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THE EFFECT OF SURAMIN-PHTHALANILIDE COMPLEXES ON THE CHEMOTHERAPEUTIC ACTIVITY AND TOXICITY OF 4′,4″-*BIS* (1,4,5,6-TETRAHYDRO-2-PYRIMIDINYL)TEREPHTHALANILIDE (NSC 57153)*

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Abstract—Suramin forms water-insoluble complexes with 4′, 4″-*bis* (1,4,5,6-tetrahydro-2-pyrimidinyl)terephthalanilide (NSC 57153). The mole ratio of Suramin–drug for maximum complex formation is 0·3 : 1. Excess Suramin dissolves this precipitate whereas excess drug does not. The uptake of NSC 57153 by P815 mast cell leukemic cells *in vitro* is affected by the presence of Suramin. Excess moles of Suramin relative to drug inhibit uptake, whereas excess moles of drug enhance the uptake.

Although Suramin administration has no effect on drug uptake by tumor cells *in vivo* and does not affect the antileukemic activity of NSC 57153, it does delay the onset of NSC 57153 toxicity in mice. Three drug components (lipid-bound, hydrophilic-bound, and "free" drug) are extracted from tumor cells and the ratio of the three drug components is not affected by the extracellular Suramin–drug ratios after the cells have been exposed to drug either *in vitro* or *in vivo*. Suramin lowers the concentration of NSC 57153 in both kidney and liver but not in muscle. The drug is extracted primarily as a lipid complex from all tissues, whether the animals have been treated with NSC 57153 or with Sumarin–NSC 57153 complexes.

MANY SUBSTITUTED phthalanilides have potent activity against a broad spectrum of transplanted murine leukemias and lymphomas.[1–7] However, several of these compounds cause renal toxicity and oculomotor paralysis which have minimized the clinical usefulness of the phthalanilides.[8–11] The acute and chronic toxicity of several phthalanilides can be prevented by complexing the phthalanilides with sulfonic and phosphoric compounds, but all of these agents, except Suramin, also blocked the antileukemic activity of the phthalanilides.[12–14] Suramin, a polysulfonate compound, reduced toxicity without preventing the chemotherapeutic efficacy of 2 terephthalanilides, NSC 50469 and NSC 66761†.[12–14]

The present study characterizes the Suramin complex with 4′,4″-*bis*(1,4,5,6-tetrahydro-2-pyrimidinyl)terephthalanilide (NSC 57153), which causes delayed renal toxicity,[11] quantitates the effects of Suramin on the uptake of NSC 57153 by P815 mast cell leukemia *in vitro* and *in vivo*, and evaluates the effects of Suramin on chemotherapeutic efficacy and delayed toxicity of NSC 57153 *in vivo*.

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† NSC 50469 is 2-amino-4′,4″-di(2-imidazolin-2-*yl*)terephthalanilide dihydrochloride, and NSC 66761 is 2-amino-4′,4″-*bis*(4-methyl-2-imidazolin-2-*yl*)terephthalanilide dihydrochloride.

MATERIALS AND METHODS

The phthalanilide, NSC 57153, was obtained from the Clinical Branch, Collaborative Research, National Cancer Institute, United States Public Health Service.* Suramin (trade name, Antrypol) was purchased from Imperial Chemical Industries Ltd., Pharmaceutical Division, Wilmslow, Cheshire, England.

In the chemotherapeutic studies, drug or its Suramin complexes were administered i.p. consecutively on days 1–10 to tumor-bearing BDF_1 hybrid mice which had been inoculated i.p. with 10^6 P815 mast cell leukemia cells on day zero. The sex of the mice is given with the experimental results. Drug uptake by P815 cells *in vivo* was evaluated after drug or drug complex was administered as a single dose to tumor-bearing mice on day 6 or 7, and the cells were harvested from 10 mice on day 7 after drug exposure of 0·5 or 24 hr. The doses are given with the experimental results.

In drug uptake studies, cells were harvested from at least 10 mice on day 7, centrifuged at 700 *g* for 15 min, washed twice with saline, and resuspended in Fischer's medium[15] containing 10% horse serum. The drug or its Suramin complex was added to 15-ml aliquots of a cell suspension (3–5 $\times$ 10^7 cells/ml) and incubated with stirring at 37° for 4 hr.

