[CANCER RESEARCH 35, 2651-2656, October 1975]

Immunosuppression in Primary Liver and Colon Tumor Induction with *N*-Hydroxy-*N*-2-fluorenylacetamide and Azoxymethane

R. Kroes,[1] J. M. Berkvens,[2] and J. H. Weisburger[3]

National Institute of Public Health, Bilthoven, Netherlands [R. K., J. M. B.]; National Cancer Institute, Bethesda, Maryland 20014 [R. K., J. H. W.]; and American Health Foundation, Valhalla, New York 10595 [J. H. W.]

SUMMARY

The question was examined as to whether immunosuppression in a rat model system would affect the carcinogenic processes leading to tumors in the liver and the large bowel. The protocols were designed to detect an increased incidence or a shorter latent period stemming from a change in immune status.

Groups of rats were given injections prior to initiation of the carcinogen regimen and continuously thereafter with a purified γ fraction of antilymphocytic serum (ALG). Appropriate controls received the γ fraction of normal rabbit serum, or 0.9% NaCl solution. Permanence of skin allografts showed that ALG was an effective immunosuppressive treatment. For liver cancer induction, rats were fed 120 ppm *N*-hydroxy-*N*-2-fluorenylacetamide in the diet for 16 weeks, then were continued on control diet. The animals given ALG developed liver tumors at a rate similar to that of controls.

For cancer of the large bowel, rats received a single s.c. dose of 7.5 mg azoxymethane per kg per week for 16 weeks and were then held on control diet. With an identical ALG treatment, there were fewer intestinal tumors in the early part of the treatment, because of the important early development of liver angiosarcoma, not seen in control rats given injections of 0.9% NaCl solution. At a later time, the incidence of intestinal cancer was similar in rats on ALG or on 0.9% NaCl solution.

Thus, immunosuppression had little effect on the rate of liver tumor formation with a liver carcinogen. Also, ALG led to the precocious development of liver angiosarcomas, but failed to affect intestinal cancer induction in animals given azoxymethane.

INTRODUCTION

The induction of tumors by chemicals in animals and humans is a complex process extending over a portion of the life-span of the species, and in which host factors play an important role (1, 5, 13, 23, 30). One of those elements relates to immunological competence. In humans, immunosuppressed through several modalities to maintain integrity of tissue, especially kidney transplants, there appeared an increased risk of tumor formation, especially reticulum cell sarcomas and neoplasms of mesenchymal tissue (11, 12, 14, 15, 18, 20, 22, 24).

The effect of immunosuppression on chemical carcinogenesis was studied mainly in mice, and also in rats and hamsters, a subject recently reviewed (2, 14, 18, 24). The immune status was altered by ALS[4], neonatal thymectomy, or drugs such as azathioprine, prednisone, or others. Overall, ALS or neonatal thymectomy were more or less effective, in that they evoked an earlier appearance of tumors and/or higher incidence, whereas other immunosuppressants showed such an effect only rarely.

We investigated the problem of whether effective immunosuppression could change the incidence of N-OH-FAA-induced liver tumors and AOM-induced intestinal tumors. Both of these are epithelial tumor types that have not yet been extensively investigated in this respect. Neoplasms in these tissues are important in man (13, 29, 33, 34).

MATERIALS AND METHODS

Rats. Six-week-old male Fischer 344 rats were housed, 5 to a cage, in stainless wire mesh cages. Water and appropriate diets were available *ad libitum*. The animals were weighed weekly throughout the experiment, and were observed daily.

Treatment. The carcinogen N-OH-FAA (courtesy of Dr. Elizabeth Weisburger, National Cancer Institute) was added to the basal diet of Wayne Lab Meal (Allied Mills, Chicago, Ill.) at a level of 120 ppm. Fresh batches of the diet were prepared weekly and were stored in air-tight buckets at 5° until delivered to feeding cups. AOM (Ash-Stevens, Detroit, Mich.) was prepared weekly as a 3.75-mg/ml

[1] This work was undertaken at the National Cancer Institute during a tenure of Research Training Fellowship (awarded by the International Agency for Research on Cancer, Lyon, France), while the recipient was on leave of absence from the Laboratory of Pathology, Rijksinstituut voor de Volksgezondheid, Bilthoven, Netherlands (his present address).

