as much as those from mice receiving virus alone. Since the Friend virus preferentially replicates in B-cells, one wonders if the increased weight of the spleen was due to an increasing number of B-cells which might occur if the drug selectively destroyed T-cells (an overabundance of B-cells is seen in some children with thymic hypoplasia); or an increased susceptibility of B-cells to the infection.

Since LEVINSON and his collaegues had shown that MIBT (in the presence of diavalent copper) was able to change the physical configuration of nucleic acids (Sect. F.III), possibly accounting for the biologic activity of the drug, they decided to determine if adenyl cyclase which utilizes adenosine triphosphate to form cyclic adenosine monophosphate (cAMP) was also inhibited. Their initial approach was to establish the amount of cAMP in human peripheral blood lymphocytes after incubation in the presence of drug (WEBB et al. 1974). Since the level of cAMP increased significantly after exposure to MIBT, they questioned if this was due to increased adenyl cyclase or a decrease in the specific phosphodiesterase which hydrolyzes cAMP to AMP. They found that MIBT blocked phosphodiesterase activity. Since changes in cAMP levels in lymphocytes had previously been shown to influence the immune response, WEBB et al. (1974) felt that the possibility existed that MIBT might suppress the host's natural defenses.

Little has been published on the pharmacology and toxicology of MIBT. The acute oral LD_{50} of MIBT in mice and rats is in excess of 2,000 mg/kg (BAUER 1977). MIBT is poorly absorbed from the gastrointestinal tract. The extent of adsorption is greatly influenced by particle size. When given to rats in the form of a suspension in sucrose syrup of 3 μm diameter particles, between 40% and 50% of the dose can be recovered in the feces. With 100 μm particles the recovery is near 80% (AXON 1972). The highest nonlethal dose of MIBT when given subcutaneously or intraperitoneally in mice is 2,000 mg/kg body weight and 235 mg/kg, respectively. The highest nonlethal doses of the derivative, *N*,*N*′-bis-(β-thiosemicarbazone-methylisatin)-2-methylpiperazine, when given subcutaneously and intraperitoneally in mice are 2,000 mg/kg and 475 mg/kg, respectively (BORYSIEWICZ and WITALINSKI 1979). In chronic toxicity studies in which rhesus monkeys were given 250 mg/kg daily by mouth for 1 month there was some evidence of liver damage, but the liver was unaffected at the end of similar treatment in rats and dogs (BAUER 1977).

Methisazone has an embryotoxic effect in very high doses. In mated rats given 2,000 mg/kg daily by mouth for 12 days, there were no implantations in the majority of cases, and animals treated later in pregnancy showed an increase in resorptions in comparison to untreated controls. Similar effects were found in rabbits and fetal malformations were occasionally produced (BAUER 1977).

The duration of sleep after administration of sodium pentobarbital is greatly prolonged in mice given 25 mg/kg MIBT, probably owing to competition for detoxifying systems in the liver. In rats with unrestricted access to food and water, the stomach emptying time is greatly prolonged by the oral administration of 500 mg/kg MIBT. The delay in emptying in fasted rats was much less, indicating that methisazone should be given on an empty stomach (BAUER 1977).

IV. Isatin-β-4′,4′-Dialkylthiosemicarbazones

When studying the ability of MIBT to inhibit the immunologic response of the mouse to sheep red blood cells McNeill also found that granulocyte-macrophage colony formation in vitro could be prevented (Sect. G.III). This drug and a series of 4′,4′-dialkylthiosemicarbazones would inhibit when added directly to cells flushed from bone marrow prior to use in a semisolid suspension cell assay. The dialkylthiosemicarbazones could be divided into three groups, depending on the concentration range necessary to prevent 50% of the colonies from forming. The concentration of MIBT required for this degree of inhibition was 3 μg/ml, whereas 4′,4′-morpholino or 4′,4′-tetramethylene substituents in the side chain when associated with N_1-ethyl or N_1-methyl substitutions on isatin were very active (less than 0.5 μg/ml required for 50% colony inhibition). Seven other dialkyl-TSC side chain substituents (dimethyl, diethyl, pentamethylene, hexamethylene, 3-oxopentamethylene, 2-methylpentamethylene, and diallyl) with the same isatin substitutions gave 50% inhibition of colony formation in the 0.7–1.0 μg/ml range. Methoxy substitutions at the 5 position of the aromatic ring (Fig. 8) resulted in compounds of intermediate activity (1.0–5.0 μg/ml), while azo or iodo substitutions at the 5 or 7 positions yielded compounds of very low activity (greater than 20 μg/ml being required). The immunologic implications of these findings are discussed in Sect. G.III.

V. Isatin-β-Isothiosemicarbazones

Using N_1-ethylisatin-*S*-*n*-butylisothiosemicarbazone (Fig. 13) at 150 mg/kg body weight (a concentration that would spare 50% of mice infected with 10^3 LD_{50} vaccinia virus) Zgorniak-Nowosielska et al. (1978) investigated the compound's ability to suppress both humoral and cell-mediated responses in the mouse. The humoral, or B-cell response, was measured exactly as described in Sect. G.II. The primary response to sheep red blood cells was reduced by 30%–60% depending on the strain of mouse used in the experiment. The secondary response to the red blood cells, as measured in BALB/c mice was reduced by 60% (twice as much as after the initial injection of cells). The cell-mediated, or T-cell response was measured by the degree of allergic contact dermatitis provoked by oxazolone (Sect. G.II). There was no indication that the compound diminished the cellular response.

VI. Thiazole Thiosemicarbazones

3-Methyl-4-bromo-5-formylisothiazole-TSC (Fig. 14) has been the only compound in this series to receive attention concerning its effect on bodily functions. Chronic toxicity was evaluated in rats and monkeys which were given the drug by mouth in daily doses of 250–1,000 mg/kg (5–20 times the human dose) for 3 months. The lower dose schedule had no deleterious effect on the animals (Rao et al. 1965). In rats, there was no evidence of liver or kidney dysfunction at the higher dose schedule, but there was some toxic suppression of bone marrow (see Sects. G.III, IV) and testicular damage (Bauer 1972). In monkeys, there was no kidney dysfunction, but there was some fatty change in the liver and moderate testicular atrophy (Rao et al. 1965; Bauer 1972).