All treated cells were centrifuged at 700 *g* for 5 min and were washed twice with cold 0·9% NaCl. The drug content of washed cells was extracted into chloroform: methanol (2 : 1, v : v) and partitioned between a water : chloroform : methanol biphase.[16] Total drug in the aqueous phase was chromatographically differentiated into hydrophilic-bound and "free" drug and quantitated by u.v. spectrophotometry.[17–19] The drug associated with lipid in the non-aqueous phase was determined by the acid-displacement method.[16, 20]

In the toxicity studies, drug or its Suramin complex was administered i.p. on days 1–10 to BDF_1 mice. Weights of the mice were recorded every fifth day. Two animals were sacrificed every fifth day and selected tissues were excised, frozen in liquid nitrogen, and stored at — 20° until analyzed for total drug content by the solvent method.[16, 20]

Aqueous solutions of NSC 57153 and Suramin were mixed to form the Suramin–drug complexes. Suramin–drug mole ratios 1 : 1 to 1 : 8 gave water-insoluble complexes, and the quantity of precipitate was determined by light-scattering at 660 mμ with the Zeiss PMQ II spectrophotometer. The solubilization of complexes by Suramin was determined as follows: a Suramin–drug complex was formed at a mole ratio of 0·3 : 1; the resultant precipitate was washed twice with water by centrifugation and resuspended in 10 ml of Suramin solutions to yield final Suramin–drug ratios of 1·3 : 1 to 9·6 : 1. After standing for 10–30 min at room temperature, the remaining quantity of precipitate was determined by the light-scattering technique.

RESULTS

Effect of Sumarin on the uptake of NSC 57153 *by* P815 *cells*, in vitro *and* in vivo

The antileukemic activity of phthalanilides can be antagonized by several sulfonic and phosphoric analogs, but Suramin, a polysulfonate compound, did not prevent the chemotherapeutic effectiveness of several phthalanilides.[12–14] Burchenal *et al.*[12]

* The phthalanilide was synthesized by the Research Institute of Dr. A. Wander, S. A., Berne, Switzerland.

reported that NSC 50469 and NSC 66761 were found in P815Y cells after exposure to Suramin–drug complexes *in vitro*, but was not found in cells which were exposed to the sulfonic and phosphoric analogs. In our studies the quantitative aspects of Suramin–NSC 57153 complex formation and uptake by P815 cells *in vitro* and *in vivo* were determined.

The extent of complex formation between Suramin and NSC 57153 is shown in Fig. 1. Maximum complex (precipitate) formation occurred at a mole ratio of Suramin–NSC 57153 of approximately 1 : 3·3 (0·3 : 1). This complex could not be solubilized by excess drug (Fig. 1) or by 0·1 N HCl, but could be solubilized by excess

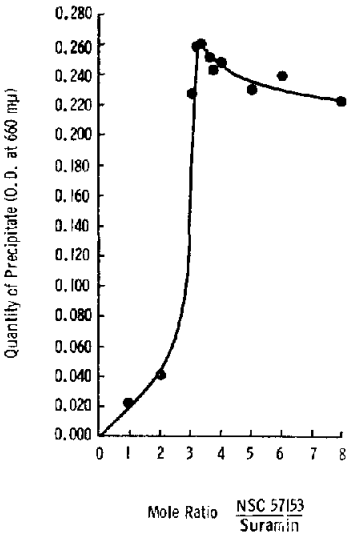

FIG. 1. Complex formation of NSC 57153 with Suramin. One μmole Suramin (1 ml) was mixed with NSC 57153 (0–8 ml) and diluted to 10 ml with water. The quantity of precipitate was determined by light-scattering. Other experimental details are in Methods.