[2] Research assistant, Rijksinstituut voor de Volksgezondheid, Bilthoven, Netherlands.

[3] Supported in part by Grants CA-12376, CA-14298, CA-15400, CA-17613, and Contract CP-33208 from the National Cancer Institute, NIH, USPHS. Present address: Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, N. Y. 10595. To whom reprint requests should be addressed.

Received November 21, 1974; accepted June 12, 1975.

[4] The abbreviations used are: ALS, antilymphocytic serum; N-OH-FAA, *N*-hydroxy-*N*-2-fluorenylacetamide (or *N*-hydroxy-2-acetylaminofluorene; *N*-2-fluorenylacetohydroxamic acid); AOM, azoxymethane; ALG, γ fraction of ALS; NRG, γ fraction of control normal rabbit serum.

solution in water. The rats received 1 s.c. injection weekly at dosages of 0.3 to 0.5 ml depending on body weight (7.5 mg/kg). ALG, prepared according to the method of Levey and Medawar (19), and NRG were obtained from Microbiological Associates, Bethesda, Md.

The ALG was tested in a separate allogenic skin graft experiment (Buffalo rat to Fischer rat) with 10 rats/group, following a dosage schedule similar to that used in the carcinogenicity study (see below), but terminating the treatment at Day 7. The average skin survival time for ALG-treated rats was 33.2 days, whereas grafted skin in 0.9% NaCl solution-treated controls and NRG-treated rats was rejected after 10.4 and 9.5 days, respectively. The same experiment, but with a double dose of NRG, led to an average survival time of 11.1 days.

Experimental Design. Details are listed in Table 1. Past experience has shown that feeding 160 ppm N-OH-FAA for 16 weeks yields a high incidence of liver tumors in male Fischer rats in 26 to 30 weeks (32). With AOM, a s.c. dose of 10 mg/kg/week for 16 weeks induces mainly intestinal tumors (and secondarily a few neoplasms in ear ducts and liver, and more rarely in kidneys) in many of the rats at risk in 26 to 32 weeks (8, 28, 29). We deliberately selected lower dosages for these tests, and scheduled necropsies on a part of the rats at various earlier intervals, in order to detect an enhanced effect owing to immunosuppression. Thus, rats were sacrificed under sodium pentobarbital anesthesia at Weeks 20, 26, or 28 and 32 (experimental weeks) after the initial carcinogen treatment. Careful gross examination was performed. Liver, kidneys, and spleen from each rat were weighed and a complete histological examination was performed on these organs. In the control and AOM-treated rats, the entire intestinal tract was slit lengthwise, washed, inspected for lesions, cut into anatomical segments, and rolled so as to present the greatest possible length on 1 microscope slide. The location of all tumors in the intestinal tract was recorded. Tissues were preserved in buffered 10% formalin, trimmed, and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin for microscopic study.

RESULTS

The means of the weekly body weights are presented in Chart 1 for the 1st 26 weeks, since many rats were sacrificed at the 26-week interval. The control rats given ALS showed lower weight gains than those on NRG. The ALS groups were sensitive to the development of pulmonary disease, which resulted in early mortality.

