

See Table II for analytical data. Ir (KBr) indicated the enol form: 3.2-3.6 (broad), 6.10, 6.20, 6.35, 6.50, 7.35, 8.44  $\mu$ ; nmr (DMSO- $d_6$ )  $\tau$  7.12 (s, 3, NCH<sub>3</sub>), 2.80 and 2.40 (d,  $J = 4.5$  cps, 1 each, thiazole protons), 1.9-2.2 (m, 4, aromatic protons), a broad, weak peak from -2.0 to -0.6 (2, the enol OH and the NH, exchanges D<sub>2</sub>O); mass spectrum parent ion  $m/e$  337 (calcd 337) with major ions of  $m/e$  273, 173, 174, 145, 144, 117, 104, and 100. A sample of 1 gave a deep red-brown color with 5% FeCl<sub>3</sub> reagent. A very stable hemichloroform solvate of 1 was isolated when chloroform was used on one occasion to recrystallize 1. Infrared spectra obtained on all compounds in Table II indicated the enol form in every case. All of the compounds gave a deep red color with dilute FeCl<sub>3</sub> solution.

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#### References

- (1) J. G. Lombardino, E. H. Wiseman, and W. M. McLamore, *J. Med. Chem.*, 14, 1171 (1971).
- (2) (a) J. G. Lombardino and E. H. Wiseman, *ibid.*, 15, 858 (1972); (b) J. G. Lombardino, U. S. Patent 3,591,584 (1971).
- (3) (a) H. Zinnes, N. Lindo, and J. Shavel, Jr., U. S. Patent 3,646,021 (1972); (b) H. Zinnes, N. Lindo, J. C. Sircar, M. L. Schwartz, and J. Shavel, Jr., *J. Med. Chem.*, 16, 44 (1973).
- (4) E. H. Wiseman and J. Chiaini, *Biochem. Pharmacol.*, 21, 2323 (1972).
- (5) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1962).

## Effects of a Series of New Synthetic High Polymers on Cancer Metastases<sup>†</sup>

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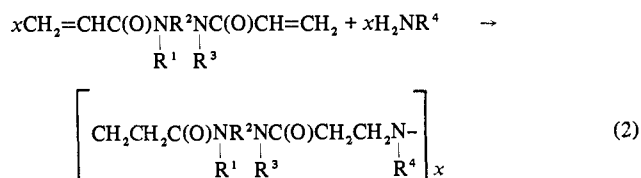
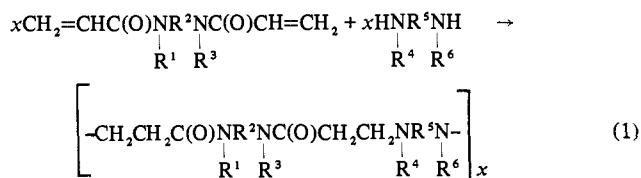
Sixteen new synthetic high polymers were studied for a possible effect on the dissemination of cancer cells from Sarcoma 180 implanted intracerebrally (ic). The efficacy in reducing the formation of lung metastases from Lewis lung carcinoma implanted intramuscularly (im) and of lymphnodal metastases from Ehrlich carcinoma implanted intratibially (it) was also determined after treatment with the polymeric substances. Some compounds tested showed almost the same activity, in the same experimental conditions, of several well-known antitumor agents, such as, for instance, cyclophosphamide and Triton WR 1339. The structure of most of the polymers is such that they may show simultaneously both cationic and tensioactive properties. The structure-activity relationship is discussed and a suggestion is made concerning the properties of polymers which should be taken into account when designing new macromolecular drugs with a potential antimetastatic activity.

A considerable amount of information is available concerning the importance of the cell membrane in affecting the various aspects of cancer cell kinetics, such as motility, invasion of normal tissue, vascular and lymphatic dissemination, and metastases formation.<sup>1-4</sup> Consequently, any possible physicochemical and biochemical interaction of the cancer cell membrane with foreign compounds may be regarded as a possible method for controlling cancer growth and dissemination. To this end high polymers may prove to be of particular interest. Polyfunctional polymers, in fact, are expected to have a stronger overall power of interaction with the cell membrane than small molecules of similar structure. This power of interaction may be due to a synergistic effect of many functional groups, either of the same kind or chemically different, attached to the same macromolecular backbone. Recent results from this<sup>5,6</sup> and other laboratories<sup>7-9</sup> seem to be encouraging in pursuing this approach. Several high polymers were tested in the present study for their possible effect on cancer dissemination and metastases.

