Biochemical Pharmacology, 1962, Vol. 11, pp. 1163–1173. Pergamon Press Ltd., Printed in Great Britain.

PERMANENT CURE OF SOME SPONTANEOUS MAMMARY CANCERS OF MICE WITH ANALOGS OF 1,2-DIMETHYL-4,5-DIAMINOBENZENE

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(*Received* 1 *March* 1962; *accepted* 27 *July* 1962)

Abstract—A mixture of 1,2-dimethyl-4-(*p*-carboxyphenylazo)-5-hydroxybenzene (CPA) and 1,2-dichloro-4-benzenesulfonamido-5-nitrobenzene (DCBN), when fed to white mice of the SPFS strain bearing spontaneous mammary cancers, cured permanently about one-third of the animals. In others, transient cures were observed, but the cancers eventually returned. The maximal number of cures was obtained with 6 g CPA and 0·5 g DCBN per kg of ration. Additional amounts did not increase the percentage of cures. A related compound, 1,2-dimethyl-4,5-*bis*(benzenesulfonamido)benzene (DMDB), also cured some cancers but seemed less potent than the combination of CPA and DCBN. Some other relatives were apparently inactive. In C3H mice, bearing spontaneous mammary cancers, CPA plus DCBN caused transient regressions of the neoplasms but did not result in permanent cures. Several substances such as aminopterin, 5-fluorouracil and 5-fluorodeoxycytidine, which inhibit the growth of certain transplanted cancers of mice, did not exert any detectable effect on these *spontaneous* cancers. The effective compounds were conceived on the basis of the demonstration that the spontaneous cancers differed from normal host tissues in that they synthesized vitamin B_{12}. The new compounds are members of a series of antimetabolites which have been shown to inhibit the biosynthesis of this vitamin in microorganisms. However, there was no direct proof that the effect on the cancers was the result of the relationship to vitamin B_{12} synthesis. The compounds exerted no detectable harmful effects on the host mice at the doses used to bring about cure of the cancers. This selective toxicity was predicted from knowledge of the relationship of the analogs to the biosynthesis of vitamin B_{12} and riboflavin.

PRECEDING papers have presented evidence indicating that certain spontaneous mammary cancers of mice differed from normal mouse tissues in that the cancers synthesized vitamin B_{12}.[1, 2] This metabolic difference allowed the prediction and realization of antimetabolites which were poisonous to the cancers but harmless to the host mice.[3, 4] These antimetabolites were structural analogs of 1,2-dimethyl-4,5-diaminobenzene, a compound that has been shown to serve as a precursor for the biosynthesis of vitamin B_{12} in microbial species.[5, 6]

Although the antimetabolites described earlier were able to harm the cancers selectively, they were not able to cure spontaneous mammary cancers permanently. All that was observed was transient regression in some of the tumors and transient obliteration of a few of them. In about half the animals no effect on the growth of the tumors was observed.[3, 4] In those that were affected, the effect was transient. After

* With the technical assistance of C. Maugé. A summary of this paper was read at the First International Pharmacological Meeting, Stockholm, 1961, and abstracted in *Biochem. Pharmacol.* **8**, 72 (1961).

periods ranging from a few weeks to a few months, the cancers returned and were then resistant to attack by the analogs.

The effects of these same analogs on transplanted cancers were more marked. Permanent cures were produced[4] in about half the cases, but this was true only when the cancers were within five passages of the original donors. With repeated transplantation they became completely resistant to the action of the analogs. In the "early passage" transplants, where the drugs were curative, a study of the relationship between dose and response indicated that all the cancers might be suppressed if a large enough dose could be used. The results with the spontaneous cancers likewise suggested that if a larger dose or a more active analog could be used it might be possible to inhibit all the cancers. Because a larger dose was not practicable, a search for more active congeners was made.

