THE EFFECT OF AVIDIN INJECTED PERITONEALLY

ON THE COURSE OF LEUKEMIA IN THE MOUSE

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THESIS

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TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Wildiers (44), in 1901, found that yeast needed a certain growth substance in addition to the nutrients then known to be required. He called this substance bios. In 1922 Fulmer and Nelson (13) demonstrated the multiple nature of bios. In 1924 Lucas (28) separated bios into two components, bios I and bios II; later that year, Miller (31) fractionated bios into three substances. Bios I was identified as meso-inositol by Eastcott (11) in 1928. In 1933 Miller (29) showed again that bios II contained at least two factors, bios II A and bios II B. Kogl (25) purified bios II B and named it biotin in 1935; and, in 1936, Miller (30) identified bios II A. In 1939 Robbins and Schmidt (37) reported a microbiological assay method for biotin. Snell, et al. (39), in 1940, devised a refined assay method and listed some particularly rich sources.

Allison, Hoover, and Burk (1), in 1933, discovered a factor necessary for the respiration of certain legume root nodule bacteria. They had extracted this factor from commercial sucrose, but suggested a wide occurrence in nature, since one species of Azotobacter synthesizes it. They stated

that their factor had not yet been purified but was not bios, and they named the factor "coenzyme R."

The discovery that large amounts of raw egg white in the diet could produce symptoms of dietary disorder was made in 1916 by Bateman (2), using dogs, cats, mice, and rabbits. Boas (4), in 1927, found that feeding dried egg white caused dermatitis and death in young rats, but that certain foods could prevent this. Boas suggested the presence in these foods of a protective organic compound which she called "factor X." In 1931 Gyorgy (15) postulated the existence of a protective factor for man--an egg-eating animal--and named his factor "vitamin H."

In 1933 Parsons and Kelly (32) showed that denaturation did not affect the toxicity of egg white, but that peptic digestion did have some effect. Later that year Parsons and Kelly (33) stated that injury due to raw egg white appeared to involve an interrelation between a positive toxicity and a relative absence of a protective factor. Findlay and Stern (12), in 1929, had suggested a deficiency rather than a toxin as the cause of "egg-white injury" (40). Salmon and Goodman (38), in 1934, stated that their data indicated a positive harmful factor in egg white, which was antagonized by the protective substances in certain foods, rather than the existence of a deficiency. In the same year, Parsons and Lease (34) suggested that the injurious effect of egg white

was not dependent on the destruction of a protective factor in other foods, but that the amount of factor in foods varied widely. Gorter (14), in 1935, concluded that certain foods contain an insoluble protein factor, the absence of which permits egg-white injury.

Partial isolation of the curative factor from liver and kidney was announced in 1936 by Lease (27). In 1937 Booher (5) succeeded in extracting vitamin H, in association with other B-complex components, from whey powder and rice polishings; and Gyorgy, et al. (21), listed three dermatoses in the rat which could be cured nutritionally by vitamin H, vitamin B, and lactoflavin, respectively. In 1939 Gyorgy, et al. (17), isolated vitamin H from liver; Birch and Gyorgy (3) investigated its physicochemical properties; and Gyorgy (16) investigated its distribution in foods previously shown to have curative effect on egg-white injury. Gyorgy (16) also stated that vitamin H appeared to be part of a compound of higher molecular weight, which was insoluble in water and in fat. The vitamin, however, could be freed in a water-soluble form by the action of yeast in the presence of toluene. Gyorgy and Rose (19) in 1940 found that vitamin H was three to five times more effective in curing egg-white injury when given parenterally rather than orally.

In 1939 West and Wilson (41) compared the effect of biotin and coenzyme R on the growth rate of yeast. In 1940

Porter and Pelczar (35) noted the similarity between biotin and vitamin H; and, in that same year, Gyorgy, et al. (18), suggested that biotin, coenzyme R, and vitamin H were identical.

In 1941 du Vigneaud, et al. (6), purified biotin (vitamin H, coenzyme R) and announced the empirical formula, $C_{11}H_{18}O_3N_2S$.

Eakin, et al. (8), in 1940, observed that amounts of biotin ordinarily sufficient were apparently unavailable to the tissues of animals with egg-white injury and suggested that egg white caused injury by making the biotin unavailable. Eakin, et al. (10), followed this with a demonstration of biotin-inactivation <u>in vitro</u> by a partially purified constituent of egg white which they suggested as the cause of egg-white injury. They named the constituent "avidalbumin." Gyorgy, et al. (20), then demonstrated <u>in vivo</u> that avidalbumin was the toxic factor of egg white and stated that egg-white injury is a biotin deficiency caused by the unavailability of biotin, since biotin forms a stable compound with avidalbumin. In 1941 Eakin, et al. (9), announced progress in purification of the injurious protein from egg white, which they renamed "avidin."