**Chemistry.** The polymers tested, which are listed in the Table I, have cationic properties and most of them contain both hydrophilic and lipophilic groups. They may be grouped into three classes: G<sub>1</sub>-G<sub>12</sub> are poly(amide amine) polymers; G<sub>13</sub> and G<sub>14</sub> are acrylamide-type polymers; and finally G<sub>15</sub> and G<sub>16</sub> are *N*-oxide polymers.

The poly(amide amine) polymers were prepared by hydrogen-transfer polyaddition of suitable primary or secondary amines to a bisacrylamide. In this kind of polyaddition, linear

polymers can be obtained with primary monoamines or secondary bisamines. This reaction was extensively studied during a research on the synthesis of *tert*-amino polymers,<sup>10</sup> and it was found to proceed very smoothly, in aqueous or alcoholic solution, without catalysts, according to the general eq 1 and 2.


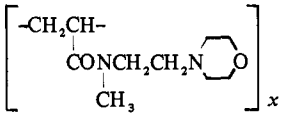
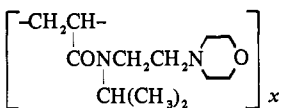
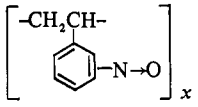
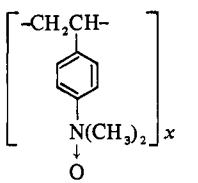


In the resulting polymers, amide and amine bonds regularly alternate along the main chain. In a previous investigation<sup>10-12</sup> it was found that the reactivity of the amines toward polyaddition of bisacrylamides is mainly dependent from the steric hindrance on the  $\alpha$  carbon, provided their basicity is not too different.

On this ground, the reactivities of the amines employed in the synthesis of the poly(amide amines) listed in the Table I cannot be expected to be very different. Hence, in the case of copolymeric products, such as are the polymers G<sub>1</sub> and G<sub>12</sub>, no rule may be expected to exist in the distribution of the different amine units among themselves, possibly with the only exception of G<sub>4</sub>. In all cases, however, amide and amine

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Table I. List of Compounds Tested

Code no.	Polymer formula	Dose, mg/kg	Route of administration <sup>a</sup>	Tumor tested for	
				Dissemination	Metastases
(A) Poly(amide amines) of General Formula $\left[ -(\text{CH}_2)_2\text{C}(\text{O})\text{N} \begin{array}{c} \diagup \diagdown \\ \diagdown \diagup \end{array} \text{NC}(\text{O})(\text{CH}_2)_2\text{N} \begin{array}{c} \diagup \diagdown \\ \diagdown \diagup \end{array} \text{R} \right]_x$					
G <sub>1</sub>	R = (CH <sub>2</sub> ) <sub>11</sub> CH <sub>3</sub> (10%) and (CH <sub>2</sub> ) <sub>2</sub> OH (90%)	20	iv	S 180	Lewis-Ehrlich
G <sub>2</sub>	R = (CH <sub>2</sub> ) <sub>11</sub> CH <sub>3</sub> (20%) and (CH <sub>2</sub> ) <sub>2</sub> OH (80%)	20	iv	S 180	Lewis
G <sub>3</sub>	R = (CH <sub>2</sub> ) <sub>11</sub> CH <sub>3</sub> (30%) and (CH <sub>2</sub> ) <sub>2</sub> OH (70%)	10	iv	S 180	Lewis-Ehrlich
G <sub>4</sub>	R = CH <sub>2</sub> COOH (50%); 50% of -N- is substituted by 	200	iv	S 180	Lewis-Ehrlich
G <sub>5</sub>	R = CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> (50%) and (CH <sub>2</sub> ) <sub>2</sub> OH (50%)	20	iv		Lewis
G <sub>6</sub>	R = CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> (10%) and (CH <sub>2</sub> ) <sub>2</sub> OH (90%)	20	iv		Lewis
G <sub>7</sub>	R = CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> (30%) and (CH <sub>2</sub> ) <sub>2</sub> OH (70%)	10	iv		Lewis
G <sub>8</sub>	R = CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub> (30%) and (CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub> (70%)	0.5	iv		Lewis
G <sub>9</sub>	R = (CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub> (50%) and (CH <sub>2</sub> ) <sub>2</sub> OH (50%)	10	iv	S 180	Lewis
G <sub>10</sub>	R = (CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub> (10%) and (CH <sub>2</sub> ) <sub>2</sub> OH (90%)	20	iv	S 180	Lewis
G <sub>11</sub>	R = (CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub> (30%) and (CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub> (70%)	2	iv	S 180	Lewis
G <sub>12</sub>	R = (CH <sub>2</sub> ) <sub>4</sub> CH <sub>3</sub> (10%) and (CH <sub>2</sub> ) <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub> (90%)	1	iv		Lewis
(B) Poly-N-(β-morpholinoethyl)acrylamides					
G <sub>13</sub>		200	iv		Lewis
G <sub>14</sub>		200	iv	S 180	Lewis-Ehrlich
(C) N-Oxide Polymers					
G <sub>15</sub>		200	iv	S 180	Lewis
G <sub>16</sub>		200	iv	S 180	Lewis