In groups with N-OH-FAA in the diet, the ALG-treated

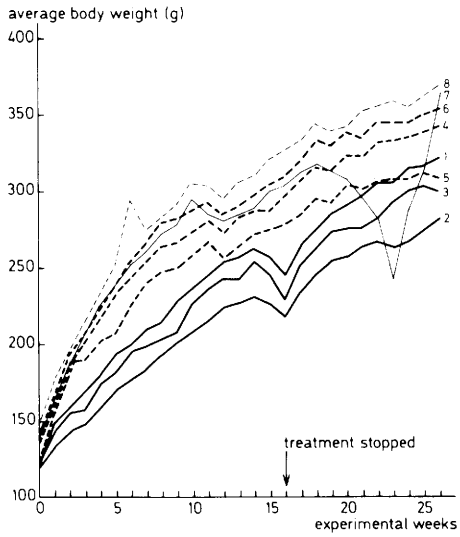

Chart 1. Average weekly body weights of male Fischer rats that received ALG, NRG, or 0.9% NaCl solution alone or in combination with N-OH-FAA or AOM. Group *1*, 0.9% NaCl solution + N-OH-FAA; *2*, ALG + N-OH-FAA; *3*, NRG + N-OH-FAA; *4*, 0.9% NaCl solution + AOM; *5*, ALG + AOM; *6*, NRG + AOM; *7*, ALG; and *8*, NRG. The carcinogen treatment was stopped at Week 16, but the injections of serum fractions or 0.9% NaCl solution continued throughout the experiment.

Table 1
Experimental design

Carcinogen	Treatment[a]	No. of rats	No. of rats sacrificed at		
			20 wk	26–28 wk	32 wk
N-OH-FAA in diet, 120 ppm for 16 wk	0.9% NaCl solution	30	15	15	
	NRG	40	20	20	
	ALG	50	25	21	
AOM, 7.5 mg/kg body wt s.c. 1 time/wk for 16 wk	0.9% NaCl solution	35	(8)[b]	12	13
	NRG	40	(8)	10	22
	ALG	50	(28)	12	6
None	NRG	30	10	5	15
None	ALG	30	(26)		4

[a] The dosage level and schedule of 0.9% NaCl solution, ALG, or NRG given i.p. 2 times a week until time of sacrifice, with Day 0 being the time carcinogen was started [Day −7, −3, 0, 3, 7, etc.; dose: 2.0, 2.0, 0.5, 0.5, and 0.5 ml, respectively, etc., (0.5 ml thereafter)].

[b] Numbers in parentheses, number of animals that died or were sacrificed in moribund state between Week 16 and Week 28.

rats had the smallest gains, and the NRG-treated rats in turn had a lesser increase than those given 0.9% NaCl solution.

With azoxymethane, the gain of NRG-treated rats was slightly higher than that of rats given 0.9% NaCl solution, whereas the ALG-treated rats had the smallest increases.

The mean body and organ weights of the rats sacrificed at each interval are summarized in Table 2. Increased liver weights were seen in the rats fed N-OH-FAA. The weights of the spleens and kidneys were generally proportional to body weights, and there were no marked differences between the groups.

In the N-OH-FAA-treated rats, multiple liver tumors were noted at 20 and at 26 weeks (Table 3). In addition, there were many hyperplastic nodules, some with atypia (16, 17, 32). Some tumors were "mixed," with both neoplastic hepatocytes and neoplastic bile ducts, as well as transitional elements.

After 20 weeks there was some trend for higher tumor multiplicity in the ALG-treated rats, compared with the 0.9% NaCl solution-treated group, but the difference was not statistically significant. After 26 weeks, the incidences and multiplicity between the groups did not differ.

The neoplasms in rats treated with AOM are listed in Table 4. The morphology of AOM-induced intestinal tumors has been described (28). Polypoid lesions, sometimes showing atypical epithelium, seen in 2 rats in each group, were not included.

ALG and, less so, NRG affected the type and yield of tumors induced by AOM. As per design, in 0.9% NaCl solution-treated rats, only intestinal tumors were found at a relatively low incidence. Liver angiosarcomas were induced, with an incidence about 4 times higher in the ALG-treated, compared to NRG-treated, rats. Fewer intestinal tumors were found in the ALG-treated rats than in 0.9% NaCl solution- or NRG-treated rats, presumably because many of

Table 2

Average terminal body weights and organ weights for male Fischer 344 rats that received the treatment outlined below