That same year West and Woglom (43), searching for a difference in the metabolism of normal and malignant cells, reported that, in every case they studied, the biotin content

of tumor tissue deviated sharply from the normal adult values in the same direction as that of the corresponding embryonic tissue. From this and other work, Laurence (26) hypothesized that raw egg white or avidin could be used therapeutically against malignancies by denying these rapidly dividing cells the biotin necessary for their growth. The patient's resultant biotin deficiency, Laurence suggested, could be controlled at any stage by administering biotin.

In 1942 du Vigneaud, et al. (7), added biotin to a ration previously shown to be protective against butter-yellow-induced hepatoma in rats. The rats showed a higher incidence of tumor formation. This lent support to Laurence's hypothesis concerning avidin as a possible therapeutic. West and Woglom (42) then induced biotin deficiency in a group of mice by feeding avidin, and planted fragments of sarcoma under the skin of these animals and a control group. All tumors grew large and were healthy. Kensler, et al. (23), in 1943, reported that feeding avidin was ineffective in treating mice with spontaneous mammary carcinoma. That same year Rhoads and Abels tested avidin as a therapeutic in humans, using one case of mammary carcinoma and one of lymphatic leukemia. Feeding these two patients 16 to 40 times the avidin necessary to combine with their dietary biotin for 30 weeks brought about no apparent improvement. However, no biotin deficiency symptoms appeared, and the urinary excretion of biotin was not reduced.

In 1944 Kaplan (22) confirmed the inability of avidin to affect the course of cancer by feeding the whites of 36 to 42 eggs daily to cancer patients who had been placed on a diet low in biotin, without effecting a cure, although there was some improvement in certain cases. Finally, in 1945, Kline, et al. (24), were unable to duplicate the original work of du Vigneaud, et al. (7), using biotin to increase the incidence of butter-yellow-induced hepatoma.

In all cases, negative results were obtained when avidin was fed to experimental animals or human patients; yet Gyorgy and Rose (19), in 1940, had demonstrated that biotin administered parenterally was more effective against egg-white injury than biotin given orally. Apparently, the stable avidin-biotin complex forms more readily and more completely in a parenteral medium. Therefore, the current work was undertaken, testing the ability of avidin to affect the course of leukemia when administered intraperitoneally.

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CHAPTER II

METHODS AND MATERIALS

Animals

When the experiment was begun, mice of the AKR strain were used. Normal and tumor-infected AKR mice were obtained from Inbred Mice Company, Houston, Texas, and Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. In this strain, 90 per cent of the mice develop lymphoid leukemia VII spontaneously between five and eight months of age. For these animals, death usually occurs at ten months of age. If tumor cells from one mouse are transferred to others, however, roughly 75 per cent of those injected will develop leukemia in about 14 days and will die in about 21 days (1).

Because only 75 per cent of the transfers are successful, mouse strain C3H/HeJ was later used. Normal and tumor-infected mice were obtained from Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This strain develops lymphosarcoma 6C3HED Gardner on transfer only. Here, 100 per cent of the transfers are successful. Death occurs in about 21 days.

Tumor Transfer

All tumor transfers were accomplished the same way, regardless of type of mouse. The donor mouse, which had been injected intramuscularly in the thigh, was killed; and the spleen, inguinal lymph nodes, and occasionally the thymus, were removed aseptically to a sterile tissue homogenizer. Because of the route of injection, there was always caseous swelling of the body wall in the area of injection. Some of this material was also taken. These tissues were then diluted in Gey solution, homogenized, and injected into the recipient mice, 0.2 ml. to each mouse, intramuscularly in the thigh.

Experimental Groups

At first the recipient mice were divided into two experimental groups. The first group, a control, received no further injections, but the individuals in the second group received on the same day 1 unit avidin (Nutritional Biochemicals Corporation) in 1.0 ml. distilled water. This second injection was given intraperitoneally in the side opposite to that previously injected with tumor suspension.

Later, the recipients were divided into three groups. The first two groups were treated as formerly, but the third group received a second injection of 10 micrograms biotin (Nutritional Biochemicals Corporation) in 1.0 ml. distilled

water, given intraperitoneally in the side opposite to that injected with tumor suspension.

Smaller experiments were also devised in which the second injection received by the experimental group was one of the following: anti-avidin guinea pig serum, anti-mouse tumor guinea pig serum, distilled water alone.

There was also a small experiment in which mice received only avidin or biotin, no tumor.

Finally, an experiment was set up in which each of twenty mice received 0.2 ml. tumor suspension and 0.5 ml. avidin. Subsequently, they received 0.5 ml. avidin every other day.