H. Clinical Studies

On the basis of studies with mice and rabbits infected with various poxviruses, two of the thiosemicarbazones, N_1-methylisatin-β-thiosemicarbazone and 3-methyl-4-bromo-5-formylisothiazole thiosemicarbazone reached clinical trials. I will not describe them in any great detail and the interested reader should consult the concise and comprehensive discussions of this subject by BAUER (1972, 1977). Even though these trials are an important part of the history of antiviral chemotherapy, the success of the World Health Organization's campaign for the eradication of smallpox has resulted in a virtual disappearance of indications for treating poxvirus infections of humans (ARITA and BREMAN 1979). However, the thiosemicarbazone's potential efficacy against poxvirus infections should not be forgotten, especially in the case of isolated laboratory outbreaks or germ warfare. In this vein, studies on thiosemicarbazone-resistant poxvirus mutants will be described. Six features of the virus have made the eradication of smallpox possible (FENNER 1979): (a) the disease was severe enough to warrant quarantine precautions; (b) there was a safe and effective vaccine; (c) the virus did not cause persistent disease or recurrent infection in humans; (d) there appeared to be no animal reservoirs; (e) subclinical but infectious cases did not occur, and cases were not infectious (or rarely so) before eruption occurred, thus making isolation of cases an important part of control; and (f) there were no major barriers of eradication, either social (as with venereal diseases) or financial (as with tuberculosis).

I. N_1-Methylisatin-β-Thiosemicarbazone

As in the laboratory animal studies (Sect. D.III), human infections, other than with poxviruses, have not been convincingly shown to respond to MIBT (HUTFIELD and CSONKA 1964; SANDEMAN 1966). If given in the proper dosage schedule, MIBT appears to have definite merit in the prophylaxis of smallpox and alastrim, and unquestionable value in the complications of vaccination.

Smallpox occurs in two clinical variants, variola major and variola minor or alastrim. Variola major is typically a very severe illness with a mortality around 30%, although its course may be greatly modified by previous vaccination. Alastrim used to occur in South America, mainly in Brazil, and was a much milder illness with a mortality of 0.5%. The initial site of multiplication of the virus after entry is not known, but is probably somewhere in the respiratory tract. After an incubation period of about 12 days the virus escapes into the bloodstream, and further cycles of multiplication take place in the dermis and also in the internal organs. The viremic phase is associated with severe prodromal illness with extreme backache, headache, and prostration. The severity of the clinical course is directly related to the amount of virus liberated into the bloodstream during viremia, and also to the degree of preexisting immunity (BAUER 1977). Because MIBT has been found to lower the attack rate 25-fold among household contacts (a contact being an individual exposed to a person with clinical smallpox and thus likely to become infected) during a smallpox epidemic, and 8-fold among contacts during an alastrim epidemic, it is assumed that the drug arrests virus multiplication during the

12-day incubation period (BAUER 1977). MIBT has not proven effective in the treatment of clinical smallpox (BAUER 1965, 1972, 1977). Since people admitted to the hospital with characteristic lesions of smallpox are in the late stage of disease (the lesions usually erupt simultaneously over the entire body, in distinction to chickenpox, which shows multiple crops of lesions), it is too late for the drug to prevent spread of the infection to target organs. The main side effect of MIBT administration is vomiting, usually occurring almost immediately or 4–6 h later (BAUER 1965). This disconcerting side effect has been repeatedly observed (LANDSMAN and GRIST 1964; HUTFIELD and CSONKA 1964; BAUER 1972, 1977). The percentage of patients in which this occurred varied from approximately 20% to as high as 100%. The effect of the drug on the prophylaxis of smallpox appears to be inversely related to the percentage of people vomiting and thus losing the drug. For adults, 6 g/day MIBT for 4 days has proven prophylactic activity, but it must not be administered in a single daily dose. The best schedule is every 3–4 h but twice a day will also be effective (BAUER 1972).

The efficacy of MIBT in the complications of vaccination have been repeatedly confirmed (BAUER 1965, 1977; MCLEAN 1977). The virus used for vaccination is always vaccinia. Its origin is uncertain: it may have derived from Jenner's original strain of cowpox virus or it may have evolved from recombination between cowpox and smallpox. Its importance lies in the fact that intradermal inoculation of it into humans produces a localized infection in the skin followed by the development of antibodies capable of neutralizing related poxviruses, including smallpox.

If persons suffering from eczema are vaccinated against smallpox, the infection may not remain confined to the site of inoculation, but may spread to involve the eczematous areas and other parts of the body, producing a generalized eruption resembling smallpox in many ways. This condition known as eczema vaccinatum may be fatal in the absence of treatment. It commonly occurs in infants with infantile eczema. Vaccination is usually witheld in such cases. The recommended course of treatment is an initial loading dose of 200 mg/kg, followed by 50 mg/kg at 6-h intervals to a total dose of 600 mg/kg.

In primary smallpox vaccination, the course of infection is brought to a halt by humoral and cell-mediated immunity. In certain cases these mechanisms may be impaired or absent, as in congenital agammaglobulinemia and hypogammaglobulinemia, and in patients undergoing immunosuppressive treatment. In such cases the lesion fails to heal and undergoes indefinite enlargement. Metastatic lesions arise as a result of viremia and can occur in almost any organ of the body. The condition is known as vaccinia gangrenosa, and is invariably fatal in the absence of treatment with antivaccinial gamma globulin or MIBT. The recommended drug dosage schedule is the same as for eczema vaccinatum. The course may be repeated as necessary after an interval of 7 days. It appears that patients who can be cured are those having a single immunologic defect. In patients with multiple defects treatment is ineffective.

APPLEYARD and WAY (1966) were the first to show that IBT-resistant poxviruses could arise in both IBT-treated tissue culture and mice. Mice (20 g body weight) were injected intranasally with 10^7 plaque forming units (pfu) rabbitpox virus. At the first pass the mice received 0.5 mg MIBT twice a day for 4 days; at the second pass 1 mg MIBT was injected daily for 4 days; and at the third pass

treatment was 1 mg of MIBT for only two days. After each pass virus obtained from the lungs of one mouse was grown in HeLa cells to produce a suspension with sufficient titer to be used for the next passage. Plaque reduction tests were then carried out in HeLa cells using an IBT-containing agar overlay and virus obtained from mouse lungs at the various passage levels. A gradual resistance to IBT developed with each successive passage. Furthermore, the virus had lost its sensitivity to MIBT when tested in mice. The virus was also passed 16 times in HeLa cells in the presence of IBT. The concentration of drug in the medium for the first five passages was 0.05–0.1 μg/ml, but it was gradually raised to 20 μg/ml at the tenth and subsequent passages. Again, a plaque reduction test was used to assess resistance. About 100 pfu were used to infect HeLa cells monolayers with the agar overlays containing up to 10 μg/ml IBT. The passaged virus become increasingly resistant to IBT, until about the tenth passage, after which there was no significant change (plaque numbers were almost identical with or without 10 μg/ml IBT in the overlay).