In this paper we wish to report the finding of some analogs of dimethyldiaminobenzene which were able to cure permanently some of the spontaneous mammary cancers of mice. These drugs are not able to cure all the cancers in a group of mice and hence they leave much to be desired, but they represent an advance over the ones previously described, in that they will bring about permanent cures of some spontaneous cancers. This advance came from finding compounds with higher potency as well as from improvements in the mode of administration. Because the problem of chemotherapy revolves around the cure of spontaneous cancers, not transplants mentioned above, all the testing was done in mice bearing spontaneous cancers, even despite the difficulties of obtaining large numbers of such animals.

The analogs that showed greatest potency in the present study were 1,2-dimethyl-4-(*p*-carboxyphenylazo)-5-hydroxybenzene or CPA, 1,2-dichloro-4-benzenesulfonamido-5-nitrobenzene or DCBN, and 1,2-dimethyl-4,5-*bis*(benzenesulfonamido) benzene or DMDB. The chemical structures of these compounds are shown in Fig. 1.

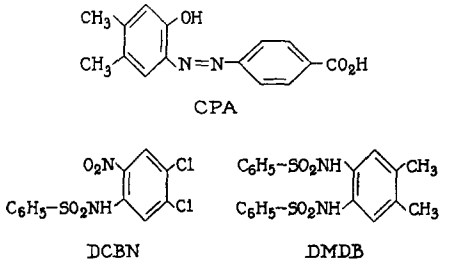

FIG. 1. Structures of the active agents.

As was described in the preceding papers of this series,[5, 7–9] the present compounds would not be expected to be reversed in their biological effects by vitamin B_{12}. They were designed with the express purpose in mind of avoiding such antagonism, because it was realized that if such antagonism did exist the vitamin in the food and tissues of the animals would prevent the therapeutic effect. Consequently, no effort was made to demonstrate reversal of the therapeutic effect on cancers; experiments with microorganisms had already demonstrated the failure of such reversal.[7, 8] The failure of vitamin B_{12} to reverse the therapeutic action is thus comparable to the failure of folic

acid to reverse the action of sulfanilamide in bacterial infections of animals. Because of this feature, which had intentionally been built into the oncolytic compounds, there was no direct demonstration that their effects on cancers were the result of interference with the biosynthesis of vitamin B_{12}. There was only the probability that this may have been so.

MATERIALS AND METHODS

Antimetabolites

CPA was synthesized according to the method of Woolley.[7] DCBN, DMBD, and their relatives were synthesized by methods to be described in a separate paper. Aminopterin was the product sold by Lederle Laboratories; 6-mercaptopurine was from the Burroughs-Wellcome Co. 5-Fluorouracil and 5-fluorodeoxycytidine were kindly supplied by Dr. R. Duschinsky of Hoffmann-La Roche, Inc.

Each analog to be administered orally was added in the desired amount to powdered stock ration (Purina Foxchow) together with enough $NaHCO_3$ or NaOH to yield the sodium salt of those compounds that were acids.* This mixture was then powdered in a ball mill and fed *ad libitum*. For parenteral administeration each compound was suspended by ball milling in Ringer's solution, or dissolved in enough NaOH to yield a neutral aqueous solution. The solutions were sterilized by heating and injected once daily intraperitoneally.

Animals

Two strains of mice bearing spontaneous mammary cancers were used (SPFS and C3H). The scarcity of *spontaneous* cancers limited the size of the experimental groups. The SPFS were Rockefeller-Institute-Swiss mice, all derived from three original litters which had been delivered by Cesarian section and maintained in isolation as a colony free of certain infectious, endemic diseases of mice (SPF means "specific pathogen-free"). The females of this colony were examined weekly, and whenever a small tumor was found in any mouse she was started on an experiment. The nature of the tumors was determined from time to time by excision of the neoplasms and histological examination of sections of them. The C3H mice were purchased from the Jackson Memorial Laboratories and were from their inbred colony. Individuals bearing spontaneous tumors less than 1 cm in greatest dimension were selected and used.

