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LOYOLA UNIVERSITY

A STUDY ON THE EFFECT OF A SPINDLE-CELL
SARCOMA ON THE LECITHINASE ACTIVITY OF THE ORGANS
OF THE ALBINO RAT.

A THESIS

SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

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IN

THE DEPARTMENT OF

PATHOLOGY

BY

ALFRED H. BENSON

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INTRODUCTION

During the past few years several papers have appeared in the literature, dealing with the newly demonstrated enzyme which is capable of hydrolyzing lecithin. Kay (1926) was the first to prove the presence of this enzyme in the kidney. King (1931) put the preliminary findings of Kay on a firmer basis by demonstrating the presence of this enzyme in a number of animal tissues.

As it had been found by McJunkin and Henry (1935) that lecithin inhibits cell growth, it was decided to study further the physiological and pathological function of this enzyme by determining whether or not the presence of a tumor would effect the concentration of lecithinase in the various organs of the albino rat, and to determine the concentration of this enzyme in the tumor tissue.

As far as could be ascertained by an examination of the literature, no comparable work has been attempted.

METHODS AND MATERIALS

The method and material used for analysis of tissues for lecithinase in this study is essentially that described by King (1931). A few modifications were introduced and the values of these changes will be indicated.

The sample of tissue to be analyzed is first freed of any adherent tissue and then weighed accurately, ground up with sand and transferred with twenty times its weight of water to an Erlenmeyer flask. King used chloroform saturated water for the dilution of the tissue with a drop of toluol, and autolyzed at room temperature for 48 hours and then filtered through cotton wool. It was found in this laboratory that uniform results could not be obtained in this manner because the autolysate was not clear or uniform throughout. Instead of using chloroform saturated water for the dilution, sterile distilled water was used, and a cotton pleget saturated with chloroform was wired to the under side of the cork. A drop of toluol was added to the flask. Instead of autolyzing at room temperature, the material was autolyzed in the incubator at 37.5° for 48 hours and then centrifuged. The supernatant fluid was then drawn off and mixed well, the two cc. sample of extract being withdrawn from this mixed fluid. The supernatant fluid was mixed because several determinations demonstrated that the top strata of fluid contain a higher concentration of the enzyme than do the lower strata. This modification of King's method provided a clear autolysate that gave more uniform results.

After the extract is obtained a set of two incubation tubes is prepared for each extract. The one tube contains 5 cc. of a borate buffer solution of pH 7.5, 5 cc. of a permanent lecithin emulsion containing 0.5 mg. of phosphorus, and 2 cc. of the autolyzed tissue extract. The other tube, the control, contains only 5 cc. of buffer with 2 cc. of extract. A drop of toluol and chloroform is added to each tube to prevent bacterial growth. Another control tube containing only buffer and lecithin was run as a control in the first few experiments but as these showed a negligable amount of phosphorus this control was omitted from all subsequent experiments. The tubes were then placed in the incubator at 37.5° for 48 hours. At the end of this time hydrolysis was stopped by the addition of 25% trichloroacetic acid. This acid precipitates the lecithin and any protein material carried over in the extract. Following this precipitation, the material was removed and placed in 25 cc. volumetric flasks and analyzed for phosphorus by the method described by King (1933).

King's method, in brief, is as follows: To each of the 25 cc. volumetric flasks is added 5/6 cc. of 0.2% amino-naphthol-sulphonic acid solution, 1 2/3 cc. of 5% ammonium molybdate solution and 2 cc. of a 60% perchloric acid. The color produced is then compared in the colorimeter with of a phosphorus standard solution containing 0.1 mg. of phosphorus.

The tumor used in this study is a highly malignant rapidly growing spindle-cell sarcoma which arose spontaneously in the uterus of an albino rat in this laboratory some four years ago

and has since been successfully kept alive by subcutaneous transplantation into the groin of the rat. In these experiments, after the tumor had become well established the animal was sacrificed by the isolation of the common carotid artery and bled to death. In this manner, sufficient blood was obtained in many cases for an analysis of the lecithinase activity of the serum. The normal control animals were sacrificed in a similar manner.

A few experiments were performed where dibenzanthracene ("DBA") was added to the kidney extracts. This was accomplished by rubbing up the "DBA" in a mortar and slowly adding the fluid. The fluid was colored yellow by this procedure but much of the "DBA" soon settled to the bottom.

RESULTS

First, a series of lecithinase analyses on the liver, spleen, kidney and serum of normal albino rats was obtained. The concentrations of the enzyme found in these tissues, with the exception of the serum which he did not attempt, approximated the concentrations found by King. A compilation of these results is presented in Table I.

A series of rats were then transplanted with a groin sarcoma and when the tumors were well established they were sacrificed, and an analysis made on corresponding tissues and in addition, the tumor tissue. Results of this series appears in Table II.

The results of these two series of experiments reveal a marked decrease in the lecithinase activity of the tissues of the tumor-bearing animals as compared to the normal series, as well as a low degree of enzyme activity in the tumor tissue.