All C3H/HeJ mice infected with tumor died. Some AKR mice infected did not die. In figuring the mean average of days from injection to death, mice that did not die were disregarded, since in these cases tumor transfer may have been unsuccessful, even though some symptoms might exist. On the other hand, some C3H/HeJ mice died within seven days after injection. These, too, were disregarded, since death from tumor transfer should not occur this soon. Probably, in these cases transfer was not accomplished aseptically, and death was due to infection.

The antigenicity of avidin in the guinea pig was also investigated by testing for antibody presence in blood serum from infected animals. Complement titers (C'4 only)

were obtained according to the method of Kabat and Mayer (2). Four guinea pigs were bled; 2.0 ml. each on day 0 and day 4. C'4 was titered and the results for each pig were averaged. On days 5, 7, and 9, each pig received an injection of 2 units of avidin in 2.0 ml. distilled water. On day 19 (14 days after the first injection) the pigs were bled and C'4 was titered. On day 23 this was repeated, and the titers were averaged.

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CHAPTER III

RESULTS

Mice infected with tumor and injected with avidin repeatedly showed a longer average life span than mice infected with tumor only, regardless of strain or source. AKR mice obtained from Houston, Texas, received only tumor or tumor plus avidin. These results, summarized in Table I, show fewer deaths, as well as lengthened survival, for mice in the experimental (avidin) group.

TABLE I

EFFECT OF AVIDIN ON THE SURVIVAL OF AKR MICE OBTAINED FROM INBRED MICE COMPANY, HOUSTON, TEXAS

Type of Injection	Number of Animals	Number Killed	Percentage Killed	Mean Average Survival Time in Days
Tumor Only	25	14	56	14.92
Tumor Plus Avidin	25	8	32	31.375

AKR mice obtained from Bar Harbor, Maine, were used in a wider variety of experiments. These are summarized in Table II. Here the smallest percentage of deaths occurred

TABLE II

EFFECT OF AVIDIN ON THE SURVIVAL OF AKR MICE OBTAINED FROM ROSCOE B. JACKSON MEMORIAL LABORATORY, BAR HARBOR, MAINE

Type of Injection	Number of Animals	Number Killed	Percentage Killed	Mean Average Survival Time in Days
Tumor only	36	28	77.77	19.03
Tumor plus avidin	35	20	57.14	27.00
Tumor plus biotin	34	18	52.95	21.94
Avidin only	5	0	...	...
Biotin only	5	0	...	...
Tumor plus anti-avidin serum	3	2	66.66	10.50
Tumor plus anti-tumor serum	3	3	100.00	7.66
Tumor plus distilled water	2	1	50.00	2.00

in the biotin group, although the percentage of deaths in the avidin group was less than 5 per cent greater, and the longest average survival time was in the avidin group: 5-plus days longer than the biotin group and 8 days longer than the control group.

Single injections of avidin or biotin produced no visible effect upon the mice receiving them. The results with tumor plus "anti-avidin" guinea pig serum, anti-lymphoid

leukemia VII guinea pig serum, or distilled water indicate very little. Too few animals were used, deaths occurred too soon to be ascribable to tumor formation, and previous work (1) indicates anti-lymphoid leukemia VII guinea pig serum has a repressive effect on tumor growth.

Total results on AKR mice from both Houston and Bar Harbor receiving tumor only and tumor plus avidin are summarized in Table III.

TABLE III

EFFECT OF AVIDIN ON THE SURVIVAL OF AKR MICE OBTAINED FROM INBRED MICE COMPANY, HOUSTON, TEXAS, AND ROSCOE B. JACKSON MEMORIAL LABORATORY, BAR HARBOR, MAINE

Type of Injection	Number of Animals	Number Killed	Percentage Killed	Mean Average Survival Time in Days
Tumor only	61	42	68.86	17.66
Tumor plus avidin	60	28	46.67	28.25
Tumor plus biotin	34	18	52.95	21.94

Results with Bar Harbor AKR's receiving tumor plus biotin are repeated for comparison. Here the avidin group shows the smallest percentage of deaths and the longest survival time. But the biotin group shows a percentage of

deaths smaller and an average survival time longer than those of the control group.

Results using C3H/HeJ mice are summarized in Table IV.