Carcinogen	Treatment	Body or organ	Wt (g) at time of sacrifice		
			20 wk	26 wk	32 wk
N-OH-FAA in diet, 120 ppm for 16 wk	0.9% NaCl solution	BW[a]	285 (15)[b]	322 (15)	
		Spleen	0.70	0.92	
		Liver	16	17	
		Kidneys	1.8	1.9	
	NRG	BW	271 (20)	300 (20)	
		Spleen	0.68	0.79	
		Liver	17	16	
		Kidneys	1.7	1.9	
	ALG	BW	258 (25)	269 (21)	
		Spleen	0.66	0.56	
		Liver	16	14	
		Kidneys	1.7	1.7	
				26–28 wk	
AOM, 7.5 mg/kg BW s.c. 1 time/wk for 16 wk	0.9% NaCl solution	BW		324 (5)	355 (10)
		Spleen		0.90	0.88
		Liver		10	10
		Kidneys		2.0	2.2
	NRG	BW		3.46 (10)	338 (21)
		Spleen		0.85	0.94
		Liver		9.7	10
		Kidneys		2.0	2.2
	ALG	BW		298 (10)	2.97 (5)
		Spleen		0.72	0.73
		Liver		9.1	8.1
		Kidneys		2.1	2.1
				26 wk	
None (control)	NRG	BW	333 (10)	368 (5)	372 (15)
		Spleen	0.74	0.85	0.92
		Liver	8.6	13	12
		Kidneys	2.0	2.4	2.3
	ALG	BW	315 (10)		373 (4)
		Spleen	0.61		0.80
		Liver	8.4		12
		Kidneys	1.9		2.4

[a] BW, body weight.

[b] Numbers in parentheses, actual number of rats.

Table 3

Effect of ALG (after 20 and 26 weeks) on the liver tumor incidence in rats that received 120 ppm N-OH-FAA in the diet for 16 weeks

Group	Time (wk)	No. of rats in group	No. of rats with hyperplastic nodules[a]	No. of rats with hepatomas	No. of rats with mixed tumors[b]
0.9% NaCl solution-treated	20	15	15 (2.3)[c, d]	6 (1.5)	5 (1.0)
	26	15	15 (3.2)	10 (2.0)	5 (1.2)
NRG-treated	20	20	20 (2.8)	4 (1.0)	4 (1.0)
	26	20	18 (3.4)	14 (1.9)	10 (1.2)
ALG-treated	20	25	24 (3.2)[d]	15 (1.6)	15 (1.2)
	26	21	19 (3.4)	15 (1.6)	11 (1.1)

[a] With atypia; classification of these and other liver tumors, according to Ref. 32.
[b] Mixed tumors include hepatocellular and bile duct tumors.
[c] Numbers in parentheses, average number of lesions per animal per group.
[d] The multiplicity of hyperplastic nodules between these 2 groups is not significantly different by t test ($0.2 > p > 0.1$).

Table 4

Effect of ALG on the incidence of intestinal cancers in rats that received 7.5 mg AOM per kg per week for 16 weeks

There were no tumors in 20 control rats given NRG and 30 rats given ALG.

Group	From 16–18 wk				After 28 wk				After 32 wk				Incidence in all rats
		Intestinal cancer				Intestinal cancer				Intestinal cancer			
	No. of rats	Small intestine	Large intestine	Total incidence	No. of rats	Small intestine	Large intestine	Total incidence	No. of rats	Small intestine	Large intestine	Total incidence	
0.9% NaCl solution-treated	8	1	1	2 (25)[a]	12	3	3	6 (50)	13	3	6	9 (69)	17 (51)
NRG-treated	8	1	2	3 (38)	11	1	4	5 (45)	22	5	7	10[b] (45)	18 (43)
ALG-treated	28	1	1	2 (7)	12	0	1	1 (7.5)	6	0	3	3 (50)	6 (11)

[a] Numbers in parentheses, percentages.
[b] Two rats had a carcinoma of both the small and large intestines.

Table 5

Effect of ALG on the occurrence of liver angiosarcomas in rats that received 7.5 mg AOM per kg per week for 16 weeks

There were no tumors in 20 rats on NRG and 30 rats on ALG.

Treatment	From 16–28 wk		After 28 wk		After 32 wk		Total incidence in all rats
	No. of rats	Rats with angiosarcoma	No. of rats	Rats with angiosarcoma	No. of rats	Rats with angiosarcoma	
Plus 0.9% NaCl solution	8	0 (0)[a]	12	0 (0)	13	0 (0)	0 (0)
Plus NRG	8	1 (12.5)	10	3 (30)	22	1 (4)	5 (12.5)
Plus ALG	28	14[b] (50)	12	7[c] (58.5)	6	1 (16)	22 (48)

[a] Numbers in parentheses, percentages of angiosarcoma-bearing rats.
[b] One rat with 2 tumors of the same type.
[c] Two rats with 2 tumors of the same type.

them died early with a competing lesion, hepatic angiosarcoma. No tumors were found in the NRG- and ALG-treated control rats, which did not receive any carcinogen. Early mortality in the ALG-treated rats was due to death with pneumonia.