TABLE I

Normal Rat Lecithinase

Lecithinase activity expressed in gammas of free P. liberated:

Exp. No.	1	2	3	4	5	6	7	8	9	10	Average
Kidney	24	29	22	31	55	45	36	19	47	30	34
Spleen	15		18	14	19	19	14	11	10	14	15
Liver				14	4	10	7	3		8	7.7
Serum	8	10		0	8	16	6	0	4	0	4.3

TABLE II

Tumor Rat Lecithinase

Exp. No.	1	2	3	4	5	6	7	8	9	10	11	12	13	Average	% inc. over N.
Kidney	6	20	29	24	21	16	14	24	11	37	14	15	19	19	44.12
Spleen	0	11	6	12	12	6	2	0	8	0	0	14	6	6	60.00
Liver	0	16	7	0	7	10	16	0	8	5	0		5	6.2	2208
Serum		0	0	0	0	0			4	6	0			1.2	72.1
Tumor	0	0	25	2	0	1	5	6	3	6	1	11		4	

Four rats resisted all efforts to tumor transplantation. These rats were then sacrificed and their organs analyzed for lecithinase activity. The results, together with the percentage of increase over the normal series, are recorded in Table III.

The next series of experiments performed, was to determine the effect, if any, of "DBA" on the lecithinase activity of the

kidney autolysate. 10 mg. of "DBA" was added to the contents of the incubation tubes in a manner already described. A set of normal incubation tubes was also included as a control. The results are seen in Table IV.

An attempt at determining the relation of the age of the tumor to the change of concentration of lecithinase in the organs of the rat was made without any conclusive results.

TABLE III

Resistant Rat Lecithinase

Exp. No.	1	2	3	4	Average	% inc. over N.
Kidney	29	37	38	37	35	2.9
Spleen	15	18	24	19	19	26.6
Liver	13	4	6	9	8	4.0
Serum	0	11	0	7	4.5	4.6

TABLE IV

Effect of "DBA"

Exp. No.	1	2	3	4	5	6	7	8	9	10	11	12	Average	
Kidney	12	7	31	8	10	18	12	10	0	4	13	23	14	
Kidney "DBA"	25	17	23	71	8	15	9	9	0	11			19	35.7 inc. over the normal controls

DISCUSSION

Lecithin is present in every living cell. Since it has been found by McJunkin and Henry (1935) that lecithin inhibits cell growth, this protoplasmic constituent must be a factor in controlling the growth of the individual cell. All cell activity

is accomplished by chemical changes which are in turn promoted by organic catalysts or enzymes. The concentration of lecithinase in any given tissue should then be an indication of the degree of controlling action that lecithin exercised in that tissue.

Every tumor growth is in its essence an uncontrolled growth of cells. Since this is true, the basic cause of all tumor growth must be on one hand a decrease of the inhibiting factors of cell growth, or on the other hand an increase of the stimulating factors, or a combination of the two.

The results of the experiments performed indicate that there is a substantial decrease in the lecithinase activity of all of the tissues of a tumor-bearing animal as compared to the normal animal. In addition, there was found to be a very low concentration of this enzyme in the tumor tissues. An interpretation of the meaning of these experimental findings can only at best be placed on a hypothetical basis. It must be understood that the following discussion regarding the role of lecithinase in tumor formation is purely theoretical.

The low concentration of lecithinase in the tumor tissue indicates that there is a low degree of inhibition exerted by lecithin in the tumor cells thus allowing them to progress in uncontrolled or blastomatous growth. The low lecithinase activity of the liver, spleen, etc. in a tumor-bearing animal may be an expression of a mobilization of enzyme from these organs to the tumor area, in an attempt to retard its growth.

In the few tumor resistant animals that were analyzed, an increase over the normal lecithinase was found. This may be an explanation as to why they were tumor resistant. To explain the results of the "DBA" experiments is a little more difficult. In view of the fact that "DBA" is a carcinogenic agent, this substance in the light of the above theory would be expected to decrease the amount of lecithinase activity. However, the experimental results showed that "DBA" materially increased the activity of the lecithinase of kidney tissue. The fact that the "DBA" was strictly an in vitro addition may explain the discrepancy, or another theory may be ventured to explain this. Perhaps the "DBA" stimulates the lecithinase to further activity, thus necessitating an increase of the stimulating factors of growth in order to maintain a normal rate of growth. Continued action of the "DBA" may then so increase the stimulating factors that control of growth is lost and a tumor results.

CONCLUSIONS

1. Sarcomatous tissue has a low concentration of lecithinase.
2. The spleen, liver, kidney and serum of a tumor-bearing albino rat have a lower concentration of lecithinase than do the corresponding organs of a normal animal.
3. The organs of tumor resistant animals have a higher than normal concentration of lecithinase.
4. Addition of "DBA" in vitro, to a kidney extract, materially increases the lecithinase activity of that extract.

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