Studies on the *in Vitro* Antitumor Activity of Fatty Acids
I. 10-Hydroxy-2-decenoic Acid from Royal Jelly*

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SUMMARY

Whole royal jelly, when premixed with tumor cells before inoculation, has been shown to inhibit completely the development of transplantable AKR leukemia and of three lines of mouse ascitic tumors. Fractionation studies have established that all, or nearly all, of the *in vitro* antitumor activity of whole royal jelly can be accounted for in the major component of the ether-soluble fatty acid fraction, 10-hydroxy-2-decenoic acid. Concentration studies have shown that 1.5 mg. of 10-hydroxy-2-decenoic acid or 40.0 mg. whole royal jelly per ml. of cell suspension completely prevented tumor formation with all four types of tumors. The *in vitro* antitumor activity has been shown to be demonstrable only at low pH values. No activity with either compound could be shown above pH 5.6. Time studies have shown that the reaction between the tumor cells and 10-hydroxy-2-decenoic acid was completed within 6 minutes. Some variations in susceptibility to royal jelly or 10-hydroxy-2-decenoic acid have been demonstrated within the four test tumors.

Royal jelly is a thick milky material secreted by the pharyngeal glands of young worker bees which, when fed as the sole nutrient to larvae, causes the development of sexually mature queen bees. The unusual nature of this material has prompted many investigations into its pharmacological properties (7), but no direct evidence for specific activity has yet been obtained. As part of a long-term study on the chemistry and biological activity of royal jelly (15), an investigation of its possible antitumor properties has been carried out (16). It is the purpose of the present communication to report that admixture of royal jelly with tumor cells *before* inoculation completely suppressed the development of a transplantable mouse leukemia and the formation of ascitic tumors in mice. Fractionation studies established that this *in vitro* antitumor activity was associated with the main fatty acid component of royal jelly, 10-hydroxy-2-decenoic acid. The pH of the reaction mixture was shown to be a determining factor in promoting the antitumor activity *in vitro*.

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MATERIALS AND METHODS

Mouse leukemia studies.—Antileukemia studies were carried out by an early method of Burchenal and associates (3), using female mice, 5–6 weeks of age, of the AKR strain.[1] Cells were suspended in Gey's balanced salt solution (5) by mincing the spleen with a ground glass homogenizer. The suspension was then filtered by gentle suction through cotton and diluted to the approximate cell concentration required. The first transfer was made from a mouse dying from spontaneous leukemia, and successive transfers were made as the mice showed signs of dying from leukemia. To these cell suspensions were added whole royal jelly or various fractions of royal jelly. In pH experiments, the cell suspensions in Gey's solution were adjusted to acid ranges with 0.1 M citric or phosphoric acid. The pH of each mixture was measured with a Beckman pH meter. The mixture was then shaken and injected subcutaneously into the right side at a dose of 0.2 ml/mouse. Each mouse received 2–5 million tumor cells, as measured by

[1] The AKR and C3H mice used in these experiments were purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The Connaught mice were purchased from the Connaught Medical Research Laboratories, University of Toronto.

direct count in a hemocytometer. The number of cells administered per mouse was constant within any one experiment. Injections were normally completed within 2 minutes of preparing the test mixtures. Control mice received either tumor cells alone or royal jelly alone at the experimental levels.

The criterion used in these experiments was survival: the mice either developed leukemia or were protected. Each dead mouse was examined grossly; sections were then prepared from the liver and spleen to determine the extent of leukemia infiltration. First transfers from mice with spontaneous leukemia caused death in approximately 50 days, but successive transfers caused death more rapidly, in some cases within 14 days. The experiments were considered terminated at the end of 90 days in order to distinguish between transplanted leukemia and spontaneous leukemia. In most cases the mice actually lived more than 150 days.

