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A Study of the Changes in Serum Ethyl Butyrase in Experimental Malignancy of the Albino Rat

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A STUDY OF THE CHANGES IN SERUM ETHYL BUTYRASE IN EXPERIMENTAL MALIGNANCY OF THE ALBINO RAT

BY

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B. PURPOSE OF THE PROBLEM

The original intention was the study of the changes in serum ethyl butyrase in experimental malignancy of the albino rat. Later, however, the effects of the various constituents of tissues and of carcinogenic substances were studied, both in vivo and in vitro.

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C. OUTLINE

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I. METHODS AND MATERIALS

The animals used were the albino rats of the Wistar Institute strain. The blood was drawn directly from the heart using a 5 cc. syringe and a 26 gauge needle. Usually, no anesthesia was used. Injections of macerated tumor tissue were made by opening the abdominal cavity as aseptically as possible and injecting through the incision. Other materials were injected subcutaneously by the use of a syringe and trocar needle.

Ethyl butyrase determinations were made in the manner outlined by Green.[1] To 20 cc. of M/80 phosphate buffer solution of pH. of 8.0 measured in a 100 cc. Erlenmeyer flask, 0.25 cc. of phenophthalein and 0.25 cc. of ethyl butyrate were added. Then 0.5 cc. of serum was added, and the flask was incubated for two hours at 37°. The contents of the flask were titrated against N/100 NaOH until a pink tint appeared. The amount of NaOH used was the measure of ethyl butyrase activity. A control flask containing all solutions except the serum was always run.

II. EXPERIMENTAL

PART 1. IN VIVO EXPERIMENTS

In all experiments, a control animal, which was bled along with the experimental animals, was used. The average for all control animals is given in Table I.

TABLE I.

No. of animals	1st butyrase determination	2nd butyrase determination	% decrease
30	29.5	28.2	4.2

The effect of the second bleeding, 24 to 48 hours after the first was to cause a decrease of 4.2% in the ethyl butyrase content of the serum.

It was found by experiment that the maximum dose of fresh mashed tumor that could be injected intraperitoneally was 2.0 grams. Doses of 0.5 grams produced the same effect as those of 2.0 grams. In Table II are given the results of the injection of fresh tumor tissue intraperitoneally.

TABLE II.

No. of animals	Butyrase before injection	Butyrase 24-36 hrs. after injection	Butyrase 48 hrs. after injection	% decrease
27	24.1	14.6	14.1	39.4

A tumor was removed from the animal, placed in a flask with twice the weight of water and 1 cc. of toluene. This mixture was allowed to stand 4 days at 37° C. The mixture was then filtered, and the filtrate evaporated to dryness. The residue was dissolved in water to make a solution, 1 cc. of which was equivalent to 1 gram of fresh tumor. Up to 5 cc. was injected intraperitoneally. Table III gives the result of these injections.

TABLE III

No. of animals	Butyrase before injection	Butyrase 24 to 36 hrs. after injection	% decrease
4	24.5	19.4	24.9

A tumor was removed from the animal, crushed, mashed, and boiled in water for 30 minutes. The material was again crushed and mashed in a mortar and then reboiled for 30 minutes. After cooling, 0.5 to 2.0 grams of this material was injected intraperitoneally. Table IV gives the results of this procedure.

TABLE IV

No. of animals	Butyrase before injection	Butyrase 24 to 36 hrs. after injection	% decrease
8	27.7	17.9	35.4

An autolysate of dog's kidney was made in the same manner as the tumor autolysate. Quantities from 5 to 10 grams were injected intraperitoneally.

TABLE V.

No. of animals	Butyrase before injection	Butyrase 24 hrs. after injection	% decrease
7	23.1	21.2	8.3

To determine whether or not the decrease in ethyl butyrase after the injection of fresh or boiled tumor was due to the proteins of the tumor, injections of 0.5 to 2.0 grams of Witte's peptone and of proteose were made. These substances were dissolved in distilled water and then boiled. Usually 3 cc. of water were used for each gram of peptone or proteose.

TABLE VI.

No. of animals	Butyrase before injection	Butyrase 24 hrs. after injection	% decrease
14	20.4	15.1	26.8

A mixture of 100 mgm. of lecithin, 100 mgm. of peptone, and 25 to 100 mgm. of cholestrol was suspended in distilled water. This suspension was injected intraperitoneally.

TABLE VII.

No. of animals	Butyrase before injection	Butyrase 24 hrs. after injection	% decrease
11	19.8	14.1	28.8

Cholesterol in amounts of 50 to 200 mgm. was suspended in lecithin and water, olive oil, and water. Equal quantities of lecithin and 2 to 5 cc. of water were used. When water alone or olive oil were employed, 2 to 5 cc. were used. The lecithin was used only as a vehicle. These suspensions were always boiled and later cooled before using.

TABLE VIII.

No. of animals	Butyrase before injection	Butyrase 24 hrs. after injection	% decrease
14	22.8	14.7	35.5

Two rats were injected with 5 and 50 mgm. of dibenzantracene respectively. The ethyl butyrase decrease after 24 hours was less than 10%.

From 50 to 70 mgm. of ergosterol was dissolved in 5 cc. of olive oil and injected intraperitoneally.

TABLE IX.