The spleens of all ALG-treated rats had small follicular zones and usually a lymphocyte depletion of the periarteriolar lymphocytic sheets was present. The spleens of NRG- and 0.9% NaCl solution-treated animals were normal.

DISCUSSION

We attempted to establish whether modification of immunocompetence with ALG would alter the carcinogenic process in the liver and intestinal tract in a rat model system. The protocols were designed to permit a detection of increased cancer incidence or a shorter latent period.

ALG was active as an immunosuppressant, for there was a prolongation of allogenic skin graft survival time. Also,

characteristic histological changes in the spleen were present, similar to those described (17, 25). These rats were also more sensitive to the development of fatal pulmonary disease. Due to this infectious process in the lungs, enhanced by ALG, the rats weighed less and died prematurely, but it is believed that the ALG regimen was not unduly toxic, as such. Support for this view comes from a consideration of weight gain, which in the 1st 12 weeks was slightly lower than controls, but followed a parallel trend. Toxicity would have been revealed by an early divergence of weight curves. The late deviation seen was due to the progressive development of infectious processes, especially in the lung, in the ALG groups, witness to the efficacy of the immunosuppression.

In the ALG-treated rats, the incidence of N-OH-FAA-induced liver tumors was similar in both 0.9% NaCl solution- and NRG-treated rats. Early on there seemed to be a weak trend toward increased multiplicity of hyperplastic nodules. Friedrich-Freska and Hoffmann (10) also found an increased incidence of preneoplastic lesions when diethylnitrosamine-treated rats were given ALS in the 1st 42 days of the experiment, but under different conditions we saw only minimal effects (31). Della Porta *et al.* (6) obtained an increased incidence of liver tumors when urethan was given to neonatally thymectomized mice. However, no measurable change of tumor response was observed in rats with N-OH-FAA (9) or diethylnitrosamine (26) as carcinogens, and with azathioprine or cyclophosphamide, respectively, as immunosuppressants. Thus, the development of liver tumors does not seem to be influenced to a great extent by the immune system.

Striking differences, however, were noted in the induction of azoxymethane-induced tumors. Although intestinal cancers did occur in all groups, they were relatively rare in the ALG-treated rats, compared with rats given NRG or 0.9% NaCl solution, especially in the rats dead before 28 weeks. This is because a competing lesion, liver angiosarcoma, developed in the ALG-treated rats early and in a high frequency. The liver lesion did not occur in the rats given injections of 0.9% NaCl solution and were infrequent in the NRG-treated animals.

Similar observations were made in female rats dosed with 7,12-dimethylbenzanthracene; these rats had only mammary tumors, but those also given ALS developed nephroblastoma (3). In another study (7), the i.v. administration of *N*-methylnitrosourea led to bladder cancers only in ALS-treated rats, whereas neurogenic tumors were present in both ALS- and normal horse serum-treated rats.

Angiosarcoma was seen when rats were given low p.o. dosages of 1,2-dimethylhydrazine (*cf.* Refs. 8 and 27), closely related to azoxymethane. The interesting finding that ALG-induced immunosuppression led to a type of tumor not often seen with an intact immune status may mean that these tumors are more antigenic than the other tumor types (such as intestine) which are usually induced by the carcinogen. Under normal circumstances, such a highly antigenic tumor will not develop because of the efficient elimination by the immune system. Weakened immunosurveillance (23) by the ALG treatment thus would lead to tumor development. The presence of a low incidence of angiosarcoma in NRG-treated rats may be accounted for by a nonspecific effect of repeated NRG doses on the immune system (21).