Suramin (Fig. 2). At high mole ratios of Suramin–drug (4 : 1 and 8 : 1) most of the complex was soluble (Fig. 2) which was similar to the solubilization of *d*-tubocurarine chloride–congo red precipitates with excess congo red.[21]

The uptake of NSC 57153 by P815 cells *in vitro* was a function of the extracellular drug concentration. With extracellular drug concentrations of 20, 40, 60, and 80 μg of NSC 57153/ml medium, the intracellular drug levels were 14·9 ± 2·4 (S.D.), 24·0 ± 3·3, 37·7 ± 16·7, and 58·4 ± 23·1 μg/10^8 cells respectively. Intracellular NSC 57153 was differentiated into lipid-bound, hydrophilic-bound, and "free" drug. "Free" drug represented approximately 50 per cent of total drug; lipid-bound and hydrophilic-bound approximately 25 per cent each. This is similar to the uptake of another terephthalanilide, NSC 60339*, by P388 lymphocytic leukemia cells *in vitro*.[19, 22]

At drug concentrations of 20, 40, 60, and 80 μg/ml medium, Suramin did not affect the per cent distribution of the three drug fractions (Table 1) but did influence the overall uptake of the drug *in vitro* (Fig. 3), At Suramin–drug ratios of 4 : 1 and 8 : 1, the uptake of NSC 57153 by P815 was inhibited. At maximum complex formation

* NSC 60339 is 2-chloro-4′,4″-di(2-imidazolin-2-*yl*)terephthalanilide.

(0·3 : 1), the uptake of NSC 57153 was stimulated approximately 2-fold. These experimental results were not affected by nonspecific adsorption of precipitated Suramin–drug complexes to cells because such complexes were not soluble in the chloroform : methanol or chloroform : methanol : water (acid) solvents which were used in the determination of intracellular drug concentrations. If such complexes were present intracellularly, our methodology would not have detected them either.

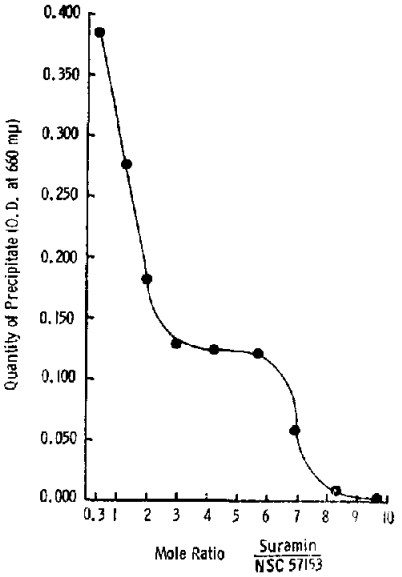

FIG. 2. Solubilization of NSC 57153-Suramin complexes with Suramin. Suramin was added to a known amount of Suramin–drug complex (0·3 : 1) which was water insoluble. The insoluble complex which was not dissolved by Suramin was quantitated by light-scattering. Other experimental details are described in Methods.

TABLE 1. EFFECT OF SURAMIN ON THE INTRACELLULAR DISTRIBUTION OF NSC 57153 IN P815 MAST CELL LEUKEMIA*

Suramin–drug ratios†	No. of expts. at each of 4 drug concentrations†	No. of determinations	Per cent NSC 57153 in P815 cells		
			Lipid-bound	Hydrophilic-bound	"Free"
Control	7	28	23·3 ± 7·7‡	25·2 ± 10·4	51·5 ± 12·0
0·1 : 1	3	12	15·9 ± 4·2	29·3 ± 11·7	56·0 ± 11·8
0·2 : 1	3	12	14·7 ± 4·2	27·1 ± 12·2	58·5 ± 13·9
0·3 : 1	6	24	16·3 ± 5·9	19·6 ± 8·2	64·8 ± 10·5
1 : 1	3	11	16·9 ± 7·9	31·3 ± 17·0	51·7 ± 14·5
2 : 1	4	16	18·8 ± 4·6	28·9 ± 11·5	52·9 ± 10·9
4 : 1	4	16	17·9 ± 6·6	23·9 ± 8·3	58·1 ± 8·7
8 : 1	4	14	21·1 ± 7·9	19·5 ± 6·7	59·4 ± 10·4

* The P815 cells were harvested from male and female mice on day 7, washed with saline, and suspended in Fischer's medium, which contained horse serum and various concentrations of drug, for 4 hr at 37°.

† In all but two experiments, the extraceullular drug concentrations were 20, 40, 60 and 80 μg NSC 57153/ml for each Suramin–drug ratio. For the Suramin–drug ratio of 1 : 1 and 8 : 1, only 2 drug concentrations, 20 and 80 μg/ml, were used in 1 experiment.