Methods

Female mice bearing spontaneous cancers were caged individually and fed either stock ration (Purina Foxchow) or stock ration to which the compounds had been added as described above. The size of each cancer was measured (length, width, and height) with calipers once each week. Body weight was also determined each week. Because large numbers of mice were never available at the same time, it was necessary to take them whenever the tumors were discovered. Consequently, the controls (untreated with the compounds) were chosen by alternately selecting individuals for control and experimental groups. In most experiments, all mice (test as well as

* As an example, 1 kg of ground Foxchow was mixed with 6 g of CPA. A solution of 500 mg of DCBN in 15 ml of 1 N NaOH and 5 ml of ethanol was spread over this mixture, and the whole was ball milled for 24 hr.

control) were fed stock ration for the first week and if any showed a decrease in size of tumor, they were discarded. The reason for this will be discussed in the section on Results. All mice were kept under examination until they died, or until they had been free of cancer for at least 5 months. Each experimental group contained six animals, and experiments were repeated at suitable intervals. Whenever a cancer disappeared, treatment with the analogs was stopped and only stock ration was fed.

This cessation of treatment seemed to be important for permanence of cure. Previous studies with less potent analogs[3, 4] had shown clearly that, if the therapeutic agents were continued after disappearance of the cancers, the neoplasms always returned. Because of this prior experience, the procedure with the new compounds was as described, even though no direct test of the phenomenon of reappearance was made with them.

Criteria of cure

A permanent cure of a cancer was judged to have taken place when the neoplasm decreased in size, disappeared, and remained undetectable for at least five months. Disappearance was judged by failure to detect it by palpation. It was not unusual to find that a tumor might decrease in size, and disappear for weeks or months, only to return and to grow until the animal died. Such behavior was termed *transient cure.* Sometimes a cancer might decrease in size, but after a few weeks would begin to grow again; this was called *transient regression.* Decreases greater than 0·2 cm in any dimension that continued for longer than 1 week were considered significant. Mere slowing of the rate of growth or failure to grow was not considered to be a regression.

When a mouse had more than one cancer, it sometimes happened that the individual tumors responded differently. One neoplasm might decrease or even disappear, while another might continue to grow. When such a response was seen it was counted as a transient regression, even though one of the tumors had completely disappeared. In cases of multiple cancers, all the neoplasms were required to disappear before the mouse was considered to be cured and counted as a permanent cure.

RESULTS

Response of controls

The cancers of every one of the C3H mice which was fed only stock ration continued to grow and eventually killed the animal. There were no cases of spontaneous regression. With the SPFS mice the situation was less clear. Some of these tumors showed regressions, and a few disappeared permanently. For this reason great attention was given to control groups.

To determine whether all the tumors were mammary cancers, histological examination of sections of the tumors from 23 SPFS mice was made.* This showed that all neoplasms larger than 1 × 1 × 0·5 cm (15 tumors) were typical, malignant mammary cancers.

With the smaller tumors (less than 1 × 1 × 0·5 cm), some proved to be abscesses or other nonmalignant growth. The number of noncancerous tumors among these small ones varied considerably from experiment to experiment. Thus, in one experiment, six of eleven small tumors were noncancerous on histological examination, but

* We are greatly indebted to Dr. John B. Nelson of this Institute for the histological examinations.

in another experiment involving ten mice with small tumors, all grew slowly to great size and killed their hosts.

The ideal way to avoid inclusion of noncancerous growths, apparently, would be to employ only large tumors, and also to take a biopsy specimen from each at the start of the experiment, in order that all doubtful cases be eliminated. The difficulty with this plan was (a) the larger the tumor the more difficult it was to attain cures with the analogs, and (b) taking biopsy specimens from a small tumor endangered its continued existence as a growing cancer.

To minimize possible errors, the control mice were chosen so that the sizes of the tumors in the experimental groups were comparable to those in the control groups. All mice were kept for 1 week on stock ration, and any one with a tumor that failed to grow during this trial period was discarded. In this way most of the growths that were merely abscesses were eliminated.