Ascitic tumor studies.—*In vitro* antitumor studies were conducted in three lines of ascitic tumors: the 6C3HED lymphosarcoma, the TA3 mammary carcinoma, and the Ehrlich carcinoma. Initially, these tumors were carried in the tumor-specific mouse strains, but in later experiments all three tumors were carried in the nonspecific Connaught mouse line, following the freezing procedure of Morgan, Guerin, and Morton (9). An exception to this procedure was found desirable with the 6C3HED lymphosarcoma. Routine serial passage was carried out in C3H mice, but the experiments were performed in Connaught mice. Careful comparisons showed that results with the 6C3HED cells were similar in C3H and Connaught mice. The stock tumors were carried by routine serial passage at weekly intervals. Ascitic cells were harvested by intraperitoneal puncture 7 days after inoculation. The cells and accompanying fluid were mixed with two volumes of deionized water to lyse any red cells present, and the mixture was centrifuged at 2000 r.p.m. for 3 minutes. The supernatant fluid was decanted and discarded, and the packed tumor cells were resuspended in Hanks' balanced salt solution (6) to the desired cell count, as measured by a hemocytometer. This suspension was mixed with appropriate amounts of whole royal jelly or fraction of royal jelly, and the pH was determined with a Beckman pH meter. The reaction mixture was then administered intraperitoneally to groups of at least ten mice in 0.5-ml. doses. Each mouse normally received 5–8 million tumor cells. Inoculation of the test animals was completed within 30 minutes of preparing the reaction mixtures. Male mice, 20–22 gm. in weight, were used throughout these studies.

As in the antileukemia experiments, the criterion was survival: control mice died from ascitic tumors in less than 14 days, while mice receiving appropriate mixtures of cells and royal jelly failed to develop tumors. One hundred per cent death of the control mice in approximately 14 days was obtained consistently with all three ascitic tumors. Mice protected by royal jelly were kept under observation for 90 days from the start of the experiment. This period was found necessary to distinguish between compounds which protected the mice completely and those which afforded only partial protection by slowing the rate of tumor development. At the end of the 90-day period, the experiments were terminated, and random mice were autopsied to confirm the absence of tumors. In one case, protected mice were kept under observation for 15 months and showed no tumors at the end of this period.

Royal jelly.—The royal jelly used in these studies was collected at the Department of Apiculture of the Ontario Agricultural College. To supply the amounts required, special methods for large-scale production were devised (14). The antitumor activity *in vitro* was found to be approximately equivalent in lots produced over a 3-year period. The royal jelly was stored in a deep freeze immediately after production, and small portions were thawed shortly before use. Fractionation of fresh royal jelly was carried out by the procedures of Abbott,[2] whereas lyophilized royal jelly was fractionated by the methods of Townsend and Lucas (15).

RESULTS

In vitro *antitumor activity of whole royal jelly.*—Initial experiments indicated that admixture of whole royal jelly with tumor cells prior to inoculation completely prevented the development of transplanted mouse leukemia or ascitic tumors. To determine the minimal effective concentration, graded amounts of royal jelly were mixed with tumor cells and aliquots of the mixtures inoculated into groups of mice. Typical data from these experiments are presented in Tables 1 and 2.

It is evident (Table 1) that concentrations of whole royal jelly equal to or greater than 30.0 mg/ml of cell suspension have completely prevented development of the transplantable leukemia. In the case of the 6C3HED ascitic tumor (Table 2), concentrations of whole royal jelly greater than 40.0 mg/ml of cell suspension have

[2] O. D. Abbott. Royal Jelly—unpublished data, College of Agriculture, University of Florida, Gainesville, Florida.

completely prevented development of the tumor. Half this concentration has afforded partial protection, while smaller quantities were found to have no demonstrable effect. Similar studies with the Ehrlich and TA3 tumors showed that this same concentration (40.0 mg/ml of cell suspension) was completely protective in both cases.