‡ Mean ± S.D.

The effect of Suramin on the uptake of NSC 57153 by P815 cells *in vivo* was also determined (Table 2). All three drug fractions were extracted from these P815 cells and, as in the experiments *in vitro*, Suramin had little or no effect on the relative distribution of the three drug components. On the other hand, Suramin did not affect the uptake of drug by P815 cells *in vivo*. This latter finding was further supported by the observations that the chemotherapeutic effectiveness of NSC 57153 was unaffected

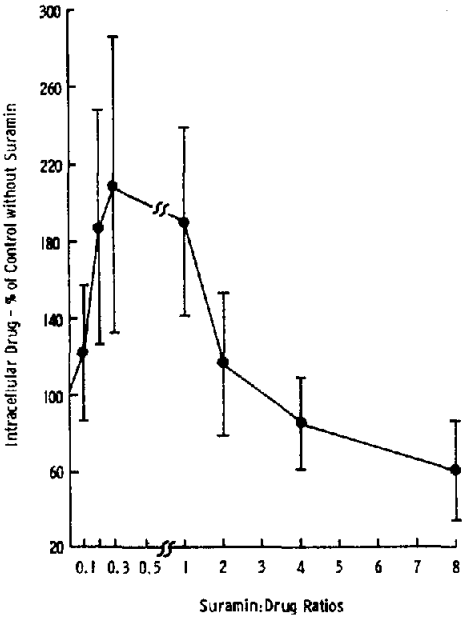

FIG. 3. Effect of Suramin on the uptake of NSC 60339 by P815 cells *in vitro*. Drug concentrations were 20, 40, 60, and 80 μg/ml medium. The data represent averages ± S.D. for these drug concentrations at each Suramin–drug ratio. Experimental details are in Methods.

TABLE 2. NSC 57153 CONCENTRATIONS IN P815 CELLS, GROWING I.P. IN MICE, AFTER A SINGLE I.P. DOSE OF NSC 57153 OR SURAMIN–DRUG COMPLEXES

	NSC 57153 (μg/10⁸ cells)*							
	Total		Lipid bound		Hydrophilic-bound		"Free"	
Time after treatment (hr)	0·5	24	0·5	24	0·5	24	0·5	24
Suramin–drug ratios								
Control†	3·1	4·4	1·5	2·0	0·3	0·2	1·3	2·2
0·33 : 1	4·3	3·6	2·1	1·5	0·3	0·3	1·9	1·8
2 : 1	6·0	3·1	3·0	1·8	0·5	0·2	2·5	1·1
8 : 1	3·7	3·0	2·0	1·4	0·3	0·4	1·4	1·2

* Average of 2 experiments.

† The P815 leukemia cells were grown i.p. in BDF_1 mice (male or female). The cells were harvested on day 7, and the drug (4 mg/kg) or the same concentration of drug as Suramin–drug complexes was administered as a single dose, 0·5 and 24 hr before the cells were harvested.

by the presence of Suramin compared to drug controls in the absence of Suramin (Table 3). The chemotherapeutic effectiveness of other Suramin drug combinations—Suramin ranged from 10 to 150 mg/kg and NSC 57153 ranged from 0·5 to 32 mg/kg—did not vary significantly from controls.

TABLE 3. EFFECT OF SURAMIN ON THE CHEMOTHERAPEUTIC EFFICACY OF NSC 57153 AGAINST P815 CELLS GROWING I.P. IN MICE*

Animal group	Dose (mg/kg/day × 10)	Median survival time (days)			
		Female mice		Male mice	
Control		9·0	9·0	9·0	9·0
Suramin	40	8·0	8·5	9·0	8·5
NSC 57153	4	35·5	21·5	13·0	27·5
Suramin–NSC 57153	40 : 4†	28·0‡	20·0§	15·0§	29·0‡

* Each BDF_1 mouse was inoculated i.p. with 10^6 P815 mast cell leukemic cells on day 0, and drug or Suramin–drug was administered i.p. daily on days 1–10. Each experimental group contains 10–100 mice.

† The mole ratio of Suramin–drug was 4 : 1.