GHENDON and CHERNOS (1972) confirmed the tissue culture observations of APPLEYARD and WAY (1966) and extended them to include vaccinia virus. Chick embryo fibroblast cells were used, with the viruses being passed eight times in concentrations of MIBT increasing gradually from 0.2 to 1.0 μg/ml. Resistance to the drug was measured by a plaque reduction test or the yields of virus 24 h after infection in a liquid medium. Using virus passed in the presence of drug, no difference in virus yields were noted in either test using 1 μg/ml MIBT. The mutants appeared to be stable and retain resistance after a number of passages without the inhibitor. Furthermore, treatment with antiserum against vaccinia virus was shown to reduce the titer of both the original strains and the resulting inhibitor-resistant mutants to the same extent.

Further studies by KATZ et al. (1973 d) not only led to the isolation of IBT-resistant mutants, but also to mutants dependent on IBT for their replication. Vaccinia-infected chick embryo cells were treated with the mutagen, iododeoxyuridine (5 μg/ml) and IBT (14 μ*M*). The culture was harvested 48 h later and used for infection of new cultures in the presence of IBT alone. Virus from one of these cultures was titrated using a plaque assay containing IBT. Well-separated plaques were excised and grown into stocks in the presence of IBT. One of these stocks formed plaques only in the presence of IBT (the IBT-dependent mutant) while another formed plaques both in the presence and absence of the drug (IBT-resistant mutant). The sedimentation rates of the three strains [wild-type (WT), IBT-dependent, and IBT-resistant] were the same, as was their neutralization by WT antiserum (KATZ et al. 1973 c). The polypeptide analysis of all three strains showed a few minor differences in the relative amounts of several polypeptides. The IBT-resistant mutant appeared to lack one of the peptides, designated 6 b (Sect. F.III). The IBT-dependent mutant was found to be able to grow in the absence of IBT during mixed infection with WT or IBT-resistant viruses (KATZ et al. 1973 c). Mode of action studies (KATZ et al. 1973 a) showed that the IBT-dependent mutant was blocked in its replication cycle in the absence of IBT at the same place the WT was blocked in its presence (Sect. F.III). KATZ (1979) has recently summarized his studies on these IBT-sensitive, IBT-resistant, and IBT-dependent strains of vaccinia virus.

II. 3-Methyl-4-Bromo-5-Formylisothiazole Thiosemicarbazone

This isothiazole was very effective in preventing death of smallpox-infected mice. Unlike MIBT, it would also spare rabbits infected with rabbitpox virus (RAO et al. 1965). It was then assessed in the treatment of smallpox in humans using a controlled double-blind trial in Madras, India (RAO et al. 1966a). The total number of patients admitted to the trial was 1293, 601 receiving a placebo and 692 being treated with the drug. The usual treatment was 6 g every 6 h for 10 days. The mortality rates in these two groups were 23.3% (140 deaths) and 22.4% (155 deaths), respectively. Marks of previous vaccination were seen on 478 patients; 218 of these received the placebo and eight died (3.7%) and the remaining 260 received the drug, of whom 5 died (1.9%). The mortality rates among the 815 patients who had not been previously vaccinated were 132 of 383 (34.5%) given placebo and 150 of 432 (34.7%) given the drug. The differences in the figures were not significant. Some slight differences in favor of the drug were seen in analysis of the mean number of febrile days, the time of scabbing, and the virus content of the scabs, but the differences were not significant. The isothiazole was also assessed in the prophylaxis of contacts – contacts being persons associated with a patient who develops smallpox and who thus run a risk of acquiring the infection and developing the disease. The persons treated were all family contacts of smallpox patients and the trial was restricted to those contacts who had not been previously vaccinated (RAO et al. 1966b). The drug was given to 196 contacts while 201 received the placebo. In the drug-treated group there were 40 cases of smallpox (20.4%) with 7 deaths (17.5% of the cases). In the placebo group there were 60 cases (29.9%) with 12 deaths (20% of the cases). The mortality rates were not significantly different. As with the MIBT trials the drug was not well tolerated. Vomiting, reported in 75% of the patients, was the major side effect. This loss of drug could have accounted for the lack of chemoprophylactic activity.

IBT-resistant mutants (Sect. H.I) were found to be almost as resistant in tissue culture to 3-methyl-4-bromo-5-formylisothiazole as to IBT itself (APPLEYARD and WAY 1966), thus suggesting a similar mode of action of the two compounds (Sect. F.VI).

J. Perspectives

Of all the antiviral agents discovered in the last two decades, the thiosemicarbazones are certainly one of the most interesting groups of compounds. The thiosemicarbazones have been shown to inhibit the replication of poxviruses in both tissue culture and animal studies. Furthermore, one of these compounds, N_1-methylisatin-β-thiosemicarbazone (MIBT) has been successfully used in the prophylaxis of smallpox in humans. Even though interest in the clinical application of MIBT has disappeared as the worldwide incidence of smallpox has decreased to an undetectable level, the potential value of thiosemicarbazones in medical virology remains high. The isatin-β-thiosemicarbazones in the presence of divalent cations of the first transition series metals (copper being the most effective) can inactivate on contact virtually any type of mature virus particle. With the exception of interferon, no selective, defined substance has such broad antiviral properties. The abil-

ity of thiosemicarbazones to inhibit only the poxviruses at an intracellular stage in their morphogenesis may be attributed to the claims that poxviruses contain copper. Divalent copper, as well as other first transition series metals, complexed with various thiosemicarbazones can change the physical configuration of nucleic acids and interfere with their template function. With the use of ionophores copper–thiosemicarbazone complexes within the cell might be able selectively to prevent virus synthesis resulting from infection with fully functional virus. Furthermore, if copper–thiosemicarbazone complexes selectively contact inactivate mature virions by interfering with genome function alone, the way may be open to the rapid production of potent inactivated vaccines.