Isolation of active component of royal jelly.—With the *in vitro* antitumor activity of whole royal jelly established, attention was directed toward isolating the active component. Royal jelly was fractionated as described previously (15), and the activity of each fraction was tested against 6C3HED cells at concentrations equal to, above, and below the level normally present in whole royal jelly. The results of these experiments are summarized in Chart 1. It is evident that the *in vitro* antitumor activity of the whole royal jelly was contained almost entirely in the ether-soluble acid fraction.

TABLE 1

EFFECT OF GRADED LEVELS OF WHOLE ROYAL JELLY WHEN MIXED WITH LEUKEMIC CELLS PRIOR TO INOCULATION INTO MICE*

Royal jelly (mg/ml cell suspension)	Average survival (days)		No. survivors at 90 days
	Test mixture	Cell control	
7.5	23	21	0/7
15.0	21	21	0/7
30.0	∞†	21	7/7
45.0	∞	48	5/5
60.0	∞	48	5/5
80.0	∞	25	19/19

* Each mouse received 2–5 million leukemic cells either alone (cell control) or mixed with royal jelly (test mixture).

† Represents survival beyond the 90-day test period.

Additional experiments were carried out in which royal jelly was lyophilized and the dry powder sterilized by treatment with ethylene oxide. This material, after reconstitution in deionized water, proved fully as active as the untreated material.

In vitro *antitumor activity of 10-hydroxy-2-decenoic acid.*—The major component of the ether-soluble fatty acid fraction of royal jelly was originally isolated by Townsend and Lucas (15) and was later identified by Butenandt and Rembold (4) as 10-hydroxy-2-decenoic acid, while confirmation of the structure has been reported recently (1). Accordingly, considerable quantities of this compound were isolated from royal jelly,[3] and the activity was tested against ascitic tumors and transplantable leukemia. The results of these experiments are summarized in Table 3.

It is apparent (Table 3) that 1.5 mg. of 10-hydroxy-2-decenoic acid per ml. of cell suspension

TABLE 2

EFFECT OF GRADED LEVELS OF WHOLE ROYAL JELLY WHEN MIXED WITH 6C3HED LYMPHOSARCOMA CELLS PRIOR TO INOCULATION INTO MICE*

Royal jelly (mg/ml cell suspension)	Average survival (days)		No. survivors at 90 days
	Test mixture	Cell control	
20	19†	13	4/9
40	∞‡	13	10/10
60	∞	13	8/8
80	∞	13	10/10
100	∞	12	47/47

* Each mouse received 5–8 million tumor cells either alone (cell control) or mixed with royal jelly (test mixture).

† Calculated from times of death of five out of nine mice which failed to survive.

‡ Represents survival beyond the 90-day test period.

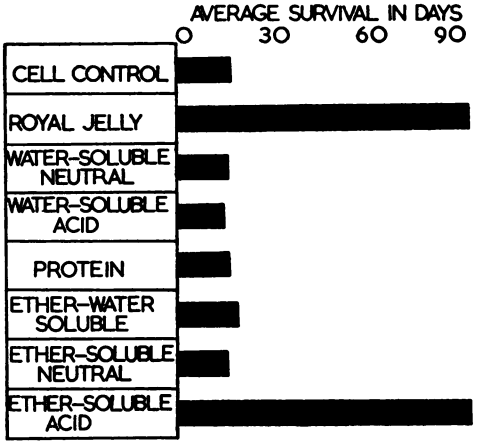

CHART 1.—*In vitro* antitumor activity of whole royal jelly and its component fractions when mixed with 6C3HED lymphosarcoma cells prior to inoculation into mice. Results expressed as average survival time in days of groups of ten to 24 mice in each test mixture. All fractions were tested at concentrations equivalent to those occurring in fresh whole royal jelly.