Even when noncancerous growths were excluded, the problem of spontaneous regression of SPFS tumors was not solved. Such spontaneous regressions were observed in some tumors which had been proved by histological examination to be malignant cancers. Thus, mouse 1528 was first observed with a tumor that measured 0·9 × 1·1 × 0·7 cm; this neoplasm grew steadily for 4 weeks and then began to decline. When the tumor had grown smaller for 6 weeks (0·6 × 0·6 × 0·6 cm), it was excised, sectioned, and examined histologically. It was a typical malignant growth. The tables of data contain several examples of similar spontaneous regressions and cures.

The variation in rate of growth of spontaneous cancers was much greater than that found in transplanted cancers. For this reason the effects of the drugs could not be judged merely from measurements of growth rate of the cancers. In the C3H animals, which were an inbred strain, the variation in growth rate of the cancers was less than in the SPFS strain, so that some useful information was gained from a study of growth rates in the C3H strain.

TABLE 1. EFFECTS OF ORALLY ADMINISTERED CPA AND DCBN ON SPONTANEOUS MAMMARY CANCERS OF SPFS MICE

CPA (g/kg ration)	DCBN (g/kg ration)	Mice	Trans. regres.	Trans. cures	Perm. cures
0	0	80	13	2	6
10	0·5	17	5	1	4
6	0·5	36	10	4	11
3	0·5	15	2	1	5
3	0·25	6	1	0	1
3	1·0	14	6	1	3
3	2·0	5	1	0	1
3	0	6	1	1	1
1·5	0·25	6	1	1	0
0	0·5	4	0	0	1
0	2·0	6	2	0	2

Effects of orally administered CPA and DCBN on cancers of SPFS mice

The effects of CPA and DCBN (either singly or together) are shown by the data in Table 1. The data suggest that in combination these compounds were able to cure permanently about one-third of the mice and to affect favorably most of them. The optimal dose seemed to be 6 g of CPA and 0·5 g of DCBN per kg of ration. Increasing

the amount of CPA to 10 g/kg did not give improved response; in fact, such an inincrease may have diminished the response somewhat, although the number of animals was not great enough to establish this last point. At the 10-g level the mice did ot eat the ration readily and, during the first week or two, ate so little that they lost v eight. With continued exposure to the ration, however they learned to eat it, and regained their lost weight. The diminished intake at the start might have been partially responsible for the relatively poor response at this dose level.

A further factor may have contributed to the failure of the 10-g dose to improve upon that obtained with 6 g. All mice which ate the rations containing CPA, up to and including the 6-g level, apparently reduced the azo group in the molecule completely. No red color was excreted in their urine and their tissues (aside from their fur) were not stained with the azo compound; however, those mice which ate the ration containing 10 g CPA/kg excreted red urine. This may have indicated that these animals had not degraded all of the CPA ingested and that some escaped into the urine. Whether or not CPA, *per se*, is an anticancer agent, or requires metabolic change to become active, is not known.

Whenever a mouse experienced a regression of its tumor, and the cancer disappeared, the administration of CPA and DCBN was stopped. This was done because, in earlier experiments with drugs of this class,[3, 4] it had been found that if administration was continued after the neoplasms had disappeared, the cancers invariably returned. It was thought that this may have been because a drug-resistant strain of the cancer had arisen in the continued presence of the drug in the animal, or that drug-destroying enzymes had arisen in the host. Experimental evidence for such points of view has been already presented.[3, 4] Nevertheless, the need for cessation of therapy when the cancers disappeared was not examined in the cases of CPA plus DCBN. To minimize risk of the development of such resistance, the discontinuance of the drugs was practised as outlined.

In several of the mice given CPA and DCBN the cancers disappeared, and administration of the drugs was stopped. After a few weeks or months the cancer again appeared. The compounds were not started again, but the growth of the new neoplasms was followed. In some cases they continued to grow and resulted in death of the hosts, but in others they receded again after 6 to 8 weeks and totally disappeared. This secondary disappearance was thus without direct aid of chemotherapy. It is possible that these animals were immune to the secondary cancer and had developed the immunity as a result of their previous temporary cure. No proof of such a possibility has been attempted. It is very difficult to provide adequate controls for experiments of this sort, because the mice which had not been treated with the drugs did not show the phenomenon.

