CARCINOGENICITY AS RELATED TO AGE[1]

◆6588

R. Schoental
Department of Pathology, Royal Veterinary College, Royal College Street, London, England

INTRODUCTION

Cancer is considered a disease of old age. The highest mortality from cancer occurs indeed after the age of 50, but the tumors are likely to have been initiated long before any symptoms of the disease become apparent, possibly in childhood or even in utero.

The majority of tumors that occur in late life may be caused by multiple carcinogenic agents; they represent great difficulties when attempting to trace the aetiological factors involved. The difficulties are becoming ever more formidable with the increasing complexities of modern life.

Though not numerous, tumors occur in the newborn, in early childhood, and even in the fetus (1–3). Childhood tumors have been reported to be one of the main causes of mortality among the young, and their frequency appears to be increasing in the last years (4). The tracing of the aetiology of tumors that occur in the fetus or in early childhood has better chances of success. Though the causative agents need not be the same as those operating in later life, the recognition and possible elimination of even a single agent would be of practical value. The combination of the remaining carcinogenic factors would obviously be less effective, and the appearance of the tumors accordingly delayed, hopefully, beyond the lifespan of the individual.

Evidence from animal experiments brought to light the fact that in order to induce tumors, exposure to carcinogenic agents does not need to be continuous; single or a few doses may be sufficient; these may give no warning in the form of a recogniz-

[1]The following abreviations are used in this review: PAH = polycyclic aromatic hydrocarbons; MCHA = 3-methylcholanthrene; 7,12-DMBA = 7,12-dimethylbenz[a]anthracene; OC = oral contraceptives; Enovid = 17α-ethynyl-19-hydroxyestren-3-one; DES = 3,4-di-(4-hydroxyphenyl)-hex-3-ene = diethylstilbestrol; AAT = N-2-fluorenylacetamide; Zearalenone =[6(10-hydroxy-6-oxo-*trans*-1-undecenyl)]-β-resorcinic acid lactone; MNU = N-methyl-N-nitrosourea; ENU = N-ethyl-N-nitrosourea; DEN = N-nitrosodiethylamine; DDT = 1,1,1-trichloro-2,2-di-(4-chlorophenyl)-ethane.

able illness for a long time. The latent period before tumors appear is to some extent related to the dose: the lower the dosage, the longer will usually be the latent period. Cancer seen in old age might have been initiated early in life.

Age is conventionally calculated from the time of birth, though the individual's existence starts at conception. However, biologically, the consignment of cells, which in time develop into the future ova, exist in the female's ovary since its organogenesis in her mother's uterus (5).

The existence of a mammalian organism can be roughly subdivided into the following stages:

1. The quiescent presence of the germ cell in the ovary
2. Maturation of the germ cell into an ovum in the mother's ovary
3. Maturation of the male germ cells into spermatozoa in the father's gonads
4. Fertilization of the ovum by the spermatozoon
5. Implantation of the fertilized ovum and embryogenesis
6. Organogenesis and the development of the fetus
7. Parturition
8. Infancy (suckling, or milk diet)
9. Childhood (with adoption of mixed diet)
10. Adolescence (hormonal influence due to maturing gonads)
11. Adulthood
12. Senescence

Assuming certain genetic susceptibility, exposure at appropriate stages to carcinogenic agents, viral, physical, or chemical, could have lethal, cytotoxic, mutagenic, or teratogenic effects, or could initiate the chain of events that result in cancer.

This paper considers the induction of tumors by chemical carcinogens in relation to age. Viral agents have been reviewed by Gross, 1970 (6); radiation carcinogenesis by Stewart, 1971 (7).

In the introduction to his classical paper "Occurrence and significance of congenital malignant neoplasms," Wells (1940) stated (1):

> In glaring contrast (to the long time usually needed for tumors to develop as a result of experimental or industrial exposure to carcinogenic agents) is the fact that certain types of malignant growth are seen almost exclusively in the very young and that sometimes a malignant growth may develop in the fetus, even producing widespread metastases, before the brief span of intra-uterine existence has been accomplished. Surely these tumors must differ in some fundamental way from the ordinary sorts of cancer, which require so long a period for development, and this difference may throw some light on the mystery of malignancy.

During the two scores of years since this was written, new carcinogens came to light, "natural" and synthetic ones, which can induce in rodents tumors similar to those seen in man (8–11); and also in the fetus, when given to pregnant females during the second half of pregnancy (12). Treatment of pregnant females during the early stages of gestation, leads to death of the embryo, its absorption, or abortion, or malformations, but rarely to tumors (4). However, even in the transplacentally treated offspring, tumors appear late in their life. Animal models, which would

result in tumors during the intrauterine existence, have still to be devised. Whether such early tumors may be the result of exposure to carcinogenic agents of the ovum or of the spermatozoa before they leave the parental gonads, requires detailed investigation. In experiments in which female mice were treated with PAH (MCHA or 7,12-DMBA) and were allowed to breed, degeneration of the ovarian follicles, reduced fertility, or sterility followed (13). Ovarian and mammary tumors developed in the treated females; their incidence varied depending on the strain of the mice used (13). However, as regards the offspring, no detailed examinations have been made.

In such experiments, any progeny should be carefully examined microscopically. Many malformations and incipient neoplasms may remain unrecognized on naked eye examination when dealing with an aborted or stillborn fetus (14).

TRANSPLACENTAL CARCINOGENESIS

Estrogens

Transplacental carcinogenesis has been the subject of a symposium sponsored by the WHO and by the International Agency for Research on Cancer in 1971, the proceedings of which have been published (12). The interest in the transplacental effects of chemicals has been stimulated by the tragic epidemic-like occurrence of malformations in Germany among babies of mothers that were given the sedative-hypnotic drug, thalidomide, during pregnancy (15, 16). More recently, a chance finding of seven cases of vaginal cancer in one hospital among young women 14–22 years of age attracted attention, because of the rarity of this type of neoplasia in the young. The tumors have been traced to be the result of treatment of the patients' mothers with large doses of diethylstilbestrol (up to 125 mg/day) or steroidal estrogens for threatened abortion (17). Since then, a number of additional cases of vaginal abnormalities and tumors in young women came to light, which are of similar iatrogenic origin (18). As yet, no reports are available whether the treatments had carcinogenic sequalae in the treated women or in their male offspring. As the latent period for the clinical appearance of the vaginal tumors in the very susceptible young was 14–22 years, a longer time interval would be expected to elapse before tumors could become apparent in the mothers. The consequences of high estronization of the male fetus require specific investigation. Masculinization of the female offspring as a result of treatment of their mothers during pregnancy with progestins or estrogens has been reported (19).

Medications involving relatively high doses of estrogens are given sporadically to women to suppress lactation (5–15 mg/day), for the treatment of mammary carcinoma (10–20 mg/day), as postcoital contraceptives (25 mg twice/day, for 5 days) (20) etc. Epidemiological investigations now in progress should pay attention to the long term effects of hormonal imbalance, not only in the treated women, but also to those that arise in their progeny, male or female, who may be conceived at any time subsequent to treatment.