Acknowledgments. The literature survey for this review was carried out while the author was on leave at the National Institute of Neurological and Communicative Disorders and Stroke. Special thanks go to PAUL DEPORTE of the Translating Service of The National Institutes of Health Library. I gratefully acknowledge Dr. EHUD KATZ for his comments after reading the manuscript. Drs. SYDNEY ARCHER, JAMES MOORE, and HARRY ROY also have helpfully reviewed specific sections. Studies conducted on the thiosemicarbazones in the author's laboratory were aided by a grant from The National Institute of Allergy and Infectious Diseases. I also thank JUDY MALONEY for typing and proofreading.

References

Agrawal KC, Sartorelli AC (1978) The chemistry and biological activity of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones. Prog Med Chem 15:321–356

Andreani A, Cavrini V, Giovanninetti G, Mannini Palenzona A, Franchi L (1975) Ricerche su sostanze ad attività antivirale. Nota II-derivati di 2-chloro-3-formilindoli N-sostituiti. Farmaco [Sci] 30:440–448

Andreani A, Bonazzi D, Cavrini et al. (1978) Ricerche su sostanze ad attività antivirale. Nota IX-sintesi ed attivita antivirale di tiosemicarbazoni di 1-acil-2-alogeno-3-formilindoli. Farmaco [Sci] 33:754–760

Appleyard G, Way HJ (1966) Thiosemicarbazone-resistant rabbitpox virus. Br J Exp Pathol 4:144–151

Appleyard G, Westwood JCN, Zwartouw HT (1962) The toxic effect of rabbitpox virus in tissue culture. Virology 18:159–169

Appleyard G, Hume VBM, Westwood JCN (1965) The effect of thiosemicarbazone on the growth of rabbit pox virus in tissue culture. Ann NY Acad Sci 130:92–104

Arita I, Breman JG (1979) Evaluation of smallpox vaccination policy. Bull WHO 57:1–9

Axon A (1972) Presentation of methisazone (an antiviral chemical) as a medicine for oral administration. J Mond Pharm 15:221–232

Bablanian R (1968) The prevention of early vaccinia-virus induced cytopathic effects by inhibition of protein synthesis. J Gen Virol 3:51–61

Bach MK, Magee WE (1962) Biochemical effects of isatin β-thiosemicarbazone on development of vaccinia virus. Proc Soc Exp Biol Med 110:565–567

Bauer DJ (1955) The antiviral and synergic actions of isatin thiosemicarbazone and certain phenoxypyrimidines in vaccinia infection in mice. Br J Exp Pathol 36:105–114

Bauer DJ (1958) The chemotherapeutic activity of compounds of copper, rhodium, and certain other metals in mice infected with neurovaccinia and ectromelia viruses. Br J Exp Pathol 39:480–489

Bauer DJ (1961) The zero effect dose (E_o) as an absolute numerical index of antiviral chemotherapeutic activity in the pox virus group. Br J Exp Pathol 42:201–206

Bauer DJ (1963) The chemotherapy of ectromelia infection with isatin-β-dialkylthiosemicarbazones. Br J Exp Pathol 44:233–242

Bauer DJ (1965a) Monocyclic thiosemicarbazones as antiviral agents. Ann NY Acad Sci 130:105–109

Bauer DJ (1965b) Clinical experience with the antiviral drug marboran (1-methylisatin 3-thiosemicarbazone). Ann NY Acad Sci 130:110–117

Bauer DJ (1972) Thiosemicarbazones. In: Bauer DJ (ed) Chemotherapy of virus diseases. Pergamon, London (Int Encyclopedia Pharmacol Ther, vol 1, sect 61, pp 35–113)

Bauer DJ (1977) The specific treatment of virus diseases. MTP Press, Lancaster

Bauer DJ, Apostolov K (1966) Adenovirus multiplication: inhibition by methisazone. Science 154:796–797

Bauer DJ, Sadler PW (1960a) The structure-activity relationship of the antiviral chemotherapeutic activity of isatin β-thiosemicarbazone. Br J Pharmacol 15:101–110

Bauer DJ, Sadler PW (1960b) New antiviral chemotherapeutic agent active against smallpox infection. Lancet 1:1110–1111

Bauer DJ, Sadler PW (1961) Derivatives of isatin β-thiosemicarbazone with antiviral chemotherapeutic activity against ectromelia infection. Nature 190:1167–1169

Bauer DJ, Sheffield FW (1959) Antiviral chemotherapeutic activity of isatin-β-thiosemicarbazone in mice infected with rabbit-pox virus. Nature 184:1496–1497

Bauer DJ, Dumbell KR, Fox-Hulme P, Sadler PW (1962) The chemotherapy of variola major infection. Bull WHO 26:727–732

Bauer DJ, Apostolov K, Selway JWT (1970) Activity of methisazone against RNA viruses. Ann NY Acad Sci 173:314–319

Bishop DHL, Shope RE (1979) Bunyaviridae. In: Fraenkel-Contrat H, Wagner RR (eds) Comprehensive virology, vol 14. Plenum, New York, pp 1–156

Bock M (1957) Thiosemicarbazon-Wirkung bei experimentellen Pocken-Infektionen der Maus. Z Hyg Infektionskr 143:480–489

Bopp LH (1976) Contact inactivation of influenza virus by isatin β-thiosemicarbazones and $CuSO_4$. Masters thesis. Rensselaer Polytechnic Institute, Troy, N.Y.