Relative effectiveness of CPA and DCBN on large and small cancers of SPFS mice

Table 2 shows the relative effectiveness of CPA and DCBN fed together to SPFS mice bearing small tumors (less than $1 \times 1 \times 0{\cdot}5$ cm) and to those bearing larger tumors (greater than $1 \times 1 \times 0{\cdot}5$ cm). It was plain that, the larger the tumors when chemotherapy was started, the less chance there was for a cure. This same result was found with C3H cancers in which, although no cures were achieved, transient regressions of small cancers, but not of large ones, were found. Likewise, the same

phenomenon of the importance of starting with small cancers was seen when drugs such as DMDB were given to SPFS mice.

Effect of injected CPA and DCBN on spontaneous cancers of SPFS mice

CPA and DCBN cured some, but not all, mice when they were injected rather than fed. For example, three of six SPFS mice permanently lost their cancers when they were injected daily with 2·5 mg CPA and 0·5 mg DCBN. DCBN by itself cured three of seven mice at 1 mg/day, and one of ten mice at 0·5 mg/day, and CPA by itself (5 mg/day) cured none of the five mice permanently. The scarcity of mice with spontaneous cancers limited the number of animals tested, but the results showed clearly that injection (rather than feeding) of the compounds would not cure all of the cancers.

TABLE 2. RELATIVE EFFECTIVENESS OF CPA AND DCBN ON LARGE AND SMALL CANCERS OF SPFS MICE

Large cancers were greater than 1 cm and small cancers less than 1 cm at the start of the experiment.

CPA (g/kg ration)	DCBN (g/kg ration)	Mice with small c.	Mice with large c.	Perm. cures
0	0	37		6
0	0		43	0
10	0·5	12		4
10	0·5		5	0
6	0·5	19		7
6	0·5		17	4
3	2		5	1
3	1	5		2
3	1		9	1
3	0·5	12		5
3	0·5	3		0
3	0·25	3		1
3	0·25		3	0
3	0	4		2
3	0		2	0
1·5	0·25	6		0
0	2	3		2
0	2	3		0
0	0·5		4	1

TABLE 3. EFFECTS OF ORALLY ADMINISTERED CPA AND DCBN ON SPONTANEOUS MAMMARY CANCERS OF C3H MICE

CPA (g/kg ration)	DCBN (g/kg ration)	Mice	Trans. regres.	Trans. cures	Perm. cures
0	0	54	0	0	0
3	0·5	17	6	1	0
6	0·5	17	10	0	0

Effect of orally administered CPA and DCBN on C3H spontaneous cancers

The data of Table 3 summarize the effects of CPA and DCBN when fed to C3H mice bearing small, spontaneous mammary cancers. No permanent cures were observed, but in one mouse a transient cure occurred. This one case seemed significant because in this strain of mouse the cancers never failed to grow steadily, and spontaneous cures were never observed. Furthermore, the compounds brought about

transient regressions of the cancers in many of the mice during the first weeks of the experiment. This indicated that these compounds are active but not active enough to succeed in this strain. Fig. 2 shows the average growth rate of the cancers in six treated mice, as compared with that in six untreated controls. The transient regressions (the dip in the curve of Fig. 2) were not just the result of feeding some noxious compound that may have made the mice sick and thus unable to support the normal growth of their cancers. A very large experience with many kinds of chemical substances fed to C3H mice during the past 14 years has shown us that the tumors continue to grow whether the host is well or ill. Furthermore, the mice fed CPA and DCBN were not ill, insofar as could be detected; they grew and reproduced normally. The results shown in Fig. 2 were confirmed in two additional experiments.