The effects of smaller dosage are likely to take longer to become clinically recognizable. The continuous use of oral contraceptives (OC), which contain small doses

of estrogens in conjunction with progestogens, would be expected to have cumulative effects, which have yet to be adequately investigated. An increased risk, tenfold of thromboembolic (21) and twofold of gall bladder diseases has been reported among the users of OC as compared with nonusers (22). Serious malformations occurred among the offspring of women who have taken OC as "pregnancy tests" or without realizing that pregnancy already existed (23).

Recently, jaundice among breast-fed infants has been correlated with the use of OC by their mothers, long before these babies were conceived (24).

In mice fetal malformations have been produced experimentally by the administration of daily doses of OC corresponding to 1–3 times the doses that prevent pregnancy in this species (25).

Since the first demonstration by Lacassagne in 1932 (26) that folliculin (oestrone) can induce mammary tumors in male mice, estrogens have been known to be carcinogenic. They are able to induce tumors in several target organs (breast, uterus, vagina, anterior pituitary, ovary, etc), in susceptible animals under appropriate conditions (27, 28). The younger the animals, the more susceptible they are to the action of estrogens (and other hormones). Newborn animals are particularly sensitive. A single dose of DES induced multiple cysts of the epididymis in male mice and tumors of the vagina, uterus, and myoblastoma in female mice (29); similar results were obtained with the OC Enovid (30). Continuous administration of large doses of Enovid in diet, induced uterine tumors in all the treated mice (30). In spite of the small scale of these experiments, the results are highly significant.

The foundations of chemical carcinogenesis have been the result of careful observations made on small numbers of patients or animals by experienced and interested scientists who could recognize the significance of the tumors found, even though these were few in numbers. It would be of interest to know whether the present tendency to use very large numbers of animals (mega-mouse experiments have been suggested) and in consequence to have to rely on observations made by unskilled technical staff and on statistical evaluation by computers, will lead to an improvement as regards the reliability of the results and the discovery of new (unprogrammed) facts.

Besides affecting target organs, estrogens have been found to induce renal tumors in male hamsters (*Cricetus auratus*) (31); whether they play some role in diseases of the kidneys in other species remains to be investigated. Sex hormones can greatly modify the response to carcinogenic agents. They are responsible for the differences in the incidence and/or in the localization of tumors observed in response to the same treatment, depending on age, sex, and pregnancy. Most hepatocarcinogens are usually more effective in males than in females, and castration or treatment with the respective sex hormones appropriately modify the response. This has been found to be so in the case of N-2-fluorenylacetamide (AAF) and its congeners (32–34), azo-dyes (35), N-4-(4'-fluorobiphenyl)acetamide (36), pyrrolizidine alkaloids (10), cycasin (11), and aflatoxins (8). However, the cirrhogenic action of carbon tetrachloride (37) and the carcinogenic response of the liver to diethylnitrosamine have been reported to be more accentuated in the females (38). Estrogens have a modifying

effect on the carcinogenic action of polycyclic aromatic hydrocarbons (MCHA and 7,12-DMBA) on mouse gonads (13); they also enhance the induction of tumors of the mammary gland in Sprague-Dawley rats (39); when ovariectomized the rats have a very low incidence of mammary tumors, but develop renal tumors (40).

The modifying effects of sex hormones is no doubt related to their multifarious actions: on food consumption, metabolism, induction of enzymes (41); on development and growth, etc. The normal liver is usually able to metabolize, conjugate, and inactivate estrogenic agents, but in certain pathological conditions, it might be unable to cope with an excessive intake; the circulating estrogens can then affect the gonads and other organs.

An interesting observation has been recently reported from Australia: C3H A^{vy} mice, which have a high incidence of mammary and hepatic tumors in the Laboratories of the National Cancer Institute (NIH) in USA, have been found to develop fewer tumors when imported to Australia; the incidence declined strikingly in the subsequent generations bred in Australia. However, when the mice were given the American diet, the incidence of their mammary tumors increased and was restored to the American level when their bedding was replaced by shavings of red cedar (*Juniperus virginiana*), which is used at NIH (42). The authors suspect that the red cedar shavings contain carcinogens. This is indeed likely, in view of the carcinogenic activity of 3,4,5-trimethoxycinnamaldehyde, a derivative of certain α,β-unsaturated aldehydes, which are normal constituents of wood lignins (43, 44). Other specific carcinogenic compounds may also be present in red-cedar wood. However, the enhancing effect of the American diet on the induction of mammary cancer may be related to its content of estrogens (see below). This remarkable instance of modification of the incidence of "spontaneous" tumors, believed to be genetically determined, by environmental factors, appears of great importance and warrants closer examination.

Synthetic estrogens, mainly DES, have been used as additives to livestock diets in order to accelerate their growth and fattening-up and to obtain a better food conversion. The Food and Drug Administration of the USA has prohibited the addition of DES to animal fodder or the implantation of its pellets into the ears of livestock (45); however, estrogenic material, "Ralgro," has been developed to replace DES. This preparation has similar estrogenic activity, but is of different structure (46); it consists of two stereoisomers, the hydroxylic reduction products of zearalenone, a secondary metabolic product of *Fusarium graminearum (Gibberella zeae)* and certain related fungi, which often contaminate cereals and other foodstuffs. The estrogenic activity of zearalenone has been discovered during investigation of a pathological condition in pigs, known as "vulvo-vaginitis," which has been traced to contamination of the pig's diet with Fusaria (46).

Plants used as food also contain substances that have estrogenic activity. In Figure 1 are given the chemical structures of representative compounds of several types of known estrogenic agents. The physiological female sex hormones are steroidal compounds, Figure 1 (*I*); estrone has been found also in plant materials, e.g. date palm kernels (47). Genistein (Figure 1 *III*), the 5,7,4'-trihydroxyisoflavone, is

present in red clover, *Trifolium subterraneum,* (Leguminosae) and in related plant species. Though its estrogenic activity is only 10^{-5} of that shown by DES or estradiol, it has been found to be responsible for sterility in sheep that grazed on subterranean clover in Australia due to the high content of this constituent (48). A number of related isoflavonoids present in plants are also estrogenic (49). Coumestrol (Figure 1 *IV*) and its congeners are coumarin derivatives, which have a higher estrogenic activity than the isoflavonoids, and are present in soya beans and other plants used as food (50). There are still others (51).

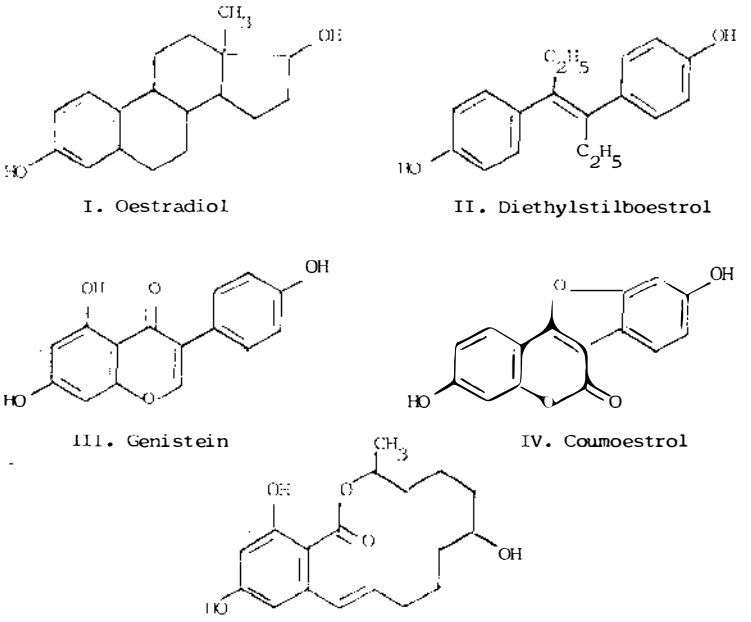


Figure 1 Structures of compounds that have estrogenic activity.