<sup>a</sup>iv = intravenously.

groups must regularly alternate along the macromolecular chain.

The acrylic polymers G<sub>13</sub> and G<sub>14</sub> were prepared by radical polymerization of the corresponding monomers.<sup>13</sup> Poly(2-vinyl)pyridine *N*-oxide (G<sub>15</sub>) was also prepared by radical polymerization of the monomers.<sup>14</sup> Poly(4-dimethylamino)-styrene *N*-oxide (G<sub>16</sub>) was prepared by *N*-oxidation of poly(4-dimethylamino)styrene with peracetic acid.<sup>15</sup>

The average molecular weights of the polymers were not exactly determined. However, they were estimated through intrinsic viscosity measurements. It is tentatively suggested that the molecular weights of poly(amide amines) G<sub>1</sub>-G<sub>12</sub> were in the order of a few tens of thousands, while that of the other acrylic and *N*-oxide polymers G<sub>13</sub>-G<sub>16</sub> were in the order of a hundred thousands.

### Biological Activity and Discussion

The results obtained with the polymers on (1) the inhibition of several tumors<sup>‡</sup> in different strains of mice, (2) the

reduction of the number of cancer cells disseminated in blood and lung,<sup>§</sup> and (3) the reduction of the number and of the weight of metastases located in lung<sup>#</sup> and lymph nodes<sup>\*\*</sup> are summarized in Table II. Only compounds which had shown a significant activity in at least one test are reported in Table II. Each figure in the table represents the percentage of the mean value obtained in treated mice in respect to that of controls. The effect on the primary tumor at the site of implantation was evaluated according to the inhibition of tumor weight in the case of subcutaneous (sc) Sarcoma 180 (5 × 10<sup>6</sup> cells) following treatment with polymers on days 1, 3, 5, 7, and 9 after tumor implantation; in the case of Ehrlich carcinoma intratibially (it) following treatment with polymers on days

<sup>§</sup>Sarcoma 180 cells (10<sup>5</sup>) were injected intracerebrally (ic) in the thalamic area on the medium line, according to Rosso, *et al.*<sup>16</sup> The presence of neoplastic cells in the blood and lungs of mice bearing ic tumor was evaluated 7 days after tumor implantation by a bio-assay procedure described by Rosso, *et al.*,<sup>17</sup> and Donelli, *et al.*<sup>18</sup>

<sup>#</sup>Metastatic nodules on the surface of the lung of C<sub>57</sub>B1/10 J/Sel mice bearing Lewis lung carcinoma intramuscularly (im) were counted and measured 25 days after tumor implantation according to Wexler.<sup>19</sup>

<sup>\*\*</sup>Ehrlich carcinoma cells (10<sup>5</sup>) were injected intratibially (it), as described by Franchi, *et al.*<sup>20,21</sup> Macroscopic metastases, mainly localized in the lymphatic system, were counted and weighed at autopsy after natural death.