Borysiewicz J, Lucka-Sobstel B (1978) The effect of certain Mannich N-bases, derivatives of isatin β-thiosemicarbazone, on the replication of vaccinia virus in in vitro studies. Acta Microbiol Pol Ser A Microbiol Gen 27:111–121

Borysiewicz J, Tadeusiewicz R (1976) Computer evaluation of antiviral activities of some thiosemicarbazones in experiments in vivo. Acta Virol 20:402–410

Borysiewicz J, Witalinski W (1979) Effect of N,N′-bis(methylisatin-β-thiosemicarbazone) on vaccinia virus replication in vitro and in vivo. Arch Virol 62:83–86

Borysiewicz J, Porebska A, Potec Z (1973) Activity of isatin-β-thiosemicarbazone derivatives against vaccinia virus studied in chick cells by the methyl-cellulose assay. Acta Microbiol Pol Ser A Microbiol Gen 22:205–207

Borysiewicz J, Mizerski J, Pryjma J (1977) Effect of methisazone on immune response in mice. Chemotherapy 23:276–281

Brockman RW, Sidwell RW, Arnett G, Shaddix S (1970) Heterocyclic thiosemicarbazones: correlation between structure, inhibition of ribonucleotide reductase, and inhibition of DNA viruses. Proc Soc Exp Biol Med 133:609–614

Brownlee KA, Hamre D (1951) Studies on chemotherapy of vaccinia virus. I. An experimental design for testing antiviral agents. J Bacteriol 61:127–134

Buu-Hoi NP, Saint-Ruf G, Perche JC, Bourgeade JC (1968) Nouvelles thiosemicarbazones, oxo-4Δ^2-thiazolinylhydrazones-2 et thiazolidinones-4 potentiellement antivirales. Chim Ther 3:110–115

Campaigne E, Thompson RL, Van Werth JE (1959) Some heterocyclic thiosemicarbazones possessing anti-viral activity. J Med Chem 1:577–599

Caton MPL, Jones DH, Slack R, Squires S, Wooldridge KRH (1965) Antiviral chemotherapy. II. Structure-activity relationships in a series of isothiazolealdehyde and ketone thiosemicarbazones. J Med Chem 8:680–683

Caunt AE (1967) The effect of marboran on the growth of varicella virus in tissue culture. In: Spitzy KH, Haschek H (eds) 5th international congress of chemotherapy. Verlag der Wiener medizinischen Akademie, Vienna, pp 313–317

Cho CT, Bolano CR, Kamitsuka PS, Wenner HA (1970) Methisazone and monkey pox virus: studies in cell cultures, chick embryos, mice, and monkeys. Am J Epidemiol 92:137–144

Cooper JA, Moss B, Katz E (1979) Inhibition of vaccinia virus late protein synthesis by isatin-β-thiosemicarbazone: characterization and in vitro translation of viral mRNA. Virology 96:381–392

Domagk G, Behnisch R, Mietzsch F, Schmidt H (1946) Über eine neue, gegen Tuberkelbazillen in vitro wirksame Verbindungsklasse. Naturwissenschaften 33:315

Easterbrook KB (1962) Interference with the maturation of vaccinia virus by isatin-β-thiosemicarbazone. Virology 17:245–251

Fenner F (1979) Portraits of viruses: the poxviruses. Intervirology 11:137–157

Fox MP, Bopp LH, Pfau CJ (1977) Contact inactivation of RNA and DNA viruses by N-methyl isatin β-thiosemicarbazone and $CuSO_4$. Ann NY Acad Sci 284:533–543

Francis RD, Bradford HB (1976) Some biological and physical properties of molluscum contagiosum virus propagated in cell culture. J Virol 19:382–388

Franke R, Labes D, Tonew M, Zschiesche W, Heinisch L (1975) Antivirale Thiosemikarbazone und verwandte Verbindungen. III. Quantitative Beziehungen zwischen Struktur und antiviraler Aktivität von Isatin-β-isothiosemikarbazonen gegen Mengovirus. Acta Biol Med Germ 34:491–499

Freund M, Schander A (1902) Thiosemicarbazid als Reagens auf Aldehyde und Ketone. Chem Ber 35:2602–2606

Gabriel R (1971) 1-Methylisatin 3-thiosemicarbazone treatment of NZBxNZW hybrid mice. Br J Exp Pathol 52:271–275

Gerzon K (1965) Discussion-thiosemicarbazones. Ann NY Sci 130:128–130

Ghendon YZ, Chernos VI (1972) Mutants of poxviruses resistant to acetone and N-methyl-isatin-beta-thiosemicarbazone. Acta Virol 16:308–312

Giannella M, Gualtieri F (1970) Ricerche nel campo della sostanze ad azione antivirale carbammati, tiosemicarbazoni e semicarbazoni di-α-ossimminochetoni ciclici. Farmaco [Sci] 25:509–518

Giannella M, Gualtieri F, Melchiorre C, Pigini M (1973) Ricerche nel campo sostanze ad attività antivirale. Farmaco [Sci] 28:597–610

Gladych JMZ, Hunt JH, Jack D, Haff RF, Boyle JJ, Stewart RC, Ferlauto RJ (1969) Inhibition of rhinovirus by isatin thiosemicarbazone analogues. Nature 221:286–287

Haase AT, Levinson W (1973) Inhibition of RNA slow viruses by thiosemicarbazones. Biochem Biophys Res Commun 51:875–880

Hamre D, Bernstein J, Donovick R (1950) Activity of p-aminobenzaldehyde, 3-thiosemicarbazone on vaccinia virus in the chick embryo and in the mouse. Proc Soc Exp Biol Med 73:275–278

Hamre D, Brownlee KA, Donovick R (1951) Studies on the chemotherapy of vaccinia virus. J Immunol 67:305–312

Hanson AG (1977) Contact inactivation of some RNA viruses by thiosemicarbazone derivatives and copper ions. Master's thesis. Rensselaer Polytechnic Institute, Troy, N.Y.

Hapel A, Gardner I (1974) Appearance of cytotoxic T cells in cerebrospinal fluid of mice with ectromelia virus-induced meningitis. Scand J Immunol 3:311–319

Harford CG, Rieders E, Osborn R (1972) Inhibition of arc-like fragments of immature forms of vaccinia virus by methisazone. Proc Soc Exp Biol Med 139:558–561

Heinisch L, Kramarczyk K (1972) Antivirale Thiosemicarbazone und verwandte Verbindungen. I. Synthese und Struktur substituierter Isatin-Thiosemicarbazone und -isothiosemicarbazone. J Prakt Chem 314:682–698

Herrlich A, Stickl H, Munz E (1965) Kann man den Ablauf der Pockenschutzimpfung medikamentös beeinflussen? Dtsch Wochensch 90:69–74

Herrman EC (1968) Sensitivity of herpes simplex virus, vaccinia virus, and adenoviruses to deoxyribonucleic acid inhibitors and thiosemicarbazones in a plaque suppression test. Appl Microbiol 16:1151–1155