Though the various estrogenic compounds differ in their chemical structures, they show marked similarity as regards the positions of the hydroxyl groups, which are essential for the estrogenic action. Indeed, of the two stereoisomeric reduction products of zearalenone, which differ in the configuration of the hydroxyl at C-6 (Figure 1 *V*), one is much more active than the other (46).

The various plant and fungal estrogenic compounds have yet to be tested for carcinogenic action. However, their effects on the target organs are likely to be similar to those found with DES and with the steroidal estrogens. The activity of both the steroidal and the synthetic estrogens depends on the presence of hydroxyl-groups at the extremities of their molecules (estrone has only about 1/10th of the activity of estradiol, to which it is metabolically reduced in the animal body). The

hydroxyls are involved in the binding with specific receptor proteins present in the cytosol of cells in the target organs. The resulting complexes are then carried into the nucleus where they become tightly bound to acceptor proteins. This process can be detected in vivo and in vitro; it is susceptible to inhibition by thiol reagents (52).

In the environment of animals and man, the load of estrogenic agents has been steadily increasing. Some of the consequences, which may include changes in sexual and social behavior, are not easy to evaluate in animal experiments. Attempts should be made to restrict the present liberal uses of estrogenic substances to the unavoidable minimum.

The high incidence of mammary (53) and/or uterine tumors in women all over the world is a sufficient indication that man is a species susceptible to the action of estrogenic agents.

Chemical Carcinogens

Most of the chemical carcinogens, especially in their activated form, have cytotoxic, mutagenic, teratogenic, as well as carcinogenic action; there is, however, no direct parallelism among these activities.

Cytotoxic effects are the least specific and can be caused by a variety of compounds acting at appropriate concentration. The relation between mutagenesis and carcinogenesis (54, 55) will not be fully understood, until the active forms of indirectly acting carcinogens become known.

Teratogenic action (56) causing morphological and/or functional abnormalities in the fetus implies that the carcinogen when given to pregnant females at an appropriate stage of gestation can cross the placenta or exert its effect indirectly, e.g. by interference with blood supply etc. Severe malformations are usually not compatible with survival beyond the intrauterine existence; these can be detected by removing the fetus from the uterus a few days after treatment, before it is lost due to resorption or abortion. A number of carcinogens show teratogenic effects under such conditions, including certain polycyclic aromatic hydrocarbons, azo-dyes, 2AAF, antitumor agents, estrogens (57), pyrrolizidine alkaloids (58), cycasin (59), various nitroso- and related compounds (60). The effects depend on many factors, including genetic susceptibility, the species and strain of the test animal, also on the stage of development of the fetus at the time of administration of the compound. Thus, aflatoxin B_1 induced malformations in hamsters and in C3H mice when treated at the most sensitive period between the days 8–12 of gestation, but induced only growth retardation in rats (60).

The concentration of the acting substance appears to be critical for teratogenic effects; repeated small doses are not additive, in contradistinction to carcinogenic responses, which are the result of summation or potentiations of even very small individual doses. Many teratogens are not carcinogenic (56); the evidence for thalidomide is equivocal (61).

Neoplastic transformation is a very rare cellular event, which affects only a few among the billions of cells that come in contact with a carcinogen introduced into the animal body. Evidently, a cell has to be in a very specific receptive stage for the "fateful" interaction to take place.

In the majority of studies on the induction of tumors by chemical agents weanling or young adult rodents were used, and the treatments were either continuous or repeated over long periods (62). More recently, newborn animals given such treatments proved to be more susceptible than adults to the action of many carcinogens. These include polycyclic aromatic hydrocarbons, aromatic amines, azo-compounds, urethane and certain nitroso and other compounds (compare 63–65), pyrrolizidine alkaloids (66) cycasin (59), aflatoxins (8), N-4-(4'-fluorobiphenyl)acetamide (36) and others. The reviews by Toth in 1968 (63) and by Della Porta & Terracini in 1969 (64) illustrate the difficulties involved in trying to evaluate the many variables (number of doses and their size, frequency and route of administration, solvents used, etc) inherent in such experiments. Comparisons *sensu stricto* of susceptibility to carcinogens at various ages became possible with the recognition that certain compounds are able to induce tumors with a *single* dose. The first indication of this has been obtained in experiments with a pyrrolizidine alkaloid, lasiocarpine (67), soon confirmed in the case of dimethylnitrosamine (68), nitroso-N-methylurethane (69), and retrorsine (70). However, the incidence of tumors in these cases was rather low; among the rats surviving for more than 1 year after a single dose of retrorsine, the incidence of hepatomas did not exceed 20% (70).

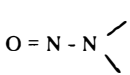
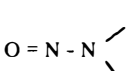
Using pure strains of rats, inbred for 50–100 generations (71) and having more than 70 nitroso and related compounds (mostly synthesized by Preussmann), Druckrey and his co-workers were able to extend greatly the studies of single dose carcinogenesis (72, 73).

The finding that a single dose of MNU will induce tumors in a variety of organs including the brain (72) had particularly significant consequences. MNU is more effective when given to newborn animals than to weanlings (74); when given intravenously it will induce in the rat preferentially tumors of the nervous system (75). When given intravenously to pregnant rats during the second week of gestation, the fetuses develop malformations (76, 77), while the treated mothers may develop tumors of the gonads (78). Methylnitrosourea probably does not require enzymic activation and though very unstable at alkaline pH, it evidently retains its activity for the short time required to be carried in the blood stream throughout the animal body.

The higher homologue, ENU, which is more stable, proved more effective and convenient for comparative studies of its transplacental and postnatal action (79–83) and has been used extensively by many workers (84–89). The examples given in Table 1 illustrate the effects in rats of single doses of MNU and ENU in relation to the age at the time of treatment, the size of the dose, and the route of administration. When a single dose of MNU was given orally, mainly gastrointestinal and kidney neoplasias developed (90), but intravenously it induced also neurogenic tumors (72).