Prolonged immunosuppression in humans is not uncommon, since transplantation of tissues and organs has been introduced. This extended treatment seems to have an influence on the development of several types of tumors in man (18, 20, 22). Since most of the tumors arising in humans are believed to be induced by chemical agents (4, 30, 33, 34), it seems important to investigate the influence of immunosuppression on the effect of chemical carcinogens in animals in greater depth. The finding of this study that other tumor types may develop due to immunosuppression seems important in relation to tumor development in man.

REFERENCES

1. Alexander, P. Escape from Immune Destruction by the Host through Shredding of Surface Antigens: Is this a Characteristic Shared by Malignant and Embryonic Cells? Cancer Res., *34:* 2077–2082, 1974.
2. Baroni, C. D., Scelsi, R., Peronace, M. L., and Uccini, S. Heterologous Antilymphocyte Serum Hastens the Growth of 7,12-Dimethylbenz(a)anthracene-induced Tumors in Mice. Brit. J. Cancer, *28:* 221–226, 1973.
3. Bourgoin, J. J., Cueff, J., Bailly, C., and Dargent, M. Incidence de Néphroblastomes Chez le Rat Sprague-Dawley Immuno-déprimé Soumis au DMBA. Bull. Cancer, *4:* 429–434, 1972.
4. Boyland, E. The Correlation of Experimental Carcinogenesis and Cancer in Man. Progr. Exptl. Tumor Res., *11:* 222–234, 1969.
5. Coggin, J. H., Jr., and Anderson, N. G. Cancer, Differentiation and Embryonic Antigens: Some Central Problems. Advan. Cancer Res., *19:* 106–165, 1973.
6. Della Porta, G., Colnaghi, M. I., and Parmi, L. Influenza della Timectomia, della Splenectomia e del Cortisone Sulla Cancerogenesi. Tumori, *56:* 121–135, 1970.
7. Denlinger, R. H., Swenberg, J. A., Koestner, A., and Wechsler W. Differential Effect of Immunosuppression on the Induction of Nervous System and Bladder Tumors by *N*-Methyl-*N*-nitrosourea. J. Natl. Cancer Inst., *50:* 87–93, 1973.
8. Druckrey, H., and Lange, A. Carcinogenicity of Azoxymethane Dependent on Age in BD Rats. Federation Proc., *31:* 1482–1484, 1972.
9. Frankel, H. H., Yamamoto, R. S., Weisburger, E. K., and Weisburger, J. H. Chronic Toxicity of Azothioprine and the Effect of this Immunosuppressant on Liver Tumor Induction by the Carcinogen *N*-Hydroxy-*N*-2-fluorenylacetamide. Toxicol. Appl. Pharmacol., *17:* 462–480, 1970.
10. Friedrich-Freska, H., and Hoffmann, M. Immunological Defense against Preneoplastic Stages of Diethylnitrosamine-induced Carcinomas in Rat Liver. Nature, *223:* 1162–1163, 1969.
11. Good, R. A. Immunodeficiency and Malignancy. *In:* R. Doll, I. Vodopija, and W. Davis (eds.), Host Environment Interactions in the Etiology of Cancer in Man, pp. 265–274, Lyon: International Agency for Research in Cancer, WHO, 1973.
12. Hellström, I. and Hellström, K. E. Cell-mediated Immune Reactions to Tumor Antigens with Particular Emphasis on Immunity to Human Neoplasms. Cancer, *34:* 1461–1468, 1974.
13. Higginson, J. Metabolic Aspects of Host-environmental Relationships: An Overview. *In:* R. Doll, I. Vodopija, and W. Davis (eds.), Host Environment Interactions in the Etiology of Cancer in Man, pp. 201–212. Lyon: International Agency for Research on Cancer, WHO, 1973.