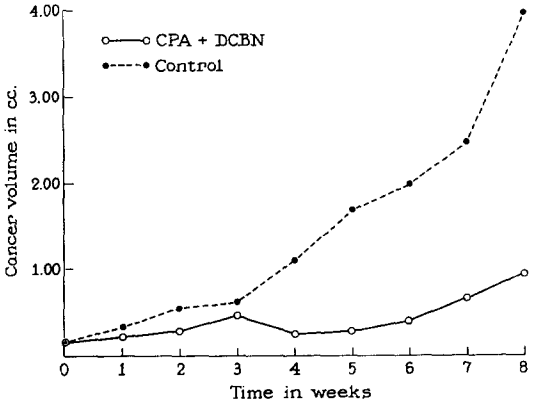

FIG. 2. Average growth rates of spontaneous mammary cancers of C3H mice fed CPA + DCBN or control rations. Average was obtained from 6 mice in each group. The drug ration contained CPA at 6 g/kg and DCBN at 0·5 g/kg.

Effect of DMBD on SPFS spontaneous mammary cancers

Table 4 shows the results of feeding or injecting DMDB into SPFS mice bearing spontaneous mammary cancers. There was some curative action, but it was less than with CPA plus DCBN. The addition of CPA (3 g/kg ration) to DMDB (5 g/kg) did not increase the percentage of cures.

Effect of DMDB on spontaneous C3H cancers

C3H mice bearing small spontaneous mammary cancers were treated with DMDB in the manner described for the SPFS mice. Both oral and intraperitoneal routes were studied. The results summarized in Table 4 show that this compound caused transient regressions but no permanent cures. The indication was that it is probably a more potent inhibitor of tumor growth in C3H than in SPFS cancers but is not active enough to cure significant numbers of either strain.

Lack of toxicity to the host animals

When given by the oral route CPA, DCBN, or DMDB did not exhibit significant toxicity, either for normal mice or for the cancerous ones. Except for those getting 10 g of CPA plus 0·5 g of DCBN per kg of ration, all animals ate the rations readily and suffered no loss of body weight or other signs of illness from the compounds.

Even those animals receiving 10 g of CPA plus 0·5 g of DCBN learned eventually to eat the ration, and then showed no signs of toxic effects; their lack of toxicity was indicated further by their ability to bear normal litters. The achievement of selective toxicity, such that the drugs would harm the cancers without at the same time harming the host animals, has been the object of the present approach to the chemotherapy of this disease, as outlined in the earlier papers.

TABLE 4. EFFECTS OF FED OR INJECTED DMDB ON THE SPONTANEOUS MAMMARY CANCERS OF SPFS AND C3H MICE

DMDB p.o. (g/kg ration)	DMDB i.p. (mg/mouse/day)	Mice	Trans. regres.	Trans. cures	Perm. cures
		SPFS mice			
0	0	80	13	2	6
5	0	7	2	0	1
0	3	16	3	0	2
		C3H mice			
0	0	54	0	0	0
5	0	19	6	0	0
0	2·5	31	16	1	0

TABLE 5. EFFECTS OF RELATED DRUGS ON SPONTANEOUS MAMMARY CANCERS OF MICE

Drug*	p.o. (g/kg ration)	i.p. (g/mouse/day)	SPFS mice	C3H mice	Trans. regres.	Trans. cures	Perm. cures
DCDB	0	2·5	0	11	5	0	0
DCDB + CPA	2 + 3	0	0	12	7	4	0
DCDB + CPA	2 + 3	0	6	0	1	0	1
DCBA	0	1	9	0	3	0	2
DCBA + CPA	1 + 3	0	6	0	1	2	0
DCBS	0	4	0	8	2	0	0
DCBS + CPA	2 + 6	4	0	6	3	0	0
DCBS + CPA	2 + 6	4	7	0	2	0	2
DCBT	0	5	12	0	1	0	0
DCBT	0	5	0	12	1	0	0
DMBA	0	2·5	0	6	1	0	0
DMBA	0	2·5	6	0	3	0	0
DMBS	0	5	7	0	5	2	0

* DCDB was 1,2-dichloro-4,5-*bis*(benzenesulfonamido)benzene; DCBA was 1,2-dichloro-4-benzenesulfonamido-5-aminobenzene; DCBS was 1,2-dichloro-4-benzenesulfonamido-5-succinamidobenzene; DCBT was 1,2-dichloro-4-benzenesulfonamido-5-phthalimidobenzene; DMBA was 1,2-dimethyl-4-benzenesulfonamido-5-aminobenzene; DMBS was 1,2-dimethyl-4-benzenesulfonamido-5-succinamidobenzene.