When the carcinogenic efficacies of MNU and ENU were directly compared by giving one equimolecular dose of each (0.14 m*M*/kg body weight) to rats of the same strain at the end of pregnancy, the incidence of neurogenic tumors in the offspring was 39.7% (with MNU) and 97.4% (with ENU) respectively (86). This may be due to the instability and higher toxicity of MNU. Its toxicity (possibly

Table 1 Tumors and/or malformations induced in rats with a single dose of carcinogen in relation to the age at the time of treatment

Compound	Strain	Age in days	Dose mg/kg body wt	Route[a]	Tumors present in				Malform.	Refs
					Nervous System	Stomach	Kidney	Others		
N-methyl-N-nitrosourea	BD	(-13)→(-8)	10, 20	iv					++	76
	White	(-14)→(-10)	10	ip					++	77
	White	(-1)	20, 40	iv	+				++	4
	BD	>60	70-100	iv	+	++	+	++		72
	White	>60	90	ig		++	+	+		90
N-ethyl-N-nitrosourea	BD IX	(-8)	40, 80	iv					++	79
		(-8)	5-80	iv	+++					79-81, 83
		(-11)→(-1)	60	iv	+++					80
		(-1)	5-60	iv	+++					81
		1	5-80	sc	+++		+	+		81
		10	10-80	sc, o	+++	+		+		81
		30	20-80	iv	+++			++		81
		>60	60-200	iv	++	+	+	++		81
	10 various BD strains	(-8)	50	iv	+++			+		82
	Long Evans	(-6)	10	iv	+++					84
	Sprague Dawley	(-3)	50	iv	+++		+			85

[a] iv = intravenous; ig = intragastic; sc = subcutaneous; o = oral.

related to the alkylating action) prevents the use of doses high enough to give an "effective" concentration of the carcinogenic entity in the fetus after passage through a fully developed placenta. The barrier characteristics of the placenta are likely to vary with the progress of pregnancy; compounds may pass more freely before the placenta is fully developed and at the end of gestation when it is undergoing degeneration (96).

With alkylnitrosobiurets (91, 92) the results are similar to those obtained with alkylnitrosoureas, but higher dose levels were required. After a single dose, given to adult rats, intragastrically, stomach tumors preferentially developed and also tumors of the kidneys and other organs. However, when N-ethyl-N-nitrosobiuret was given to pregnant rats, exclusively neurogenic tumors were found in the offspring. The effective dose was about one fifth of that required for the adult rat (92).

Examples in Table 2 illustrate the differences in the response to azoxy compounds (59, 93–95). The younger the animals, the smaller is the dose that is required to induce neoplasias. The respective hydrazo and azo compounds are readily oxidized in the body to azoxyderivatives and accordingly give similar results (94).

ENU proved to be a very suitable carcinogen for the transplacental induction of almost exclusively neurogenic tumors in the rat (80). A dose that corresponds to 2% of the LD_{50} for the adult rat will induce neurogenic tumors in the majority of the offspring, when given on the 15th day of gestation (the –8 day of age) or later.

The neoplasias thus induced often represent tumors of considerable size in the hemispheres, in the cerebellum, the spinal cord, the cranial or peripheral nerves; they include oligodendrogliomas, astrocytomas, mixed gliomas, ependymomas, neurinomas (97). Similar neurogenic tumors were obtained by a number of workers in different laboratories who gave ENU to other strains of rats at various times during the third week of pregnancy (84–87). However, the incidence of the tumors can vary, depending on the strain of the rats (82).

Hamsters develop also neurogenic tumors when given ENU during the second half of pregnancy (4, 80), but mice similarly treated respond with lung and lymphoid tumors (88); mice of the strains A and C3Hf that are liable to "spontaneous" hepatomas also develop liver neoplasias. It thus appears that as a result of transplacental action of ENU, the neoplasias in the offspring develop mainly in organs that respond with tumors when the animals are treated postnatally. Mice of many strains appear to be resistant to neurogenic tumors, but a few mice (about 10%) of the strains IF and DBA developed neurogenic tumors including medulloblastomas, when given as newborns a single, subcutaneous dose of ENU (10–120 mg/kg of body weight) (89). No such tumors were found in similarly treated mice of the strains A or C57BL (89).

Similar species and strain differences have been observed in transplacental and/or postnatal studies of various carcinogens: cycasin, or its aglycone, methylazoxymethanol (11, 95, 98), urethane (99–104), pyrrolizidine alkaloids (105, 106), and others. The striking differences in response to carcinogens of different animal species, different strains, and even among animals of the same strain when kept under varied conditions are usually referred to genetic, immunological, or viral factors, however, the exact mechanism that determines susceptibility or resistance has not been ex-

Table 2 Tumors and/or malformations induced in rodents with a single (or a few) doses of carcinogen in relation to the age at the time of treatment

Compound	Species (strain)	Age in days	Dose mg/kg body wt	Route[a]	Tumors present in				Malform.	Refs.
					Kidney	Intestine	Nervous System	Others		
Azoxymethane	Rats (BD)	(-13)→(-8)	30	iv	-	-	-	-	-	93
$CH_3 - N = N - CH_3$ ↓ O		(-13)→(-8)	30	sc	-	-	-	-	-	
		(-1)	30	sc	+		+			
		1	4	sc	+	+	+			
		3	6	sc	++	+	+			
		10	12	sc	+	+	+			
		30	20	sc	+	++	+			
		>60	40	sc	++	++				
Methylazoxymethanol	rats Fischer	(-8)→(-7)	20	iv			+		+	59, 95
		60	20	iv	++	++		+		
$CH_3 - N = N - CH_2OH$ ↓ O	golden hamster	(-13)	20	iv					+++	
Azoxyethane	rats BD1X	(-13)	50	iv					++	94
$H_5C_2 - N = N - C_2H_5$ ↓ O		(-8)	150	iv			+++			
		(-1)	150	iv			+++			
		>60	210	iv			+			

[a] iv = intravenous; sc = subcutaneous.

plained. This is one of the most important problems for investigation; understanding of the factors responsible for resistance to tumor induction may give a lead to cancer prevention.

The transplacental studies have demonstrated the greater sensitivity to carcinogens of the fetus in relation to that of its adult mother. In some instances, the presence of the carcinogen given to the pregnant female has been detected in the fetus (103, 107–109). Using labeled 3-methylcholanthrene the quantities detected in the mouse fetus represented 0.26% of the dose given to the mother. It would appear that 2 ng/mg of fetal lung tissue is sufficient to induce lung tumors in later life (109). The carcinogens appear to persist longer in the fetus than in the adult (103).

Transplacental studies are very suitable for such investigation. When direct comparison is made of the response of the fetus and the adult to the same dose of carcinogen, experimental errors are eliminated that might arise due to leakage or to licking of the material by the mother, when newborns are injected; moreover, the carcinogen can undergo activation in the mother's tissues, regardless of the metabolic competence of the fetus. The problem of the passage of carcinogens through the placenta at various stages of pregnancy remains to be investigated.

Evidence for the in vivo Formation of Nitroso Carcinogens from Precursors

In view of the very small dose of ENU required for the transplacental induction of malformations and tumors of the nervous system in rats (5 mg/kg body weight), pregnant females were used in experiments intended to detect whether ENU can be formed in the animal body from ethylurea and nitrite in sufficient concentrations to induce such effects. Positive results have been reported for both teratogenic action (110, 111) and for the induction of neurogenic tumors in the offspring (112, 113).

It is of great interest that administration of ascorbic acid prior to the nitrite prevented the development in the fetus of hydrocephalic lesions (111) in direct confirmation of the observation of Mirvish et al (114) that in the animal stomach, ascorbic acid reacts with nitrite and therefore prevents the nitrosylation of the alkylamino compounds to carcinogens.