TABLE IV

EFFECT OF AVIDIN ON THE SURVIVAL OF C3H/HeJ MICE OBTAINED FROM ROSCOE B. JACKSON MEMORIAL LABORATORY, BAR HARBOR, MAINE

Type of Injection	Number of Animals	Number Killed	Percentage Killed	Mean Average Survival Time in Days
Tumor only	19	13	68.42	16.38
Tumor plus avidin	15	11	73.33	17.90
Tumor plus biotin	15	11	73.33	18.63
Tumor plus distilled water	5	5	100.00	21.20
Tumor plus repeated avidin injections	20	20	100.00	17.00

Repeated injections of avidin showed no significant difference in effect on survival time from that shown by single injections of avidin. There is some indication that repeated injections may hasten death due to biotin avitaminosis; all mice in this group showed symptoms of dietary deficiency: loss of hair, scaling, and loss of weight.

Using this strain, the biotin group outlived the avidin group by almost one day. The avidin group outlived the control group by 1.5 days. But five mice injected with tumor and distilled water lived on the average of 2.6 days longer than the biotin group, or 7.8 days longer than the control group.

Total results from AKR and C3H/HeJ mice in the three major experimental groups are summarized in Table V.

TABLE V

EFFECT OF AVIDIN ON THE SURVIVAL OF AKR AND C3H/HeJ MICE

Type of Injection	Number of Animals	Number Killed	Percentage Killed	Mean Average Survival Time in Days
Tumor only	80	55	68.75	17.35
Tumor plus avidin	75	39	52.00	25.33
Tumor plus biotin	49	29	59.18	20.68

Over-all, the avidin group shows the smallest percentage of deaths and the longest average survival time. The biotin group, however, still shows fewer deaths and longer survival than the control group.

Table VI shows the average C'4 titers at serum dilutions of 1 : 9,000 and 1 : 10,000 of four guinea pigs before and

after injection with avidin. These results indicate avidin is non-antigenic, since injection with known antigenic materials has been seen to produce an increase in this component.

TABLE VI

GUINEA PIG COMPLEMENT TITERS EXPRESSED IN UNITS OF C'4 AT SERUM DILUTIONS OF 1 : 9,000 AND 1 : 10,000

Guinea Pig	Before Injection of Avidin		After Injection of Avidin	
	1 : 9,000	1 : 10,000	1 : 9,000	1 : 10,000
I	6129	6945	6822	7580
II	6363	7170	6642	7580
III	7596	8440	6431	7270
IV	6543	7145	6512	7580

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CHAPTER IV

DISCUSSION

These studies demonstrate a leukemia-repressing action of avidin administered intraperitoneally with these tumors. Presumably, this action was the result of temporary biotin deficiency which delayed the growth of the lymphoma, giving the tumor-plus-avidin group of mice an average of eight additional days from injection to death, and a 16 per cent decrease in mortality, from the control group.

The fact that the tumor-plus, biotin animals also survived longer and in larger numbers than the control group may be explained by the injection of biotin received, which contained only 10 micrograms of biotin, a necessary nutrient. This relatively small amount probably was utilized by the animal itself before the tumor was sufficiently established in the animal's body. Further, this 10 micrograms of biotin was in each case dissolved in 1.0 ml. distilled water, and it has previously been shown that animals of the C3H/HeJ strain receiving tumor plus 1.0 ml. distilled water lived longer than the control group. In fact, mean average survival times of the groups in question, which are reviewed in Table VII, are very similar.

TABLE VII

EFFECT OF BIOTIN IN DISTILLED WATER AND OF DISTILLED WATER ALONE ON THE SURVIVAL OF AKR AND C3H/HeJ MICE

Type of Injection	Number of Animals	Number Killed	Percentage Killed	Mean Average Survival Time in Days
Tumor plus biotin	49	29	59.18	20.68
Tumor plus distilled water	5	5	100.00	21.20

This effect, therefore, could be entirely due to the distilled water. Certain ions (for example, Cl^-) may be present in the distilled water source available in higher concentrations than in tap water. Several future experiments, therefore, suggest themselves: injections of avidin in saline, avidin in physiological salt solution, avidin in reconstituting fluid, and, of course, chemical analysis of the distilled water.

The group of mice receiving repeated injections of avidin showed almost one day less survival time than the group receiving only one injection of avidin. The mice in the former group received 0.5 units of avidin in 0.5 ml. distilled water every other day. The average number of injections received was eight. These mice showed symptoms

of tumor and biotin deficiency, and death was probably due to the combined effect of these conditions.

The guinea pig complement titers seem to indicate that avidin is non-antigenic. If this is true, parenteral injection therapy should be safe, if dosage is controlled carefully.

CHAPTER V

SUMMARY

Avidin, when injected intraperitoneally into mice which have received leukemic cell injections on the same day, acts as a therapeutic against the formation of some lymphomas. This is in contradiction to previous work by West and Woglom (4), Kensler, et al. (2), Rhoads and Abels (3), and Kaplan (1), the difference lying in route of administration and in the tumor under study. Avidin has little effect, if any, on the course of cancer when given orally, as in the work cited.

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