Hoagland CL, Ward SM, Smadel JE, Rivers TM (1941) Constituents of elementary bodies of vaccinia. IV. Demonstration of copper in the purified virus. J Exp Med 74:69–80

Hutfield DC, Csonka GW (1964) Marboran in herpes genitalis. Lancet 1:329–330

Iwasaki K, Nishimura T, Igarashi Y, Nagaki D (1955) Studies on the chemotherapy of influenza virus I. Effect of thiosemicarbazones on influenza and Newcastle disease virus multiplication. Kitasato Arch Exp Med 28:31–44

Joklik WJ (1962) The purification of four strains of poxviruses. Virology 18:9–18

Kackell MY, Schneweis KE, Spiess H (1966) Untersuchungen über die Wirkung von Marboran, Activaccine-Hyperimmun-Serum und Vorimpfung mit Vaccineantigen auf die intracutane Pockenimpfung beim Kaninchen. Klin Wochenschr 44:1199–1204

Kaptsova TI, Marennikova SS (1967) On the prophylactic activity on N-methyl isatin β-thiosemicarbazone against smallpox infection. Acta Virol 11:554–556

Kaska WC, Carrano C, Michalowski J, Jackson J, Levinson W (1978) Inhibition of the RNA dependent DNA polymerase and malignant transforming ability of Rous sarcoma virus by thiosemicarbazone-transition metal complexes. Bioinorg Chem 8:225–236

Katz E (1979) Sensitivity, resistance, and dependence on the antiviral drug isatin-β-thiosemicarbazone (IBT) in the growth of vaccinia virus in tissue culture. In: Zuckerman A (ed) Dynamic aspects of host-parasite relationships, vol 3. Israel Universities Press, Jerusalem, pp 98–107

Katz E, Felix H (1977) Synthesis of vaccinia virus antigens in HeLa cells in the presence of isatin β-thiosemicarbazone. Antimicrob Agents Chemother 11:202–208

Katz E, Margalith E, Winer B (1973 a) An isatin β-thiosemicarbazone (IBT)-dependent mutant of vaccinia virus: the nature of the IBT-dependent step. J Gen Virol 21:477–484

Katz E, Margalith E, Winer B, Goldblum N (1973 b) Synthesis of vaccinia polypeptides in the presence of isatin β-thiosemicarbazone. Antimicrob Agents Chemother 4:44–48

Katz E, Margalith E, Winer B, Lazar A (1973 c) Characterization and mixed infections of three strains of vaccinia virus: wild type, IBT-resistant and IBT-dependent mutants. J Gen Virol 21:469–475

Katz E, Winer B, Margalith E, Goldblum N (1973 d) Isolation and characterization of an IBT-dependent mutant of vaccinia virus. J Gen Virol 19:161–164

Katz E, Margalith E, Winer B (1974) The effect of isatin β-thiosemicarbazone (IBT)-related compounds on IBT-resistant and on IBT-dependent mutants of vaccinia virus. J Gen Virol 25:239–244

Katz E, Margalith E, Winer B (1975) Isolation and characterization of a γ-thiochromanone-4-thiosemicarbazone-resistant mutant of vaccinia virus. Antimicrob Agents Chemother 7:85–90

Katz E, Margalith E, Winer B (1978 a) Formation of vaccinia virus DNA-protein complex in the presence of isatin-β-thiosemicarbazone (IBT). J Gen Virol 40:695–699

Katz E, Margalith E, Felix H, Winer B (1978 b) Synthesis of vaccinia viral RNA and proteins in the presence of isatin-β-thiosemicarbazone. In: Siegenthaler W, Luethy R (eds) Current chemotherapy. Proceedings of the 10th International Congress of Chemotherapy, vol 1. American Society for Microbiology, Washington CD, pp 330–332

Katz E, Margalith E, Winer B (1978 c) Genetic recombination between a temperature sensitive mutant and an isatin β-thiosemicarbazone (IBT) resistant mutant of vaccinia virus. J Antimicrob Chemother 4:159–162

Kudriavtsev FS, Solov'ev IU V, Korovin RN, Shurchilov AF (1977) Effect of izatizon and OL-56 on the reproductive and transformational properties of Marek's disease virus (in Russian). Veterinariia 2:45–46

Landsman JB, Grist NR (1964) Controlled trial of marboran on group vaccinated against smallpox. Lancet 1:330

Levinson W (1973) Inhibition of viruses, tumors, and pathogenic microorganisms by isatin β-thiosemicarbazone and other thiosemicarbazones. In: Carter WA (ed) Selective inhibitors of viral function. CRC Press, Cleveland, Ohio, pp 213–226

Levinson W, Helling R (1976)Inactivation of λ phage infectivity and λ deoxyribonucleic acid transfection by N-methyl isatin β-thiosemicarbazone. Antimicrob Agents Chemother 9:160–163

Levinson W, Woodson B, Jackson J (1971) Inactivation of Rous sarcoma virus on contact with N-ethyl isatin β-thiosemicarbazone. Nature New Biol 232:116–118

Levinson W, Faras A, Morris R et al. (1973 a) Effect of N-methyl isatin β-thiosemicarbazone on Rous sarcoma virus, several isolated enzymes and other viruses. In: Fox CF, Robinson WS (eds) Virus research-2nd ICN-UCLA symposium on molecular biology. Academic Press, New York, pp 403–414

Levinson W, Faras A, Woodson B, Jackson J, Bishop JM (1973 b) Inhibition of RNA dependent DNA polymerase of Rous sarcoma virus by thiosemicarbazones and several cations. PNAS 70:164–168

Levinson W, Coleman V, Woodson B, Rabson A, Lanier J, Whitcher J, Dawson C (1974) Inactivation of herpes simplex virus by thiosemicarbazones and certain cations. Antimicrob Agents Chemother 5:398–402

Levinson W, Oppermann H, Jackson J (1977a) Inhibition of vesicular stomatitis virus by kethoxal bis (thiosemicarbazone). J Gen Virol 37:183–190

Levinson W, Rohde W, Mikelens P, Jackson J, Antony A, Ramakrishnan T (1977b) Inactivation and inhibition of Rous sarcoma virus by copper binding ligands: thiosemicarbazones, 8-hydroxyquinolines, and isonicotinic acid hydrazide. Ann NY Acad Sci 284:525–532