CARCINOGENS IN MILK

The great susceptibility at the perinatal period to many carcinogens indicates the need for paying particular attention to the possibility that carcinogens ingested by lactating females may be excreted in the milk and present a hazard to the suckling young. Surprisingly, in spite of the milk's vital importance as the main foodstuff of the very young few experimental data are available on this problem.

"Natural" carcinogens are of particular interest in this connection. Poisoning of livestock, cows, sheep, and chicken by plants containing the hepatocarcinogenic (Senecio) pyrrolizidine alkaloids (PA) has been known for many years (115). When given to lactating rats, the suckling young developed acute and chronic liver and other lesions; the treated mother rats did not show ill effects, and their lactation remained unimpaired (116).

The form in which the PA are excreted in the milk is not yet known. More extensive studies would be needed, in order to establish whether and under which conditions the suckling young could survive long enough and develop tumors. The carcinogenic toxin of the bracken fern, *Pteridium aquilinum* has been reported to pass into the milk when fed to cows (117).

The fungal carcinogens, aflatoxin B_1 and G_1 are excreted in the milk in traces together with their equally toxic metabolites, aflatoxin M_1 and GM_1 (8), when given to several species of animals, including rats and cows, during lactation. Similarly, when the carcinogenic cycasin and its aglycone, methylazoxymethanol, were given to nursing rats, these compounds could be detected in the milk and in the tissues of the suckling young, and also some related metabolic products, whose structure has not yet been identified (107). The suckling young of cows, pigs, or rats fed cycas meal during lactation developed liver and other lesions that were more pronounced than those induced in the treated mothers (118).

Urethane, given to lactating mice, induces lung adenomas and lymphoid leukemias in the suckling young (102).

Mice nursed by mothers treated with methylcholanthrene also develop increased incidence of lung and lymphoid tumors (119); the presence of the hydrocarbon in the milk has been detected (109, 120).

As for the carcinogenic nitrosocompounds, hamsters nursed by mothers given daily small doses of DEN during lactation developed tumors of the upper respiratory tract, including the lung, trachea, and nose; similar tumors were present also in the nursing mothers (121). Rats nursed by mothers given a few large doses of DEN intragastrically during lactation developed aesthesioneuroepithelioma of the nasal ethmo-turbinals, which spread into the brain; some had also liver tumors; among their mothers, liver and kidney tumors were found. (122). The ready induction of various tumors by the milk of rodents treated with DEN raises the problem whether the nitrosamine is excreted into the milk in sufficient concentration to account for the results or whether the milk contains also some carcinogenic metabolites of DEN, perhaps related to the hydroxyethyl-N-nitroso-N-ethylamine and the respective carboxylic oxidation products that have been identified in the rat's urine (123).

The work on transplacental carcinogenesis made it clear that pregnant women have to be protected from exposure to carcinogens. Similar protection is needed for women who nurse their babies. An appropriate surveillance of dried milk and other infant foods is also obviously needed.

In this connection the problem of formation of nitroso compounds in the stomach from nitrite and alkylamides, mentioned previously, is relevant. A variety of non-neurogenic tumors has been induced by the administration to adult animals of nitrite in conjunction with various alkylamido and related compounds (124–126). These results raise the possibility that in human life, similar formation of nitroso carcinogens may occasionally occur, e.g. when certain medicines are taken and food is consumed that contains nitrite as preservative (126).

Epidemiological investigation disclosed an association between drugs administered during pregnancy and congenital abnormalities of the fetus (127). This prob-

lem is at present receiving much attention. The discovery of the protective action of ascorbic acid (111, 114, 125) suggests a simple solution; starting meals with citrus fruit might be adopted with advantage.

ETIOLOGY OF CHILDHOOD TUMORS

The problem of the etiology of childhood tumors is of much greater significance than their incidence (2–3% of all cancer cases) may appear to suggest. Neoplasias of childhood are likely to have originated pre- or during the intrauterine existence (128). The etiological factors of childhood tumors may be easier to trace than those of adults; yet the same agents may be responsible for tumors that appear later in life, if they acted at lower dosage levels or at different stages of the individual's development.

Neoplasias are only one of the manifestations of the action of carcinogenic agents. Some of these can cause also cytotoxic, mutagenic, and teratogenic effects and may be responsible for some of the congenital malformations and other abnormalities, often considered of genetic or viral etiology (128). Experimental evidence has already been obtained that the offspring of rats given cycasin during pregnancy may develop tumors or malformations of the brain and of the retina (95) or may exhibit "intellectual deficit" demonstrable in specific tests (129).

Multigeneration studies have been reported. Their aim was to explore the possible effects of continuous exposure to traces of carcinogenic contaminants that may be present in human environment or as a result of the use of insecticides [DDT (130, 131)], of methylthiouracil (77), or of diets contaminated with aflatoxins (132), PAH (133, 134) etc.

Other investigations have begun to appear that deal with the consequences in offspring (and in subsequent generations of their progeny) of the administration of carcinogens during pregnancy or before mating (135). The tentative results so far obtained require to be extended by varying the experimental conditions and by paying attention to the causes of death of the fetuses and newborns that may occur in the F2 and F3 generations.

MECHANISM OF THE INDUCTION OF TUMORS

As this review shows, substances of a variety of chemical structures can induce tumors in young animals with a *single* dose. The localization of the tumors in particular organs and the cell type from which the tumors originate appear to depend on the concentration of the active entity that reaches the tissue and on the presence at the critical time of cells in a "receptive" stage.

The mechanism of action of many carcinogens has been interpreted by alkylations, involving nucleophilic substitution of nitrogen, carbon, or oxygen in nucleic acids, or in proteins (136–138). In the course of the last few years, however, it became evident that alkylations, especially of the bases of nucleic acids (which attracted most attention) did not correlate with the development of tumors (137, 139), or with the results of mutagenic studies (140, 141).

Reactions that are relevant for the induction of tumors and probably also for the mutagenic action are likely to involve both the nucleic acids and the proteinaceous constituents of chromatin (142).

Studies of the metabolism of various carcinogens in the animal body have shown that the parent compounds undergo mainly oxidation before their stepwise further degradation. Among the oxidation products that appear to represent the activated carcinogenic entities are epoxides, e.g. those formed at the K zone of polycyclic aromatic hydrocarbons (143); at the double bond $\Delta^{1,2}$ of the pyrrolizidine moiety of Senecio alkaloids (144); at the terminal double bond of the dihydrodifurano moiety of aflatoxins (144); at the α,β-double bond of unsaturated aldehydes (43) (Figure 2).

In the case of dialkylnitrosamines, ω- or β-oxidation products of one of the alkyls have been identified as urinary metabolites (123, 145). The hydroxymethyl derivatives probably form the respective aldehydes before their oxidation to the respective carboxylic acids. Metabolites with an aldehyde or keto function have been suggested to be the activated carcinogenic forms of dialkylnitrosamines, methylazoxymethanol, and related compounds (142).