[3] The 10-hydroxy-2-decenoic acid used in these experiments was isolated and purified by Dr. W. H. Brown, of the Department of Chemistry, Ontario Agricultural College, whose cooperation is gratefully acknowledged.

has completely prevented the development of transplantable leukemia in AKR mice. With the 6C3HED ascitic tumor, a level of 0.6 mg/ml of cell suspension showed slight activity, while 1.0 mg/ml conferred complete protection. Similar results were obtained with the Ehrlich carcinoma and the TA3 mammary carcinoma. Since whole royal jelly contains approximately 3 per cent of 10-hydroxy-2-decenoic acid, on a wet weight basis (15), the activity of this component accounts almost completely for the activity of the whole royal jelly.

TABLE 3

EFFECT OF GRADED LEVELS OF 10-HYDROXY-DECENOIC ACID WHEN MIXED WITH LEUKEMIC CELLS OR 6C3HED LYMPHOSARCOMA CELLS PRIOR TO INOCULATION INTO MICE*

10-Hydroxy-decenoic acid	Average survival (days)		No. survivors at 90 days
(mg/ml cell suspension)	Test mixture	Cell control	
	Leukemic cells:		
0.5	45	54	0/5
1.0	69	54	0/5
1.5	∞†	54	5/5
2.0	∞	54	20/20
	6C3HED cells:		
0.6	23‡	12	1/10
1.0	∞	12	10/10
2.0	∞	13	15/15

* Each mouse received 2–5 million leukemic cells or 5–8 million lymphosarcoma cells either alone (cell control) or mixed with 10-hydroxy-decenoic acid (test mixture).

† Represents survival beyond the 90-day test period.

‡ Calculated from the times of death of nine out of ten mice which failed to survive.

pH Dependency of antitumor activity in vitro.—The tumor cell suspensions used in the present studies were prepared in either Gey's or Hanks' balanced salt solutions (5, 6), which are buffered with phosphate and bicarbonate to pH 7.2. The addition of royal jelly or 10-hydroxy-2-decenoic acid caused a sharp drop in the pH of the reaction mixtures, as shown by the yellow color of the phenol red indicator. Accordingly, it became of interest to determine the relationship between pH and biological activity. A level of 10-hydroxy-2-decenoic acid previously shown to be effective against transplantable leukemia and 6C3HED lymphosarcoma cells was mixed with the tumor cell suspensions, and aliquots of the mixtures were adjusted to different pH values with either 0.1 N HCl or 0.1 N NaOH. Cell controls, without 10-hydroxy-2-decenoic acid, were also brought to the same pH values with either 0.1 N HCl or 0.1 N NaOH. Groups of mice were then given inoculations of portions of the mixtures in the usual manner, and the survival times of the mice were recorded. The results of these experiments are summarized in Tables 4 and 5.

It is evident (Tables 4 and 5) that the protective effect of 10-hydroxy-2-decenoic acid was exerted

TABLE 4

EFFECT OF PH ON THE ACTIVITY OF 10-HYDROXYDECENOIC ACID AGAINST TRANSPLANTABLE LEUKEMIC CELLS*

pH	Average survival (days)		No. survivors at 90 days
	Test	Control	
4.2	∞†	24	10/10
4.7	∞	24	10/10
5.6	∞	24	10/10
6.1	21	22	0/6
6.5	31	24	0/10
7.3	23	24	0/10

* All reaction mixtures contained 2.0 mg. 10-hydroxydecenoic acid per ml. cell suspension. Each mouse received subcutaneously 0.2 ml. of mixture containing 2–5 million leukemic cells.

† Represents survival beyond the 90-day test period.

TABLE 5

EFFECT OF PH ON THE ACTIVITY OF 10-HYDROXYDECENOIC ACID AGAINST 6C3HED LYMPHOSARCOMA CELLS*

pH	Average survival (days)		No. survivors at 90 days
	Test	Control	
3.6	∞†	16	10/10
4.0	∞	16	9/9
4.5	∞	12	10/10
5.2	24	8	0/15
5.9	11	12	0/10
6.9‡	17	13	0/10

* All reaction mixtures contained 1.0 mg. 10-hydroxydecenoic acid per ml. of cell suspension. Each mouse received intraperitoneally 0.5 ml. of mixture containing 5–8 million tumor cells.