Although the drugs were harmless when fed, they did have some toxicity when injected in large amounts. Levels double those reported in the tables occasionally caused death of the animals; however, the amounts used in the therapeutic experiments caused no loss of body weight or other toxic manifestations detectable by gross examination.

Effects of congeners of the active drugs on spontaneous cancers

Several congeners of DCBN and DMDB were tested in the ways just described. The results are summarized in Table 5. Although the numbers of animals employed with

each compound were too small to make possible an accurate assessment of the potency relative to the parent substances, the results showed clearly that none was markedly more active than the parent compounds. They did not cure all the mice. In fact, the suggestion was that most of them were less active than the parent compounds, or not active at all. The scarcity of mice with spontaneous mammary cancers made any further trials with these less potent compounds unjustifiable.

Failure of known oncolytic agents to cure spontaneous mammary cancers

Several chemical substances are known which have shown the ability to reduce the growth rate, or actually to cure, certain transplanted cancers of mice. Some of these have been tested to determine whether they would cure permanently the spontaneous cancers of SPFS and C3H mice. In every instance the compounds were administered daily by the intraperitoneal route, and the dose used was of maximal tolerance. Aminopterin (1 μg/mouse/day), 5-fluorouracil (0·3 mg/mouse/day), 5-fluorodeoxycytidine (1·0 or 1·3 mg/mouse/day), and 5-fluorodeoxyuridine (1 mg/mouse/day) each failed to cure any mice. The experience with spontaneous cancers of mice thus paralleled the results which others have found in spontaneous cancers of human beings, and did not coincide with those found with transplanted cancers of mice.

DISCUSSION

The data presented suggest that a mixture of CPA and DCBN brought about permanent cures of spontaneous mammary cancers of some mice of the SPFS strain. This mixture also caused some regressions of the spontaneous mammary cancers of C3H mice but did not permanently eradicate them. DMDB was less potent but did produce some curative effects. In the case of the SPFS cancers, the larger the tumor when treatment was begun, the more difficult it was to bring about a cure.

The fact that some of the untreated SPFS tumors exhibited spontaneous cure was disturbing and raised the question as to whether there really was any significant effect of the compounds at all. The fact that the compounds never cured all the cancers argued in favor of the idea that they really were not curative. However, opposed to such a point of view are the following considerations: (a) In each experiment with CPA plus DCBN, the number of cures was always greater in the treated than in the control groups. (b) Inactive compounds were readily distinguishable from the active ones; this would not have been the case if all compounds, including CPA plus DCBN, had been inactive. (c) With C3H cancers, in which the problem of spontaneous regressions never arose, the inhibitory action of CPA plus DCBN was still detectable; permanent cures of C3H cancers were not produced, but transient regressions and a transient cure were.

The ability of CPA plus DCBN to cause permanent cure of the spontaneous mammary cancers of SPFS mice represents the first case of the cure of such cancers by any chemical agent. Many other compounds, which have proved to be inhibitory or even curative of certain transplanted tumours, failed to affect these spontaneous ones, just as they have failed in spontaneous cancers of human beings. Nevertheless, the activity of CPA plus DCBN was not great enough to bring about cure of all cancers in a group of mice. The fact that as the dose was raised a plateau of response was reached, such that further increases in dose did not bring about any greater percentage of cures, may be interpreted in one of two ways. Either about half the mice bore cancers of a

different kind which were not susceptible at all to the compounds, or some property of these compounds, such as their insolubility in water at neutral pH, restricted their biological activity. Perhaps it will be possible to develop more potent congeners of these drugs that will cure all the mice with a reasonable dose.

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