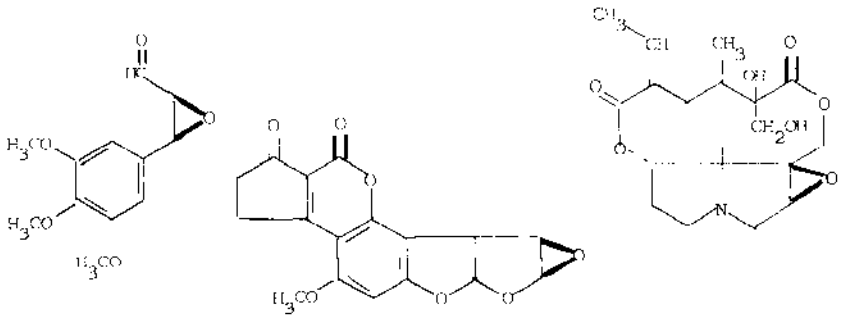


Figure 2 Suggested structures for epoxides of 3,4,5-trimethoxycinnamaldehyde (*left*), aflatoxin B_1 (*middle*), and retrorsine (*right*).

It is not unlikely that at a particular stage of the cell's existence, chromatin assumes a configuration in which a free amino group of a nucleic acid base may be present in close vicinity to two thiols of peptide chains. Such a configuration would allow the aldehydic carbonyl to condense with the amino group to form a Schiff-base type of bond, while the thiols could reduce the nitroso group and form a covalent bond. As a result, a firm bridge (possibly in the form of a six- or seven-membered ring) would bind the protein to the nucleic acid. Such binding may be irreversible and have long lasting, fateful consequences, as is the case of carcinogens which can induce tumors with even a single dose.

The particular sensitivity of the young and their actively developing tissues to the action of carcinogens may be related to their higher content of cells in the receptive state. In addition, the paucity of certain enzyme systems at the perinatal age will slow down the formation and also the degradation of the activated form of the

carcinogens. The net balance results in the higher probability that both interactants will meet in the appropriate state.

However, the interplay of other factors, genetic, viral, and immunological will determine whether and which of the cells affected by a carcinogen will die and which ones will survive, start dividing, and form the center of a new growing tissue with neoplastic characteristics.

The idea that a specific organotropism is an inherent characteristic of a carcinogen is no more tenable. It is clear from the reviewed results that a carcinogen can induce tumors in almost any organ or tissue that contains cells in appropriate receptive state at the time when it is reached by the activated carcinogenic entity in appropriate concentration.

The answers to the fundamental questions—where and how is the activated carcinogenic entity formed, and when formed, how long can it survive the passage in the blood stream and through the inter- and intracellular membranes so as to reach the recipient site in adequate concentration, and what are the essential characteristics of the activated entity—all seem to need reconsideration.

The various alkylating forms, so far proposed (136, 137) may not be relevant for the carcinogenic process. An alternative hypothesis has been suggested (149).

More than one step is likely to be involved in the activation of the parent molecules. Are these steps accomplished at the same or at different sites of the cell or possibly in different organs?

The liver may not be an over-important organ in dealing with the activation of carcinogens. Drug metabolizing enzyme systems have been reported in other organs, e.g. the lung (146).

Though we may not yet be able to give satisfactory explanations to all these questions, the demonstration that many organs are particularly sensitive to carcinogens during the perinatal period indicates that metabolic studies on the molecular level in the newborn may give better chances for the identification of the reactions relevant for the induction of tumors.

SUMMARY

The reviewed evidence derived from animal experiments performed in the last few years by many workers shows the following:

A single dose of carcinogen, which may not cause immediate ill effects, can induce tumors after a prolonged latent period.

The susceptibility of the fetus or of the newborn to carcinogens is much greater than that of weanlings or adults.

The type and the localization of the tumors induced depends not only on the characteristics of the carcinogen, its route of entry, and the size of the dose, but to a large extent on the biological age of the recipient and on the developmental and functional state of the organs at the time of treatment.

Exposure of females to carcinogens during the early stages of pregnancy has usually cytotoxic effects, which cause resorption or abortion of the embryo; during the second trimester of pregnancy malformations may be produced, while tumors

develop in the offspring when the carcinogen acts during the second half of gestation. The tumors induced in rodents transplacentally become apparent only many months later.

Hormones, especially estrogens, can induce neoplasias in their target organs; they may have, in addition, modifying effects on the action of other carcinogens.

The possibility is discussed that exposure of pregnant or lactating females to an increased load of compounds with estrogenic and other hormonal activities may present teratogenic or carcinogenic hazards to both sexes of the offspring.

Formation of carcinogenic nitroso-compounds in the stomach from noncarcinogenic constituents possibly present in food and medicines may represent an additional teratogenic and carcinogenic hazard.

In order to reproduce in animals a situation in which tumors would appear in the fetus or in the newborn (as in clinical experience), preconception exposure of the parents' gonads to carcinogens might be required, possibly in addition to intrauterine exposure of the embryo to noncytotoxic concentrations of carcinogenic agents.

Epidemiological studies in the USA disclosed an increased incidence of childhood tumors and leukemias in the offspring of parents who were exposed to irradiation long before conception, especially when the offspring was additionally irradiated prenatally or postnatally (for references see 147). Such a situation may have its counterpart also in the case of the "radiomimetic" chemical carcinogens.

ACKNOWLEDGMENTS

I wish to thank Mrs. N. P. Brewster for her excellent and devoted secretarial help.

Note added in proof: The recent finding of the very rare benign hepatomas in seven young women, 25–39 years old, possibly associated with the use of oral contraceptives for up to 12 years (148), adds to the misgivings expressed in this review as regards the use of OC.

Literature Cited

1. Wells, H. G. 1940. *Arch. Pathol.* 30: 535–601
2. Willis, R. A. 1962. *The Pathology of Tumours of Children.* Edinburgh: Oliver and Boyd
3. Morison, J. E. 1970. *Foetal and neonatal pathology.* London: Butterworth. 3rd ed.
4. Napalkov, N. P. 1973. In *Transplacental Carcinogenesis,* ed. L. Tomatis, U. Mohr, W. Davis. IARC Publ. No. 4, 1–13
5. Zuckerman, S., Mandl, A. M., Eckstein, P., Eds. 1968. *The Ovary.* London: Academic
6. Gross, L. 1970. *Oncogenic Viruses.* Oxford: Pergamon. 2nd ed.
7. Stewart, A. 1971. *Advan. Cancer Res.* 14:359–90
8. Detroy, R. W., Littlehoj, E. B., Ciegler, A. 1971. In *Fungal Toxins,* ed. A. Ciegler, S. Kadis, S. J. Ajl, 6:4–178, don: Academic
9. Magee, P. N., Barnes, J. M. 1967. *Advan. Cancer Res.* 10:163–246
10. Schoental, R. 1968. *Cancer Res.* 28: 2237–46
11. Lacqueur, G. L., Spatz, M. 1968. *Cancer Res.* 28:
12. Tomatis, L., Mohr, U., Davis, W., Eds. 1973. *Transplacental Carcinogenesis.* IARC Publ. No. 4
13. Orr, J. W. See Ref. 5, 2:533–65
14. Stewart, A. M. 1973. *Brit. J. Cancer* 27:465–72
15. Lenz, W. 1962. *Am. J. Dis. Child.* 112: 99–106
16. Karnofsky, D. A. 1965. *Ann. Rev. Pharmacol.* 5:4
17. Herbst, A. L., Ulfelder, H., Poskanzer, D. C. 1971. *N. Engl. J. Med.* 284: 878–81