14. Kripke, M. L., and Borsos, T. Immunosuppression and Carcinogenesis. A Review. Israel J. Med. Sci. *10:* 888–903, 1974.
15. Kroes, R., Weiss, J. W., and Weisburger, J. H. Immunosuppression and Chemical Carcinogenesis. Proceedings of the Fifth International Symposium on "Special Problems of Cancer Chemotherapy." Düsseldorf 1973. Recent Results Cancer Res., *52:* 65–75, 1975.
16. Kroes, R., Williams, G. M., and Weisburger, J. H. Early Appearance of Serum α-fetoprotein during Hepatocarcinogenesis as a Function of Age of Rats and Extent of Treatment with 3'-Methyl-4-dimethylaminoazobenzene. Cancer Res., *32:* 1526–1532, 1972.
17. Kroes, R., Williams, G. M., and Weisburger, J. H. Early Appearance of Serum α-Fetoprotein as a Function of Dosage of Various Hepatocarcinogens. Cancer Res., *33:* 613–617, 1973.
18. Krüger, G. R. F. Lymphoreticular Neoplasia in Immunosuppression: Facts and Fancies. Beitr. Pathol. Bd., *151:* 221–223, 1974.
19. Levey, R. H., and Medawar, P. B. Nature and Mode of Action of Antilymphocytic Antiserum. Proc. Natl. Acad. Sci. U. S., *56:* 1130–1137, 1966.
20. Mäkelä, O. Influence of Immunological Reactions on Carcinogenesis. *In:* R. Doll, I. Vodopija, and W. Davis (eds.), Host Environment Interactions in the Etiology of Cancer in Man, pp. 285–290. Lyon: International Agency for Research on Cancer, WHO, 1973.
21. Melief, C. J. M., and Schwartz, R. S. Immunocompetence and Malignancy. *In:* F. F. Becker (ed.), Cancer, pp. 121–159, New York: Plenum Press, 1975.
22. Penn, I. Chemical Immunosuppression and Human Cancer. Cancer, *34:* 1474–1480, 1974.
23. Prehn, R. T. Immunosurveillance, Regeneration and Oncogenesis. Progr. Exptl. Tumor Res., *14:* 1–24, 1971.
24. Rapp, H. J., and Hanna, M. G., Jr. Conference on Immunology of Carcinogenesis. Natl. Cancer Inst. Monograph, *35:* 1–477, 1972.
25. Röpke, C. Cytological and Histological Events Following Treatment with Anti-thymocytic Globulin in Mice. Acta Pathol. Microbiol. Scand., *79:* 23–32, 1971.
26. Schmähl, D. Investigations on the Influence of Immunodepressive Means on the Chemical Carcinogenesis in Rats. Z. Krebsforsch., *81:* 211–215, 1974.
27. Toth, B. Morphological Studies of Angiosarcomas Induced by 1,2-Dimethylhydrazine Dihydrochloride in Syrian Golden Hamsters. Cancer Res., *32:* 2818–2827, 1972.
28. Ward, J. M., Yamamoto, R. S., and Brown, C. A. Pathology of Intestinal Neoplasms and Other Lesions in Rats Exposed to Azoxymethane. J. Natl. Cancer Inst., *51:* 1029–1039, 1973.
29. Weisburger, J. H. Chemical Carcinogens and their Mode of Action in Colonic Neoplasia. Diseases Colon Rectum, *16:* 431–437, 1973.
30. Weisburger, J. H. Chemical Carcinogenesis. *In:* J. F. Holland and E. Frei, III (eds.), Cancer Medicine, pp. 45–90. Philadelphia: Lea and Febiger, 1973.
31. Weisburger, J. H., Madison, R. M., Ward, J. M., Viguera, C., and Weisburger, E. K. Modification of Diethylnitrosamine Liver Carcinogenesis with Phenobarbital, but not with Immunosuppression. J. Natl. Cancer Inst., *54:* 1185–1188, 1975.
32. Weisburger, J. H., Yamamoto, R. J., Williams, G. M., Grantham, P. H., Matsushima, T., and Weisburger, E. K. On the Sulfate Ester of *N*-Hydroxy-*N*-2-fluorenylacetamide as a Key Ultimate Hepatocarcinogen in the Rat. Cancer Res., *32:* 491–500, 1972.
33. Wynder, E. L., and Mabuchi, K. Etiological and Preventive Aspects of Human Cancer. Prevent. Med., *1:* 300–334, 1972.
34. Wynder, E. L., and Reddy, B. S. Dietary Fat and Colon Cancer. J. Natl. Cancer Inst., *54:* 7–10, 1975.