† Represents survival beyond the 90-day test period.

‡ At this pH, the concentration of 10-hydroxydecenoic acid was doubled to detect possible small effects.

only at low pH values. With transplantable leukemia cells, no protection was afforded at pH values above 5.6. With the 6C3HED lymphosarcoma cells, the protective effect was exerted at pH values of 4.5 and lower. At pH 5.2, some retardation of the rate of tumor formation was observed, but none of the test mice survived. At pH values higher than 5.2, no protective effect whatever was obtained. Even double the normally effective concentration of 10-hydroxy-2-decenoic acid was completely ineffective at neutrality. Similar experiments with whole royal jelly showed that this material, too, was active only at pH values below 5.0. The pH relationships with the Ehrlich and TA3 ascitic tumors were found to be identical with those of the 6C3HED lymphosarcoma.

In all cases, control studies were performed in which cell suspensions without 10-hydroxy-2-decenoic acid were adjusted to pH values corresponding to those of the test mixtures. These cell suspensions, at pH values ranging from 6.9 to 3.6, invariably caused death of inoculated mice within the normal 14-day period. These control experiments established that a low pH, by itself, did not interfere with the tumor-inducing capacity of the cell suspensions. The protective effect of the test mixtures, accordingly, could not be attributed to cell damage under the acid conditions of the test but must be related to an activity of 10-hydroxy-2-decenoic acid itself.

Time relationships of antitumor activity in vitro. —The early experiments were carried out with small numbers of mice and were completed within 10 minutes of mixing the tumor cells and royal jelly. With more complex experiments involving greater numbers of mice, the time intervals between preparing the test mixtures and completing the inoculations became progressively longer. It became necessary, accordingly, to determine the time relationships of the antitumor activity *in vitro*. To this end, a reaction mixture was prepared, containing 1.0 mg. of 10-hydroxy-2-decenoic acid per ml. of 6C3HED cell suspension. Three minutes after preparation of the mixture, an aliquot was removed and immediately centrifuged for 3 minutes at 2000 r.p.m. The packed cells were washed twice in Hanks' solution at pH 7.2, resuspended in fresh Hanks' solution to the original cell count, and injected into a group of ten mice. To the supernatant fluid removed from the packed cells were added fresh untreated 6C3HED cells to the original cell count, and the mixture was injected into mice. The results of this experiment, with the appropriate controls, are shown in Table 6.

It can be seen (Table 6) that the 6C3HED cells exposed to 10-hydroxy-2-decenoic for 3–6 minutes were unable to form tumors on subsequent inoculation into mice. The supernatant fluid from these cells showed only slight activity when mixed with fresh untreated 6C3HED cells. It appeared, therefore, that the reaction between the tumor cells and the 10-hydroxy-2-decenoic acid was essentially complete within 3–6 minutes and that the greater part of the 10-hydroxy-2-decenoic acid had disappeared from solution within that time. Three repetitions of this experiment yielded identical results.

In additional experiments, the reaction mixture was held at room temperature for 60 minutes, aliquots were then removed and separated as before into packed cells and supernatant fluid for inoculation into mice. The results of these experiments were in complete agreement with the previous ones and established that the reaction between the tumor cells and 10-hydroxy-2-decenoic was as complete within 6 minutes as in 60 minutes. Further experiments were carried out in which the pH of the reaction mixture was raised from 4.2 to 7.3 with 0.1 N NaOH within 1 minute of mixing the tumor cells and the fatty acid. This mixture was found to be completely ineffective in preventing tumor formation on subsequent inoculation of mice. From these results, it appeared that a time interval of from 1 to 6 minutes is required to complete the reaction between the 6C3HED tumor cells and 10-hydroxy-2-decenoic acid.