18. Herbst, A. L., Kurman, R. J., Scully, R. E., Poskanzer, D. C. 1972. *N. Engl. J. Med.* 287:1259–63
19. Wilkins, L. P. 1960. *J. Am. Med. Assoc.* 172:1028–32
20. Kuchera, L. K. 1971. *J. Am. Med. Assoc.* 218:562–63
21. Doll, R. 1971. *Brit. Med. Bull.* 27: 25–31
22. Report from the Boston Collaborative Drug Surveillance Programme. 1973. *Lancet* i:1399–1404
23. Nora, J. J., Nora, A. H. 1973. *Teratology* 7:Abstr. 24
24. Wong, Y. K., Wood, B. S. B. 1971. *Brit. Med. J.* iv:403–4
25. Andrea, F. P., Christensen, H. D., Williams, T. L., Thompson, M. G., Wall, M. E. 1973. *Teratology* 7:A11 (Abstr., Proc. Meet. Teratol. Soc., 13th)
26. Lacassagne, A. 1932. *C. R. Acad. Sci. Paris* 195:630–32
27. Gardner, W. U. 1959. *Ann. NY Acad. Sci.* 75:543–64
28. Miihlbock, O. 1972. *J. Nat. Cancer Inst.* 48:1213–16
29. Dunn, T. B., Green, A. W. 1963. *J. Nat. Cancer Inst.* 31:425–55
30. Dunn, T. B. 1969. *J. Nat. Cancer Inst.* 43:671–92
31. Kirkman, H. 1972. *Progr. Exp. Tumor Res. Basel* 16:201–40
32. Sidransky, H., Wagner, B. P., Morris, H. P. 1961. *J. Nat. Cancer Inst.* 26: 151–87
33. Firminger, H. I., Reuber, M. D. 1961. *J. Nat. Cancer Inst.* 27:559–95
34. Morris, H. P., Firminger, H. I. 1956. *J. Nat. Cancer Inst.* 16:927–49
35. Runsfeld, H. W. Jr., Miller, W. L. Jr., Baumann, C. A. 1951. *Cancer Res.* 11: 814–19
36. Stromberg, K., Reuber, M. D. 1970. *J. Nat. Cancer Inst.* 44:1047–54
37. Reuber, M. D., Glover, E. L. 1967. *J. Nat. Cancer Inst.* 38:891–99
38. Reuber, M. D., Lee, C. W. 1968. *J. Nat. Cancer Inst.* 41:1133–40
39. Huggins, C., Briziarelli, G., Sutton, H. 1959. *J. Exp. Med.* 109:25–42
40. Jasmin, G., Riopelle, J. L. 1970. *Cancer Res.* 30:321–26
41. Gillette, J. R. 1971. *Ann. NY Acad. Sci.* 179:43–66
42. Sabine, J. R., Horton, B. J., Wicks, M. B. 1973. *J. Nat. Cancer Inst.* 50: 1237–42
43. Schoental, R., Gibbard, S. 1972. *Brit. J. Cancer* 26:504–06
44. Schoental, R. 1973. *Lab. Anim.* 7: 47–49
45. Editorial. 1973. *Nature* 243:6
46. Mirocha, C. J., Christensen, C. M., Nelson, G. H. See Ref. 8, 7:107–38
47. Butenandt, A., Jacobi, H. 1933. *Hoppe-Seyler's Z. Physiol. Chem.* 218:104–2
48. Bradbury, R. B., White, D. E. 1954. *Vitam. Horm. New York* 12:207–33
49. Liener, J. E. 1969. In *Toxic constituents of plant foodstuffs*, ed. J. E. Liener, 409–48. London: Academic
50. Bickoff, E. M. et al 1957. *Science* 126: 969–70
51. Bickoff, E. M., Livingston, A. L., Hendrickson, A. P., Booth, A. N. 1962. *J. Agr. Food Chem* 10:410–12
52. Jensen, E. V., DeSombre, E. R. 1972. *Ann. Rev. Biochem.* 41:203–30
53. Campbell, H. 1972. *Proc. Roy. Soc. Med.* 65:641–45
54. Malling, H. V., de Serres, F. J. 1969. *Ann. NY Acad. Sci.* 163:788–800
55. Röhrborn, G. See Ref. 12, pp. 168–74
56. Clegg, D. J. 1971. *Ann. Rev. Pharmacol.* 11:409–24
57. Di Paolo, J. A., Kotin, P. 1966. *Arch. Pathol.* 81:3–23
58. Green, C. R., Christie, G. S. 1961. *Brit. J. Exp. Pathol.* 42:369–78
59. Spatz, M. 1969. *Ann. NY Acad. Sci.* 163:848–59
60. Di Paolo, J. A. 1969. *Ann. NY Acad. Sci.* 163:801–12
61. Roe, F. J. C., Walters, M. A., Mitchley, B. C. V. 1967. *Brit. J. Cancer* 21:331–33
62. Hartwell, J. L. 1941. *US Publ. Health Serv. Publ.* No. 149; Shubik, P., Hartwell, J. L. 1957. Suppl. 1; 1969. Suppl. 2, ed. J. A. Peters
63. Toth, B. 1968. *Cancer Res.* 28:727–38
64. Della Porta, G., Terracini, B. 1969. *Progr. Exp. Tumor Res.* 11:334–63
65. Roe, F. J. C., Walters, M. A. 1968. *Food Cosmet. Toxicol.* 6:581–82
66. Schoental, R. 1970. *Nature*, 227:401–2
67. Schoental, R., Magee, P. N. 1957. *J. Pathol. Bacteriol.* 74:305–19
68. Magee, P. N., Barnes, J. M. 1959. *Acta Unio Int. Contra. Cancrum* 15:187–90
69. Schoental, R. 1960. *Nature* 188:420–21
70. Schoental, R., Bensted, J. P. M. 1963. *Brit. J. Cancer* 17:242–51
71. Druckrey, H. 1971. *Arzneim. Forsch.* 21:1274–78
72. Druckrey, H., Steinhoff, D., Preussmann, R., Ivankovic, S. 1964. *Z. Krebsforsch.* 66:1–10
73. Druckrey, H., Preussmann, R., Ivankovic, S., Schmähl, D. 1967. *Z. Krebsforsch.* 69:103–201