<sup>‡</sup>Sarcoma 180 and Ehrlich carcinoma were transplanted in random-bred Albino Swiss mice and Lewis lung carcinoma (kindly supplied by Dr. K. Karrer, Wien) was transplanted in C<sub>57</sub>B1/10 J/Sel inbred mice, a strain of mice for which the tumor is isogenic.

Table II. Active Polymers on Cancer Cell Dissemination and/or Metastases (Controls = 100)

Polymers	Effect on primary tumors				Effect on dissemination		Effect on metastases	
	S 180 ic	S 180 sc	Ehrlich it	Lewis im	Bioassay of mice bearing S 180 ic		Lung metastases <sup>a</sup>	
					Lung	Blood	No.	Wt
G <sub>1</sub>	64 (12)	91 (2)	104 (7)	71 <sup>±</sup> (7)	33 <sup>x</sup>	120	48 (15)	33 <sup>±</sup> (9)
G <sub>2</sub>		81 (1)		84 (12)	100	160	48 (12)	40 <sup>±</sup> (14)
G <sub>3</sub>	61 (10)	106 (0.7)	123 (7)	79 <sup>±</sup> (5)	56 <sup>xx</sup>	80	47 <sup>±</sup> (10)	20 <sup>±</sup> (4)
G <sub>4</sub>	115 (26)	100 (3)	102 (7)	93 (6)	21 <sup>x</sup>	55 <sup>x</sup>	73 <sup>±</sup> (6)	67 (23)
G <sub>9</sub>		104 (4)		100 (10)	150	86	48 <sup>±</sup> (13)	93 (30)
G <sub>14</sub>	99 (7)	90 (5)	105 (17)	78 (5)	67	117	36 <sup>±</sup> (6)	16 <sup>±</sup> (4)

<sup>a</sup>Control's values: number  $\pm$  S.E. =  $7.7 \pm 1.6$ ; weight  $\pm$  S.E. =  $11.7 \pm 3.9$  mg. Each figure represents the mean value of at least ten treated mice as per cent of controls with S.E. The statistical analysis compared the original values with the relevant controls using either  $\chi^2$  (x,  $P < 0.01$ ; xx,  $P < 0.05$ ) or Student's t test (+,  $P < 0.01$ ; =,  $P < 0.05$ ).

3, 7, 12, 17, 20, 24, and 27 after tumor transplantation; and in the case of Lewis lung carcinoma intramuscular (im) following treatment with polymers on days 0, 4, 7, 11, 14, and 18 after tumor transplantation and according to the increase of mouse survival time in the case of Sarcoma 180 intracerebrally (ic) following treatment with polymers on days 1, 3, 5, and 7 after tumor transplantation. The doses employed are the maximal tolerated ones evaluated after 6 days of treatment in Albino Swiss female mice. As reported in Table II, a reduction was observed in the weight of the primary tumor in the case of Sarcoma 180 and of Lewis lung carcinoma treated with G<sub>1</sub> and G<sub>3</sub>.

However, the statistical evaluation of the results showed that only for the second tumor are the results significant. G<sub>1</sub> reduces the number of cells present in the lung of mice bearing Sarcoma 180 ic without affecting the presence of cells in the blood. It is also able to cause a decrease in the weight of lung metastases in mice bearing Lewis carcinoma im.

The same was observed for G<sub>2</sub> which, conversely, is unable to affect cancer cell dissemination in the blood and lung from the ic Sarcoma 180.

G<sub>3</sub> significantly reduces the presence of cell disseminated in the lung of mice injected with Sarcoma 180 ic and also the number and weight of lung metastases from Lewis carcinoma implanted im.

G<sub>4</sub> reduces the number of cells present in the lung (Sarcoma 180 ic). This is probably related to the decrease in the number of cells present in the blood. The number of lung metastases from Lewis carcinoma is also lowered by the treatment with the latter polymer. The same was observed for G<sub>9</sub>, although it is unable to affect cancer cell dissemination from Sarcoma 180 transplanted ic.