‡ Suramin and drug were administered i.p. separately (*ca.* 5-min delay).

§ Suramin and drug were complexed prior to i.p. administration.

Effect of Suramin on toxicity

Delayed toxicity has been found for several phthalanilides including NSC 57153.[8–11] From physiological disposition studies it was generally found that those tissues, e.g. kidney and liver, which were adversely affected by the phthalanilides, accumulated most drug.[3, 8, 11, 20, 23–25] The effect of Suramin on delayed toxicity and tissue accumulation of drug was evaluated in the following study.

As seen in Table 4, Suramin treatment increased the median survival time (MST) of female mice treated with NSC 57153. Delayed death of NSC 57153-treated mice

TABLE 4. EFFECT OF SURAMIN ON THE DELAYED TOXICITY OF NSC 57153 IN BDF_1 FEMALE MICE

Animal group	Treatment (mg/kg/day × 10)	Initial wt. (g)	Avg. wt. at MST or 80 days	MST*
Control		18	23	>100
Suramin	40	18	25	>100
NSC 57153	4	20	12	48·8
Suramin–NSC 57153	40–4†	20	15	70·5
Suramin + NSC 57153	40 + 4‡	19	16	>100
NSC 57153 + Suramin	4 + 40‡	19	14	83·5

* Median survival time in days.

† Administered as a complex.

‡ Each component was administered separately in the order shown with *ca.* 5-min wait between injections.

was associated with loss of body weight. These weight losses, which occurred by the MST or 80 days, were not significantly affected by the type of Suramin treatment. The toxicity of NSC 57153 was more apparent in male than in female mice. The MST for male mice which were treated with NSC 57153 (4 mg/kg/day × 10 days) was 34·3

days and the average weight at 34 days was approximately 13 g. Although similar weight losses occurred with Suramin–drug combinations (40 : 4 mg/kg/day × 10 days), the MST was increased significantly by Suramin and ranged from 54–66 days.

Several tissues from these animals were analyzed for drug concentration (Table 5). The kidneys of mice which received NSC 57153 (4 mg/kg/day × 10 days) had the

TABLE 5. EFFECT OF SURAMIN ON THE PHYSIOLOGICAL DISPOSITION OF NSC 57153 IN TISSUES FROM FEMALE MICE AFTER I.P. ADMINISTRATION OF NSC 57153 OR SURAMIN AND DRUG FOR 10 DAYS

Tissues	Days from start of treatment: Suramin + drug administered (mg/kg/day × 10)	5	10	15	20	25	30
		Avg. μg NSC 57153/g of tissue*					
Kidney	0 + 4	86	191	131	120	120	107
	40 + 4†	64	91	75	76	80	70
Liver	0 + 4	48	70		83		80
	40 + 4†	26	38		40		43
Muscle	0 + 4	6	8				
	40 + 4†	6	8				

* Average of duplicate samples of tissues from 2 mice.

† The mole ratio of Suramin–drug was 4 : 1 and each was administered separately in the order shown.

highest drug concentration, as found in other studies of rats, dogs, and monkeys.[20, 23] Concurrent Suramin administration resulted in lower NSC 57153 concentrations in both liver and kidney of BDF_1 mice. The drug concentration in muscle was low and was apparently unaffected by Suramin treatment. Similar but lower drug concentrations were found in kidney and liver after treatment with NSC 57153 (2 mg/kg per day × 10 days) and Suramin–drug treatment (40 : 2 mg/kg/day × 10 days). In all tissues the drug was complexed primarily (> 80 per cent) with lipids.

DISCUSSION

The phthalanilides, which are cationic at neutral pH, form ionic complexes with many biological components *in vitro*; e.g. anionic lipids,[26] nucleic acids,[27–30] and protein,[17] but evidence for these complexes *in situ* has not been demonstrated. The phthalanilides are extracted from tissues and tumor cells primarily as ionic drug–lipid complexes.[22, 26, 31] The lipids that are extracted with NSC 57153 from dog brain and with NSC 60339 from P388 and NSC 60339-resistant P388 cells are a new class of phospholipids. The phospholipids are characterized by their unusual glycerol : fatty acid : phosphorus : sulfur : nitrogen ratios and by their unidentified ninhydrin-positive components*.[26, 31] The concentration of the extracted drug complexes is related to chemotherapy[19] and is generally highest in tissues and tumors that are adversely affected by phthalanilide treatment.[8, 11, 16, 20, 22]