74. Terracini, B., Testa, M. C. 1970. *Brit. J. Cancer* 24:588–98
75. Jänisch, W., Schreiber, D., Stengel, R., Steffen, V. 1967. *Exp. Pathol.* 1:243–255
76. Kreybig, T. von 1965. *Z. Krebsforsch.* 67:46–50
77. Napalkov, N. P., Alexandrov, V. A. 1968. *Z. Krebsforsch.* 71:32–50
78. Alexandrov, V. A. 1969. *Nature* 222: 1064–65
79. Druckrey, H., Ivankovic, S., Preussmann, R. 1966. *Nature London* 210: 1378–79
80. Ivankovic, S., Druckrey, H. 1968. *Z. Krebsforsch.* 71:320–60
81. Druckrey, H., Schagen, B., Ivankovic, S. 1970. *Z. Krebsforsch.* 74:141–61
82. Druckrey, H., Landschütz, Ch., Ivankovic, S. 1970. *Z. Krebsforsch.* 73: 371–86
83. Druckrey, H., Preussmann, R., Ivankovic, S. 1969. *Ann. NY Acad. Sci.* 163:676–95
84. Grossi-Paoletti, E., Paoletti, P., Schiffer, D., Fabiani, A. 1970. *J. Neurol. Sci.* 11:573–81
85. Koestner, A., Swenberg, J. A., Wechsler, W. 1971. *Am. J. Pathol.* 63: 37–56
86. Jänisch, W., Schreiber, D., Warzok, R., Schneider, J. 1972. *Arch. Geschwulstforsch.* 39:99–106
87. Jones, E. L., Searle, C. E., Smith, W. T. 1973. *J. Pathol.* 109:123–39
88. Rice, J. M. 1969. *Ann. NY Acad. Sci.* 163:813–26
89. Searle, C. E., Jones E. L. 1973. *Nature* 240:559–60
90. Leaver, D. D., Swann, P. F., Magee, P. N. 1969. *Brit. J. Cancer* 23:177–87
91. Druckrey, H., Landschütz, C., Preussmann, R., Ivankovic, S. 1971. *Z. Krebsforsch.* 75:229–39
92. Druckrey, H., Landschütz, C. 1971. *Z. Krebsforsch.* 76:45–58
93. Druckrey, H., Lange, A. 1972. *Fed. Proc.* 31:1482–84
94. Druckrey, H. et al 1968. *Experientia* 24:561–62
95. Laqueur, G. L., Spatz, M. See Ref 12, pp. 59–64
96. Ginsburg, J. 1971. *Ann. Rev. Pharmacol.* 11:387–408
97. Wechsler, W. et al 1969. *Ann. NY Acad. Sci.* 159:360–408
98. Hirono, I. 1972. *Fed. Proc.* 31:1493–97
99. Mirvish, S. S. 1968. *Advan. Cancer Res.* 11:1–42
100. Matsuyama, M., Suzuki, H., Nakamura, T. 1969. *Brit. J. Cancer,* 23: 167–71
101. Kommineni, V. R., Greenblatt, M., Mihailovich, N., Vesselinovitch, S. D. 1970. *Cancer Res.* 30:2552–55
102. Nomura, T. 1973. *Cancer Res.* 33: 1677–83
103. Nomura, T., Takebe, H. Okamoto, E. 1973. *Gann* 64:29–40
104. Goldfeder, A. 1972. *Cancer Res.* 32: 2771–77
105. Schoental, R., Cavanagh, J. B. 1972. *J. Nat. Cancer Inst.* 49:665–71
106. Schoental, R. Unpublished results
107. Spatz, M., Laqueur, G. L. 1968. *Proc. Soc. Exp. Biol. Med.* 127:281
108. Tomatis, L. et al 1971. *J. Nat. Cancer Inst.* 47:645–51
109. Takahashi, G., Yasuhira, K. 1973. *Cancer Res.* 33:23–38
110. Alexandrov, V. A., Jänisch, W. 1971. *Experientia* 27:538–39
111. Ivankovic, S., Preussmann, R., Schmähl, D., Zeller, J. 1973. *Z. Krebsforsch.* 79:145–47
112. Ivankovic, S., Preussmann, R. 1970. *Naturwissenschaften,* 57:460
113. Osske, G., Warzok, R., Schneider, J. 1972. *Arch. Geschwulstforsch.* 40:244
114. Mirvish, S., Wallcave, L., Eagen, M., Shubik, P. 1972. *Science* 177:65–68
115. McLean, E. K. 1970. *Pharmacol. Rev.* 22:429–83
116. Schoental, R. 1959. *J. Pathol. Bacteriol.* 77:485–95
117. Evans, I. A., Jones, R. S., Mainwaring-Burton, R. 1972. *Nature* 237:107–8
118. Mickelsen, O., Campbell, E., Yang, M., Mugera, G., Whitehair, C. K. 1964. *Fed. Proc.* 23:1363–65
119. Shay, H., Gruenstein, M., Weinberger, M. 1952. *Cancer Res.* 12:29
120. Shay, H., Friedmann, B., Gruenstein, M. 1950. *Cancer Res.,* 10:797–800
121. Mohr, U., Althoff, J., Emminger, A., Bresch, H., Spielhoff, R. 1972. *Z. Krebsforsch.* 78:73–77
122. Schoental, R. Unpublished results
123. Blattmann, L., Preussmann, R. 1973. *Z. Krebsforsch.* 79:3–5
124. Sander, J. 1971. *Arzneim. Forsch.* 21: 1707–13, 2034–39; 1970. 20:418–19
125. Greenblatt, H. M., Mirvish, S. S. 1973. *J. Nat. Cancer Inst.* 50:119–24
126. Lijinsky, W., Taylor, H. W., Snyder, C., Nettesheim, P. 1973. *Nature* 244: 176–78
127. Nelson, M. M., Forfar, J. O. 1971. *Brit. Med. J.* i:523–27
128. Miller, R. W. See Ref. 12, pp. 175–80

129. Haddad, R. K., Rabe, A., Laqueur, G. L., Spatz, M., Valsamis, M. P. 1969. *Science* 163:88
130. Shabad, L. M., Kolesnichenko, T. S., Nikonova, T. V. 1973. *Int. J. Cancer* 11:688–93
131. Terracini, B., Testa, M. C., Cabral, J. R., Day, N. 1973. *Int. J. Cancer* 11: 747–64
132. Alfin-Slater, R. B., Aftergood, L., Hernandez, H. J., Stern, E., Melnick, D. 1969. *Am. Oil Chemists' Soc.* 46:493, quoted in Ref 8
133. Tomatis, L. 1965. *Proc. Soc. Exp. Biol. Med.* 119:743–47
134. Tomatis, L., Goodall, C. M. 1969. *Int. J. Cancer* 4:219–25
135. Tanaka, T. See Ref. 12, pp. 100–11
136. Druckrey, H. See Ref. 12, pp. 45–58
137. Magee, P. N. See Ref. 12, pp. 143–48
138. Culvenor, C. C. J., Downing, D. T., Edgar, J. A., Jago, M. V. 1969. *Ann. NY Acad. Sci.* 163:837–47
139. Schoental, R. 1967. *Biochem. J.* 102:5C
140. Rosenkranz, H. S., Rosenkranz, S., Schmidt, R. M. 1969. *Biochim. Biophys. Acta* 195:262–65
141. Singer, B., Fraenkel-Conrat, H. 1969. *Biochemistry* 8:3266–69
142. Schoental, R. 1973. *Brit. J. Cancer.* In press
143. Grover, P. L. et al 1971. *Proc. Nat. Acad. Sci. USA* 68:1098–1101
144. Schoental, R. 1970. *Nature* 227:401–2
145. Okada, M., Suzuki, E. 1972. *Gann* 63: 391–92
146. Fouts, J. R., Devereux, T. R. 1972. *J. Pharmacol. Exp. Ther.* 183:458–68
147. Miller, R. W. 1972. *J. Nat. Cancer Inst.* 49:1221–27 and references therein
148. Baum, J. K., Bookstein, J. J., Joltz, F., Klein, E. W. 1973. *Lancet* ii:926–29
149. Schoental, R. 1974. In press