G<sub>14</sub> was the most effective compound in decreasing lung metastases in the Lewis lung tumor. None of the compounds tested was effective in reducing the number or the weight of lymph nodal metastases. The effect on cancer dissemination and on metastasis formation showed by the active polymers in this investigation may be compared with that of well-known antitumoral agents such as cyclophosphamide<sup>22</sup> and Triton WR 1339, previously studied in this laboratory.<sup>6,22</sup>

In this connection, it should be noted that the dissemination of cancer cells is a complex chain of events including the release of cells from the implanted tumor, their transport through blood and/or the lymphatic vessels, their lodgment in normal tissues, and the metastatic growth. Therefore, the inhibition of metastasis formation may be the consequence of a direct effect of the polymers on the proliferation of cells at the primary site, as is the case of cytotoxic agents, such as cyclophosphamide,<sup>23</sup> but it may not be necessarily dependent on it. For instance, cancer dissemination and metastasis can

be affected by Triton WR 1339 without any action of this compound on tumor growth at the site of implantation.<sup>6</sup> This also seems to be the case of the polymers tested in this work, since they do not show any measurable effect on the growth of different implanted tumors. In addition, these polymers do not show cytotoxic effect on KB cells cultured *in vitro*, 48 hr after treatment with a single dose of 100  $\mu$ g/ml (L. Morasca, personal communication).

There may be other factors, however, which could explain why the polymers are ineffective on the primary tumor *in vivo*. The polymers, owing to their high molecular weight, may be unable to leave the blood stream and thus reach the tumor; or at the doses we used, their concentrations at the tumor level could be too low to affect the high number of cells growing at the site of implantation. On the other hand, considering the effect of polymers on cell dissemination and metastasis, the results reported seem to indicate that, with the exception of G<sub>4</sub>, the cell transport in the blood stream of mice bearing ic Sarcoma 180 was unaffected by the treatment. Therefore, the lower number of Sarcoma 180 cells present in the lung of treated mice in respect to controls and the decrease of lung metastases in mice injected im with Lewis carcinoma may be due to a lower capability either of cell implantation into normal tissue, or of cell survival and growth, once implanted.

It may be of interest to mention that the active polymers, with the notable exception of G<sub>4</sub>, have two important features in common when placed in an aqueous solution at a neutral pH: they are all cationic polymers, due to the presence of tertiary amino groups; and they all bear both hydrophilic and lipophilic substituents, thus exhibiting a considerable tensioactivity.

Polymer G<sub>4</sub>, however, does not exhibit any marked tensioactivity and it may, therefore, be considered as being rather exceptional among the active polymers. On the other hand, it is the only polymer tested which bears both positive and negative charges simultaneously at neutral pH, thus showing zwitterionic properties. It should be added that G<sub>4</sub> is also the only polymer which affects the number of cells present in the blood stream of mice with Sarcoma 180 implanted ic. Therefore, it is not unreasonable to suppose that it acts mainly on the phase of cell release from the tumor or during the cell transport in the blood stream.

Concerning the mechanism of action of the active polymers, it could be speculated that it is related to an effect on the membrane of the cancer cells which became more susceptible to the host rejection. Moreover, besides any modification of cancer cells membrane, it could be also speculated that the polymers are active through a nonspecific stimulation of the host response. Both the hypotheses are supported by pre-

vious data reported in the literature.<sup>24-28</sup> In this respect, however, further studies are in progress.

### Experimental Section

Intrinsic viscosities were measured at 30°. Since the yields of all the poly(amide amines) listed were always practically quantitative, elemental analysis determinations were considered to be irrelevant. This polymerization being a polyaddition, in fact, the values obtained for the products could not be expected to be different from that of the monomeric mixtures. That under the conditions used no other reaction but polyaddition takes place between amines and compounds bearing activated double bonds has been previously demonstrated.<sup>29</sup>

**Polymer G<sub>1</sub>.** To a cooled (5°) solution of 1.94 g of 1,4-bisacryloylpiperazine<sup>30</sup> in 10 ml of ethanol, 9 ml of aqueous 1 *M* ethanolamine and 1 ml of ethanolic 1 *M* *n*-dodecylamine were added under nitrogen. The mixture was thoroughly shaken and then kept in the dark at room temperature under a nitrogen atmosphere for 4 days. The solvents were then evaporated under reduced pressure and the product was dried at 45° under vacuum (0.1 mm). The yield was practically quantitative, apart from mechanical losses. The polymer had an intrinsic viscosity (in ethanol) of 0.21 dl/g.