In these studies drug could be extracted as lipid-bound, hydrophilic-bound, and "free" drug from all Suramin-treated tissues and cells even though the polysulfonate Suramin complexed with NSC 57153 *in vitro*. Although Suramin affects the uptake

* D. W. Yesair *et al.*, unpublished data.

of drug by P815 cells *in vitro*, P815 cells *in vivo* take up equivalent amounts of drug in the presence or absence of Suramin. Thus, the chemotherapeutic effectiveness of NSC 57153 is not affected by the presence or absence of Suramin. On the other hand, NSC 57153 concentrations in liver and kidney of Suramin–NSC 57153-treated mice are significantly less than the control NSC 57153-treated mice, and Suramin delays the onset of toxicity by NSC 57153. These findings are consistent with our views that there is a specific association of drug with tissue lipids[26] and that the intracellular concentration of extractable drug complexes is related to both chemotherapy[19, 22] and toxicity.[8, 20]

The uptake of drug by P815 cells *in vitro* is influenced by the relative amount of Suramin present. At maximum complex formation between Suramin and drug (mole ratio, 0·3 : 1), the uptake of drug by cells is maximal. Complexes of Suramin with excess drug (1 : >4) are taken up better than drug alone, whereas complexes with excess Suramin (4 : 1, 8 : 1) inhibit drug uptake. Since these complexes have either a zero, positive, or negative charge and since the cells have a net negative charge,[32, 33] the difference in uptake of the various drug complexes probably reflects this difference between the net charge on the complex and that on the cells.

Suramin and other anionic compounds which complex with the phthalanilides antagonize the acute and chronic toxicity of several phthalanilides.[12–14] In the present studies, Suramin also delayed the onset of toxicity by the terephthalanilide NSC 57153 in both male and female mice The weight losses after NSC 57153 administration are not affected by Suramin. The administration of Suramin does, however, result in lower drug concentrations in kidney and liver but does not change the relatively low drug concentration in muscle. The drug in these tissues is complexed primarily with lipids as in P185 cells. Since Suramin itself is not deposited in any particular tissue,[34] Suramin probably enhances the excretion of drug, as a Suramin complex before deposition of NSC 57153 as a lipid complex in tissues. Therefore, better scheduling of drug and Suramin administration may enhance their complex formation *in vivo* and thus may enhance excretion of the drug prior to its deposition in tissues. Such enhancement of drug excretion may be of value in improving the therapeutic value of the phthalanilides.[8]

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REFERENCES

1. L. L. BENNETT, JR., in *Progress in Experimental Tumor Research* (Ed. F. HOMBURGER). Jafner, New York, (1965).
2. J. H. BURCHENAL, M. S. LYMAN, J. R. PURPLE, V. COLEY, S. SMITH and E. BUCHOLZ, *Cancer Chemother. Rep.* **19**, 19 (1962).
3. C. J. KENSLER, *Cancer Res.* **23**, 1353 (1963).
4. L. W. LAW, *Cancer Chemother. Rep.* **19**, 13 (1962).
5. A. F. PITILLO, L. L. BENNETT, JR., W. A. SHORT, A. J. TOMISEK, C. J. DIXON, J. R. THOMSON, W. R. LASTER, JR., M. TRADER, L. METTIL, P. ALLAN, B. BOWDON, F. M. SHABEL, JR. and H. E. SKIPPER, *Cancer Chemother. Rep.* **19**, 41 (1962).
6. S. A. SCHEPARTZ, I. WODINSKY and J. LEITER, *Cancer Chemother. Rep.* **19**, 1 (1962).
7. J. M. VENDITTI, A. GOLDIN and I. KLINE, *Cancer Chemother. Rep.* **19**, 5 (1962).
8. C. J. KENSLER, P. E. PALM, H. M. DAY, S. P. BATTISTA, W. I. ROGERS, D. W. YESAIR and I. WODINSKY, *Cancer Res.* **25**, 1622 (1965).
9. J. C. MARSH, J. B. BLOCK, C. J. BENTZEL and R. D. POWELL, JR. *Cancer Chemother. Rep.* **36** 35 (1964).