Levinson W, Mikelens P, Jackson J, Kaska W (1978a) Anti-tumor virus activity of copper-binding drugs. In: Schrauzer GN (ed) Inorganic and nutritional aspects of cancer, chap 12. Plenum, New York, pp 161–178

Levinson W, Oppermann H, Jackson J (1978b) Induction of four proteins in eukaryotic cells by kethoxal bis (thiosemicarbazone). Biochim Biophys Acta 518:401–412

Levinson W, Idriss J, Jackson J (1979) Metal binding drugs induce synthesis of four proteins in normal cells. Biol Trace Element Res 1:15–23

Levy JA, Levy SB, Levinson W (1976) Inactivation of murine RNA tumor viruses by isatin β-thiosemicarbazone, its derivatives and analogs. Virology 74:426–431

Lieberman M, Pollikoff R, Pascale AM (1966) Effect of concomitant treatment by cortisone and N-ethylisatin-β-thiosemicarbazone on neurovaccinia virus infected mice. Proc Soc Exp Biol Med 122:484–489

Logan JC, Fox MP, Morgan JH, Makohon AM, Pfau CJ (1975) Arenavirus inactivation on contact with N-substituted isatin β-thiosemicarbazones and certain cations. J Gen Virol 28:271–283

Lovas B, Hollos I (1969) Replication of vaccinia virus in the embryonated egg in the presence of methisazone. An electron microscope study. Acta Microb Acad Sci Hung 16:47–62

Lum GS, Smith PK (1957) Experimental chemotherapy of influenza virus with particular reference to thiosemicarbazones and diphenyl compounds. J Pharmacol Exp Ther 119:284–293

Lwoff A, Lwoff M (1964) Remarques sur l'isatine β-thiosemicarbazone et sur quelques inhibiteurs du développement viral. C R Acad Sci [D] (Paris) 258:1924–1927

Magee WE, Bach MK (1965) Biochemical studies on the antiviral activities of the isatin-β-thiosemicarbazones. Ann NY Acad Sci 130:80–91

McLean DM (1977) Methisazone therapy in pediatric vaccinia complications. Ann NY Acad Sci 284:118–121

McNeill TA (1972) Effect of methisazone and other drugs on mouse hemopoietic colony formation in vitro. Antimicrob Agents Chemother 1:6–11

McNeill TA (1973) Inhibition of granulocyte-macrophage colony formation in vitro by substituted isatin thiosemicarbazones. Antimicrob Agents Chemother 4:105–108

McNeill RA, Fleming W, McClure S, Killen M (1972) Suppression of immune and hematopoietic cellular response by methisazone. Antimicrob Agents Chemother 1:1–5

Mikelens PE, Woodson BA, Levinson WE (1976) Association of nucleic acids with complexes of N-methyl isation β-thiosemicarbazone and copper. Biochem Pharmacol 25:821–828

Minton SA, Officer JE, Thompson RL (1953) Effect of thiosemicarbazones and dichlorophenoxy thiouracil on multiplication of a recently isolated strain of variola-vaccinia virus in the brain of the mouse. J Immunol 70:222–228

Morishima T, Hayashi K (1978) Meningeal exudate cells in vaccinia meningitis of mice: role of local T cells. Infect Immun 20:752–759

Moss B (1974) Reproduction of poxviruses. In: Fraenkel-Conrat H, Wagner RR (eds) Comprehensive virology vol 3, chap 5. Plenum, New York, pp 405–474

Munro TW, Sabina LR (1970) Inhibition of infectious bovine rhinotracheitis virus multiplication by thiosemicarbazones. J Gen Virol 7:55–63

Ong SG (1958) The depressive effect of certain elements on influenza virus 1 (in Chinese). Sci J (K'o Hsueh T'ung Pao, Kexue Tunghao) 3:157–159

O'Sullivan DG, Sadler PW (1961) Agents with high activity against type 2 poliovirus. Nature 192:341–343

O'Sullivan DG, Sadler PW, Webley C (1963) A study of the chemotherapeutic activity of isatin-β-4′,4′-dialkylthiosemicarbazones against ectromelia infection. Chemotherapia 7:17–26

O'Sullivan DG, Sadler PW, Russell V (1964) The influence of isatin β-thiosemicarbazone on the development of neurovaccinia virus in cells as shown by electron microscopy. Arch Gesamte Virusforsch 14:650–656

Pearson GD, Zimmerman EF (1969) Inhibition of poliovirus replication by N-methylisatin-β-4′:4′-dibutylthiosemicarbazone. Virology 38:641–650

Pennington TH (1977) Isatin-β-thiosemicarbazone causes premature cessation of vaccinia virus-induced late post-replicative polypeptide synthesis. J Gen Virol 35:567–571

Petering DH (1980) Carcinostatic copper complexes. In: Sigel H, Sigel A (eds) Metal complexes as anticancer agents. Dekker, New York Basel (Metal ions in biologic systems, vol 11, pp 197–229)

Pfau CJ (1975) Arenavirus chemotherapy– retrospect and prospect. Bull WHO 52:737–744

Pfau CJ (1977) Current status on the chemotherapy of experimental arenavirus infections. Medicina (B Aires) 37:219–224

Pillai CKS, Nandi US, Levinson W (1977) Interaction of DNA with anti-cancer drugs: copper-thiosemicarbazide system. Bioinorg Chem 7:151–157

Polatnick J (1965) Effect of chemical agents on foot-and-mouth disease virus production in cell culture. Am J Vet Res 26:1051–1055

Pollikoff R, Lieberman M, Lem NE, Foley EJ (1965) Antiviral activity of N-ethylisatin-β-thiosemicarbazone in vaccinia-infected mice. J Immunol 94:794–804

Porterfield JS, Casals J, Chumakov MP et al. (1978) Togaviridae. Intervirology 9:129–148

Rao AR, McFadzean JA, Squires S (1965) The laboratory and clinical assessment of an isothiazole thiosemicarbazone (M & B 7714) against pox viruses. Ann NY Acad Sci 130:118–127

Rao AR, McFadzean JA, Kamalakshi K (1966a) An isothiazole thiosemicarbazone in the treatment of variola major in man. Lancet 1:1068–1072

Rao AR, McKendrick GDW, Velayudhan L, Kamalakshi K (1966b) Assessment of an isothiazole thiosemicarbazone in the prophylaxis of contacts of variola major. Lancet 1:1072–1074