TABLE 6

ACTIVITY OF VARIOUS COMPONENTS OF TEST SYSTEM WHEN SEPARATED 6 MINUTES AFTER ADMIXTURE OF 6C3HED CELLS AND 10-HYDROXYDECENOIC ACID

Treatment	Average survival (days)	No. survivors at 90 days
Cell control (untreated 6C3HED cells)	12	0/10
Reaction mixture (6C3HED cells plus 1.0 mg. 10-hydroxydecenoic acid per ml. cell suspension)	∞ *	9/9
Aliquot removed 3 minutes after mixing, centrifuged 3 minutes. Packed cells washed twice in Hanks' solution, pH 7.2, resuspended in Hanks' saline, and injected into mice.	∞	10/10
Supernatant from previous step, filtered, untreated 6C3HED cells added, and injected into mice.	26	0/10

* Represents survival beyond the 90-day test period.

Variation in tumor susceptibility.—The marked effectiveness of royal jelly and 10-hydroxy-2-decenoic acid against the 6C3HED lymphosarcoma made it of interest to determine whether differences in susceptibility existed between the several tumors available for study. Accordingly, graded levels of 10-hydroxy-2-decenoic acid were tested against the various tumors, with the results summarized in Table 7.

It is evident (Table 7) that slightly lower concentrations of 10-hydroxy-2-decenoic acid are required to inhibit the transplantable leukemia and the lymphosarcoma than in the case of the other two tumors. Similar experiments with whole royal jelly showed that a concentration of 40.0 mg/ml cell suspension was completely protective with all four tumors, but that the effectiveness of lower concentrations differed, depending upon the tumor used. In general, the transplantable leukemia and the 6C3HED cells were most susceptible, followed by the Ehrlich carcinoma, whereas the TA3 mammary carcinoma cells were somewhat more resistant.

TABLE 7

DIFFERENCES IN SUSCEPTIBILITY OF EXPERIMENTAL TUMORS TO 10-HYDROXYDECENOIC ACID*

Tumor	10-Hydroxydecenoic acid (mg/ml)	Average survival (days)		No. survivors at 90 days
		Test	Control	
Transplantable leukemia (AKR)	1.0	69	54	0/5
	1.5	∞†	54	5/5
6C3HED	0.6	23	12	1/10
	1.0	∞	12	10/10
Ehrlich	1.0	25	11	2/10
	2.0	∞	11	10/10
TA3	1.0	32	15	2/10
	2.0	∞	15	10/10

* Tumor cells mixed with specified concentrations of 10-hydroxydecenoic acid at pH 4.5, and aliquots containing 5–8 million tumor cells inoculated into mice.

† Represents survival beyond the 90-day test period.

DISCUSSION

The role of royal jelly in the development of the queen bee has stimulated many investigations into its possible therapeutic use in a variety of human diseases, including leukemia (17). In most instances, such reports have been incomplete and difficult to assess. The present results appear to offer the first unequivocal demonstration of an *in vitro* antitumor activity in royal jelly. These results have been confirmed independently in two different laboratories over a 3-year period, in experiments involving several thousand mice and four different experimental tumors.

The demonstration that nearly all, if not all, of the *in vitro* antitumor activity of whole royal jelly can be attributed to 10-hydroxy-2-decenoic acid is of interest in view of the recent report that this compound exhibits antibiotic activity against a range of bacteria and fungi (2). The present studies have shown that this fatty acid is active against tumor cells only under acid conditions, and this suggests that only the undissociated fatty acid molecule is effective. There is no evidence at the present time that the *in vitro* antitumor activity of 10-hydroxy-2-decenoic acid bears any relationship to the morphogenic effect of royal jelly on bee larvae.