**Polymers G<sub>2</sub> and G<sub>3</sub>.** The same procedure was used as above, starting from the same quantity of 1,4-bisacryloylpiperazine solution, and 8 ml of 1 *M* aqueous ethanolamine and 2 ml of 1 *M* ethanolic *n*-dodecylamine (G<sub>2</sub>), or 7 ml of 1 *M* ethanolamine and 3 ml of 1 *M* *n*-dodecylamine (G<sub>3</sub>). The yields and the intrinsic viscosities of these polymers were similar to those of G<sub>1</sub>.

**Polymer G<sub>4</sub>.** To a cooled solution of 1.94 g of 1,4-bisacryloylpiperazine in 5 ml of water, 5 ml of aqueous 1 *M* piperazine and 0.375 g of solid glycine were added under nitrogen. The mixture was thoroughly shaken until all solids were dissolved and then kept in the dark at room temperature, under a nitrogen atmosphere for 1 week. The mixture was then poured into 250 ml of dry acetone and precipitated G<sub>4</sub> was collected by filtration and dried at 45° (0.1 mm). The yield was over 90%. The polymer had an intrinsic viscosity of 0.18 dl/g (in 0.1 *M* HCl/1 *M* NaCl).

**Polymers G<sub>5</sub>, G<sub>6</sub>, and G<sub>7</sub>.** were prepared in precisely the same manner as already described for G<sub>1</sub>, G<sub>2</sub>, and G<sub>3</sub>, starting with the same quantity of 1,4-bisacryloylpiperazine and the following quantities of amines: 5 ml of 1 *M* aqueous ethanolamine and 5 ml of 1 *M* ethanolic benzylamine for G<sub>5</sub>; 9 ml of 1 *M* ethanolamine and 1 ml of 1 *M* benzylamine for G<sub>6</sub>; and 7 ml of 1 *M* ethanolamine and 3 ml of 1 *M* benzylamine for G<sub>7</sub>. The yields were over 90%. The intrinsic viscosities (in ethanol) ranged from 0.20 to 0.25 dl/g.

**Polymer G<sub>8</sub>.** was prepared exactly as previously described in the case of G<sub>7</sub>, but 7 ml of 1 *M* aqueous *as-N,N*-dimethylethylenediamine was substituted for the same quantity of ethanolamine.

**Polymers G<sub>9</sub> and G<sub>10</sub>.** were prepared as previously described in the case of G<sub>5</sub> and G<sub>6</sub> by substituting ethanolic 1 *M* *n*-amylamine for the same quantity of benzylamine.

**Polymers G<sub>11</sub> and G<sub>12</sub>.** were prepared as previously described for G<sub>9</sub> and G<sub>10</sub> by substituting aqueous 1 *M* *as-N,N*-dimethylethylenedia-

mine for the same quantity of ethanolamine. The intrinsic viscosities of G<sub>9</sub>-G<sub>12</sub> (in ethanol) ranged from 0.22 to 0.26 dl/g.