10. M. V. NADKARNI, R. D. DAVIS, M. L. RAKIETEN, N. RAKIETEN and S. FRANCES, *Cancer Chem other. Rep.* **19**, 31 (1962).
11. P. E. PALM, W. I. ROGERS, D. W. YESAIR and C. J. KENSLER, *Toxi. appl. Pharmac.* **7**, 494, abstract 50 (1965).
12. J. H. BURCHENAL, H. ADAMS, S. LANCASTER and R. HIRT, *Fedn Proc.* **24**, 443, abstract 1745 (1965).
13. J. H. BURCHENAL, V. G. GREGGS, S. P. LANCASTER, R. HIRT, R. BERCHTOLD, R. FISCHER and R. BALSIGER, *Cancer Res.* **25**, 469 (1965).
14. J. H. BURCHENAL, E. SPOONER and S. LANCASTER, *Proc. Am. Ass. Cancer Res.* **6**, 9, Abstract 33, (1965).
15. G. A. FISCHER and A. SARTORELLI, in *Methods in Medical Research* (Ed. A. M. EISEN). The Year Book, Chicago (1964).
16. D. W. YESAIR, F. A. KOHNER, W. I. ROGERS, P. E. BARONOWSKY and C. J. KENSLER, *Cancer Res.* **26**, 202 (1966).
17. W. I. ROGERS, I. M. YORK and C. J. KENSLER, *Cancer Chemother. Rep.* **19**, 67 (1962).
18. A. SIVAK, W. I. ROGERS, I. WODINSKY and C. J. KENSLER, *J. natn. Cancer Inst.* **33**, 457 (1964).
19. D. W. YESAIR, W. I. ROGERS, P. E. BARONOWSKY, I. WODINSKY, P. S. THAYER and C. J. KENSLER, *Cancer Res.* **27**, 314 (1967).
20. W. I. ROGERS, D. W. YESAIR and C. J. KENSLER, *J. Pharmac. exp. Ther.* **152**, 139 (1966).
21. C. J. KENSLER, *J. Pharmac. exp. Ther.* **95**, 28 (1949).
22. D. W. YESAIR and C. C. HOFOOK, *Cancer Res.* **27**, February (1968).
23. W. I. ROGERS, S. A. SATTINGER and C. J. KENSLER, *Biochem. Pharmac.* **15**, 1225 (1966).
24. W. I. ROGERS, A. SIVAK and C. J. KENSLER, *Cancer Chemother. rep.* **38**, 19, (1964).
25. W. KREIS, R. BLOCK, D. L. WARKENTIN and J. H. BURCHENAL, *Biochem. Pharmac.* **12**, 1165 (1963).
26. D. W. YESAIR, W. I. ROGERS, J. T. FUNKHOUSER and C. J. KENSLER, *J. Lipid Res.* **7**, 492 (1966)
27. H. M. VON RAUEN, K. NORPOTH, W. UNTERBERG and H. HAAR, *Experientia* **21**, 300 (1965).
28. H. M. VON RAUEN, H. HAAR and W. UNTERBERG, *Arzneimittel-Forsch.* **16**, 533 (1966).
29. A. SIVAK, W. I. ROGERS and C. J. KENSLER, *Biochem. Pharmac.* **12**, 1056 (1963).
30. A. SIVAK, W. I. ROGERS, I. WODINSKY and C. J. KENSLER, *Cancer Res.* **25**, 902 (1965).
31. D. W. YESAIR, J. FUNKHOUSER, W. I. ROGERS and C. J. KENSLER, 9th *Int. Conf. Biochem. Lipids* **22**, abstract 4. Noordwijk aan Zee, Netherlands (1965).
32. M. ABERCROMBIE and E. J. AMBROSE, *Cancer Res.* **22**, 525 (1962).
33. A. D. BANGHAM, J. C. GLOVER, S. HOLLINGSHEAD and B. A. PETHICA, *Biochem. J.* **84**, 513 (1962).
34. A. SPINKO, *Biochem. J.* **42**, 109 (1948).