The mechanism by which 10-hydroxy-2-decenoic acid inhibits tumor formation has not yet been elucidated. Time studies have shown that the reaction between the tumor cells and the fatty acid is complete within 3–6 minutes, but is not complete after 1 minute. These studies have shown that the fatty acid disappears from solution relatively quickly in the presence of tumor cells but do not distinguish between simple adsorption on the cell surface and actual penetration within the tumor cells. It was found, however, that washing inhibited cells with buffer at neutral pH did not restore the tumor-incuding capacity of these cells, whereas raising the pH of the reaction mixture to neutrality prevented the activity of 10-hydroxy-2-decenoic acid in solution. From these observations, it appears most probable that the 10-hydroxy-2-decenoic acid either is firmly bound to the cell surface or has actually penetrated within the tumor cells. Studies with chromatography and dye exclusion methods are now in progress to investigate these possibilities.

The highly acid conditions required to demonstrate the activity of 10-hydroxy-2-decenoic acid have made it difficult to study the action of this compound on normal and malignant cells in tissue culture. The observation that the reaction between the fatty acid and the tumor cells is completed within a few minutes, however, makes possible a study of the effects of short-term exposure of tissue cultures to the active compound. These studies are now in progress and will form the basis of a subsequent communication. Tests with whole royal jelly have shown that this material is relatively nontoxic on intraperitoneal inoculation into mice. From 125 to 250 mg. per mouse was required to cause a toxic reaction, an amount 6–12 times that normally administered in the antitumor experiments. While it is generally recognized that

many substances, such as alkylating agents, are toxic to suspended neoplastic cells, these materials also exert an appreciable toxicity on the host animal. The relative nontoxicity of both royal jelly and 10-hydroxy-2-decenoic acid to the test mice is an important feature of the present experiments.

The four tumors used in these studies showed some differences in susceptibility to both whole royal jelly and purified 10-hydroxy-2-decenoic acid. Thus, the AKR transplantable leukemia and the 63CHED lymphosarcoma were relatively more susceptible than were the Ehrlich carcinoma or the TA3 mammary carcinoma. With sufficient quantities of the antitumor agents, all four tumors could be completely inhibited. It must be emphasized, however, that inhibition was obtained only of the transplantable AKR leukemia. Mice protected against this transplantable leukemia still developed spontaneous leukemia at the normal period of 10–12 months. Studies on transplantable leukemia are complicated by the possible involvement of viral agents. For this reason, each critical experiment was carried out on the three lines of ascitic tumors, as well as on the transplantable leukemia. In addition, all mice in the ascitic tumor experiments that were protected by royal jelly or 10-hydroxy-2-decenoic acid have been held under observation for a 10–12-month period beyond the 90-day experimental period. At the end of this time, random mice have been autopsied and an attempt made to demonstrate polyoma virus through inoculation of young mice with splenic extracts. Up to the present time, only negative results have been obtained.

While the present experiments have established the *in vitro* antitumor activity of royal jelly and 10-hydroxy-2-decenoic acid, they have also established two drawbacks against the therapeutic application of these findings. The first drawback is the necessity of premixing with the tumor cells before inoculation into the test mice, since both agents have so far proved ineffective in preventing tumor development if administered after the tumors have become established. The second drawback is the necessity of an acid pH to demonstrate the antitumor activity. Attempts to overcome both these objections are now in progress through tests on other fatty acids. These studies, based on 62 fatty acids of varying chain length and degree of unsaturation, have indicated that the ability to inhibit ascites tumor cells *in vitro* is possessed by a wide range of compounds. The distribution of this activity and the relationship of chemical structure to antitumor activity *in vitro* will form the basis for subsequent communications in this series.

The results presented in this communication emphasize the possible importance of fatty acids as antitumor agents. This idea is not entirely new. In a series of papers published between 1922 and 1925, Nakahara (10–13) reported the value of olive oil and of fatty acids in increasing the resistance of mice to several forms of transplantable tumors. Similar observations were reported (8) on the beneficial effect of fatty acids in retarding tumor development following application of coal tar derivatives to the skin of mice. The basic concept in these early publications is essentially similar to the present findings with royal jelly and 10-hydroxy-2-decenoic acid. It would appear, therefore, that the role of fatty acids as antitumor agents warrants intensive investigation.

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