Rapp F (1964) Inhibition by metabolic analogues of plaque formation by herpes zoster and herpes simplex viruses. J Immunol 93:643–648

Rigaudy J, Klesney SP (1979) Nomenclature of organic chemistry. Pergamon, New York, pp 305–322

Rohde W, Shafer R, Idriss J, Levinson W (1979)Binding of N-methyl isatin β-thiosemicarbazone-copper complexes to proteins and nucleic acids. J Inorg Biochem 10:183–194

Rolly H, Winkelmann E (1972) A new thiosemicarbazone active in vivo against vaccinia virus. Adv Antimicrob Antineoplast Chemother 1/1:305–307

Runti C, Collino F, Pescani G (1968) Potenziali antivirali nota II – tiosemicarbazoni di acetofenoni sostituti. Farmaco [Sci] 23:114–121

Sadler PW (1965) Antiviral chemotherapy with isatin-β-thiosemicarbazone and its derivatives. Ann NY Acad Sci 130:71–79

Sandeman TF (1966) Pilot trial of an antiviral agent in malignant disease. Br Med J 2:625–627

Sartorelli AC, Agrawal KC, Tsiftsoglou AS, Moore EC (1977) Characterization of the biochemical mechanism of action of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones. Adv Enzyme Regul 11:117–139

Schmidt-Ruppin KH (1971) Chemotherapy of cytomegalovirus infections. Further results on intraperitoneal infection of Ehrlich ascites tumor mice with viruses of low virulence in vivo: MCMV/Smith, adenovirus 2, RSV/Long, and rhinoviruses. Chemotherapy 16:130–143

Sheffield FW (1962) Inhibition of rabbitpox virus replication by isatin β-thiosemicarbazone. Br J Exp Pathol 43:59–66

Sheffield FW, Bauer DJ, Stephenson S (1960) The protection of tissue cultures by isatin β-thiosemicarbazone from the cytopathic effects of certain pox viruses. Br J Exp Pathol 41:638–647

Singh M, Sudgen JK (1971) Some pyrrolidine thiosemicarbazones as potential antiviral agents. Pharm Acta Helv 46:627–631

Smejkal F, Budesinsky Z, Sulka J, Kuchar M (1972) Study of antiviral activity of some amantadine, pyrimidine, and isatine analogs. Adv Antimicrob Antineoplast Chemother 1/2:879–883

Squires SL, McFadzean JA (1966) The possible involvement of an interfering substance in the action of thiosemicarbazones against viruses. Trans R Soc Trop Med Hyg 60:419–421

Thompson RL, Price ML, Minton SA (1951) Protection of mice against vaccinia virus by administration of benzaldehyde thiosemicarbazone. Proc Soc Exp Biol Med 78:11–13

Thompson RL, Davis J, Russel PB, Hitchings GH (1953a) Effect of aliphatic oxime and isatin thiosemicarbazones on vaccinia infection in the mouse and in the rabbit. Proc Soc Exp Biol Med 84:496–499

Thompson RL, Minton SA, Officer JE, Hitchings GH (1953b) Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse. J Immunol 70:229–234

Tonew M, Tonew E (1974) Effects of some antiviral isatinisothiosemicarbazones on cellular and viral ribonucleic acid synthesis in mengovirus-infected FL cells. Antimicrob Agents Chemother 5:393–397

Tonew E, Lober G, Tonew M (1974a) The influence of antiviral isatinisothiosemicarbazones on RNA dependent RNA polymerase in mengovirus-infected FL cells. Acta Virol 18:185–192

Tonew M, Tonew E, Heinisch L (1974b) Antiviral thiosemicarbazones and related compounds. II. Antiviral action of substituted isatinisothiosemicarbazones. Acta Virol 18:17–24

Tsunoda A, Miyazaki K, Aota T, Matsumoto S, Kumagai K, Ishida S (1971) Anti-vaccinia activity of γ-thiochromanone-1-thiosemicarbazone in vitro and in vivo. Jpn J Microbiol 16:61–66

Varma RS, Nobles WL (1967) Thiosemicarbazones derived from indanedione-1,3. J Pharm Sci 56:775–776

Veckenstedt A, Horn M (1974) Testing of antiviral compounds against mengo virus infection of mice. 1. Screening of compounds and statistical evaluation of their effects. Chemotherapy 20:235–244

Veckenstedt A, Zgorniak-Nowosielska I (1979) Antiviral activity of certain isatin-isothiosemicarbazones against mengo and vaccinia virus infections in mice. Acta Virol 23:45–51

Wang L, Levinson W (1978) N-methyl isatin β-thiosemicarbazone copper complex inhibits RNA-dependent DNA polymerase but not ribonuclease H of Rous sarcoma virus. Bioinorg Chem 8:535–540

Webb DR, Bourne HR, Levinson W (1974) A new phosphodiesterase inhibitor in human lymphocytes: N-methyl-isatin-β-thiosemicarbazone. Biochem Pharmacol 23:1663–1667

Winkelmann E, Rolly H (1972) A new thiosemicarbazone with in vivo activity against smallpox. Adv Antimicrob Antineoplast Chemother 1/1:309–311

Woodson B, Joklik WK (1965) The inhibition of vaccinia virus by isatin β-thiosemicarbazone. PNAS 54:946–953

Zak MR (1959) Experience in the combined treatment of influenza. Probl Virol 4/3:84–86

Zgorniak-Nowosielska I, Marciszewska M, Pastuszak J, Lucka-Sobstel-Stobstel B (1973) The effect of isatin-β-thiosemicarbazone derivatives on vaccinia virus multiplication in L-132 cell line (human embryonic lung). Acta Microbiol Pol Ser A Microbiol Gen 22:199–204

Zgorniak-Nowosielska I, Gatkiewicz A, Potec Z (1976) The antiviral activity of isatin-β-thiosemicarbazone derivatives on vaccinia virus infection in mice. Arch Immunol Ther Exp 24:597–601

Zgorniak-Nowosielska I, Borysiewicz J, Cwik D, Drozd E (1978) The influence of some thiosemicarbazone derivatives with antiviral activity on immune response in mice. Arch Immunol Ther Exp 26:547–552

Zwartouw HT (1964) The chemical composition of vaccinia virus. J Gen Microbiol 34:115–123