**Polymers G<sub>13</sub> and G<sub>14</sub>.** were prepared according to Danusso, *et al.*<sup>13</sup>

**Polymer G<sub>15</sub>.** was prepared according to Tamikado, *et al.*<sup>14</sup>

**Polymer G<sub>16</sub>.** was prepared according to Ferruti and Marchisio.<sup>15</sup>

### References

- (1) E. J. Ambrose in "The Biology of Cancer," E. J. Ambrose and F. J. C. Roe, Ed., Van Nostrand, London, 1966, p 65.
- (2) A. S. B. Curtiř, *Amer. Natur.*, **94**, 37 (1960).
- (3) D. R. Coman, *Cancer Res.*, **13**, 397 (1953).
- (4) I. Zeidman, *ibid.*, **17**, 157 (1957).
- (5) R. Rosso, M. G. Donelli, G. Franchi, and S. Garattini, *Eur. J. Cancer*, **5**, 77 (1969).
- (6) G. Franchi, L. Morasca, I. Reyers-Degli Innocenti, and S. Garattini, *ibid.*, **7**, 533 (1971).
- (7) H. Moroson, *Cancer Res.*, **31**, 373 (1971).
- (8) K. Kapila, C. Smith, and A. A. Rubin, *J. Reticuloendothel. Soc.*, **9**, 447 (1971).
- (9) R. F. A. Altman, L. G. Spoladore, and E. J. Esch, *Brit. J. Cancer*, **24**, 528 (1970).
- (10) F. Danusso and P. Ferruti, *Polymer*, **11**, 88 (1970).
- (11) F. Danusso, P. Ferruti, and G. Ferroni, *Chim. Ind. (Milan)*, **49**, 453 (1967).
- (12) F. Danusso, P. Ferruti, and G. Ferroni, *ibid.*, **49**, 587 (1967).
- (13) F. Danusso, P. Ferruti, and G. Peruzzo, *ibid.*, **48**, 466 (1966).
- (14) T. Tamikado, T. Sakai, and K. Sagisaka, *Makromol. Chem.*, **50**, 244 (1961).
- (15) P. Ferruti and M. A. Marchisio, *Med. Lav.*, **57**, 481 (1966).
- (16) R. Rosso, V. Palma, and S. Garattini, *Experientia*, **22**, 62 (1966).
- (17) R. Rosso, M. G. Donelli, and S. Garattini, *Cancer Res.*, **27**, 1225 (1967).
- (18) M. G. Donelli, R. Rosso, and S. Garattini, *ibid.*, **29**, 414 (1969).
- (19) H. Wexler, *J. Nat. Cancer Inst.*, **36**, 641 (1966).
- (20) G. Franchi, I. Reyers-Degli Innocenti, R. Rosso, and S. Garattini, *Int. J. Cancer*, **3**, 765 (1968).
- (21) G. Franchi, I. Reyers-Degli Innocenti, S. Garattini, O. Alfieri, G. Cademartini, and G. Ottaviani, *Lymphology*, **5**, 31 (1972).
- (22) R. Rosso, M. G. Donelli, G. Franchi, and S. Garattini, *Cancer Chemother. Rep.*, **54**, 79 (1970).
- (23) G. Franchi and S. Garattini, *Eur. J. Cancer*, **7**, 579 (1971).
- (24) A. Katchalsky, *Biophys. J.*, **4**, 9 (1964).
- (25) E. J. Ambrose, D. M. Easty, and P. C. T. Jones, *Brit. J. Cancer*, **12**, 439 (1958).
- (26) G. A. Currie and K. D. Bagshawe, *Lancet*, **1**, 708 (1967).
- (27) N. R. Di Luzio and S. J. Riggi, *J. Reticuloendothel. Soc.*, **8**, 465 (1970).
- (28) Y. Y. Maeda, J. Hamuro, and G. Chihara, *Int. J. Cancer*, **8**, 41 (1971).
- (29) F. Danusso, P. Ferruti, and G. Ferroni, *Chim. Ind. (Milan)*, **49**, 826 (1967).
- (30) F. Danusso, P. Ferruti, and G. Ferroni, *ibid.*, **49**, 271 (1967).

## N-Hydroxylation of *p*-Acetophenetidide as a Factor in Nephrotoxicity

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*N*-Hydroxy-*p*-acetophenetidide (**2**) has been synthesized by acetylation of *N*-hydroxy-*p*-phenetidine with 1 equiv of ketene. Also *N*-acetyl-*p*-benzoquinoneimine (**3**) was prepared and characterized as the cyclopentadiene adduct **13**. Both **2** and **3** give *p*-benzoquinone on hydrolysis. Intravenous injection of *N*-hydroxy-*p*-acetophenetidide, *p*-benzoquinone, and hydroquinone into rats has shown these compounds to be nephrotoxic. This study has implicated *N*-hydroxylation as a potentially nephrotoxic pathway of *p*-acetophenetidide metabolism.

The association between renal damage and excessive consumption of compound analgesic preparations is widely reported<sup>1</sup> but the underlying chemical factors are still un-

known and even the nature of the renal lesion not precisely determined.<sup>2-4</sup> A study<sup>2</sup> of the acute nephrotoxicity of *p*-acetophenetidide (phenacetin) derivatives showed that a