No. of animals	Butyrase before injection	Butyrase 24 hrs. after injection	% decrease
10	16.0	13.8	13.7

PART 2. IN VITRO EXPERIMENTS

To determine whether cholesterol and dibenzanthracene were inhibitors of the ethyl butyrase found in the blood serum, a number of in vitro experiments were made. The serum was obtained from normal, healthy rats by withdrawing the blood, centrifuging, and pooling the serum. The control flask contained 0.5 cc. of the pooled serum. To the other flasks were added various amounts of cholesterol or dibenzantracene. The flasks were incubated at 37° for 2 and 24 hours.

Heating the serum for 30 minutes at 56° resulted in total inhibition of enzyme. The flask so treated showed only the acidity due to the ethyl butyrase.

The results of these experiments are shown in Table X.

2 HR. INCUBATION PERIOD

Normal	Dibenzanthracene			Choesterol		
	5-10 mgm.	25 to 100 mgm.	over 100 mgm.	5-10 mgm.	25 to 100 mgm.	over 100 mgm.
26.2		26.4				
32.2		32.2				
21.4	22.0					
22.2					20.4	
19.0	18.8	21.4				
11.8				11.0	10.4	
24.8						26.6

24 HR. INCUBATION PERIOD

63.2		46.0				
66.2					51.8	
58.0	48.6					
56.6					49.2	
59.8	49.2					
45.2	37.4	32.8	35.6			
	33.2					
59.8				49.8		44.8

In all of the experiments at various times the animals were killed and autopsied. At no time was there any sign of peritoneal infection. Smears were made from the peritoneal fluids, and these failed to show any bacterial growth.

III. DISCUSSION AND SUMMARY

Green[1] found that the blood of the albino rat contained no fat splitting enzyme, but that it contained enzymes capable of hydrolyzing in greater or lesser degree the esters of the lower fatty acids. These esterases seem to be elaborated from the blood and tissue cells. He found that a deficency of vitamine A brings about a fall of the serum esterase. He also found a fall in the esterase due to infection, either spontaneous or experimental, but the fall was not so great as in vitamine A deficiency.

Green[2] in a subsequent report found that, during the growth of the Jensen sarcoma in an albino rat, the esterase content of the serum falls to a low level. In rats resistant to the inoculation of the sarcoma, the esterase content tends to rise. He thinks the tumor cell may produce an inhibitory substance.

Begg and Aitken[3] found that in fowls immune to cell inoculations of the Rous Sarcoma, there is a high serum lipolytic power, and this applies to fowls having received intramuscular or subcutaneous

injections of surviving tumor tissue. They used a pancreatic extract (Cole and Onslow) as a substrate.

Comfort and Osborne[4] found that an esterase is present in human blood serum, but its activity bears no consistent relationship to any disease of the pancreas. They used ethyl butyrase as a substrate.

Cherry and Crandall[5] found that obstruction of the pancreatic ducts of dogs causes no significant uniform changes in the esterase as measured by the hydrolysis of ethyl butyrate. They suggest the use of the term esterase for enzymes acting upon simple esters.

Sure[6,7] has reported twice that the blood serum esterase of rats bearing the Walker No. 256 Carcino-sarcoma was decreased 47%.

The fall in esterase cannot be attributed to the withdrawal of 2 to 3 cc. blood, since in the control animals, the second esterase determination, 24 to 48 hours after the first, was only 4.2% lower than the first. The withdrawal of 5 to 6 cc. blood had no apparent effect on the rats. The animals were, as a rule, rather quiet for the first two hours, after which they returned to their normal activities. It was noticed that pregnancy caused a decrease in the ethyl butyrase. For this reason, any rats which became pregnant during the course of an experiment were not used.

The decrease in the butyrase activity resulting from the intraperitoneal injection of fresh tumor tissue was not due to bacterial

invasion, since smears of the peritoneal fluids were sterile. Neither was the decrease due entirely to the metabolism of the tumor tissue because the injection of boiled tissue produced almost as great a decrease, 39.4% and 35.4% respectively.

That the effect of injected tumor tissue upon the blood serum ethyl butyrase was due to some constituent of the tumor was proven by the autolysate experiments. An autolysate of tumor tissue and of fresh dog's kidney when injected, produced decreases of 24.9% and 8.8% respectively. The proteins of the tumor tissue could not have caused the fall in butyrase since it required 3.0 grams of peptone or proteose to bring about a decrease of 26.8%.

The addition of choesterol in amounts up to 100 mgm. to the peptone and proteose produced a decrease in esterase nearly equal to that produced by tumor tissue. Cholesterol in amounts from 30 to 200 mgm. when suspended in lecithin or olive oil may produce a de- in butyrase of 35.5%.

The in vitro experiments show that neither choesterol nor dibenzanthracene have any inhibitory action during a 2 hour incubation period. When the incubation period was 24 hours, both cholesterol and dibenzanthracene caused a 30% decrease in the ethyl butyrase activity.

IV. CONCLUSION

The injection of two grams of fresh tumor tissue intraperitoneally causes a marked decrease in the serum ethyl butyrase.

The constituent of the tissue that diminishes or inhibits the butyrase activity is heat-stabile.

Boiled macerated kidney produces, when injected, a fall in butyrase as great as that produced by the fresh tissue.

The proteins of the tumor tissue are not responsible for the fall in serum ethyl butyrase.

Choesterol and dibenzanthracene can, during an incubation period of 24 hours, act as inhibiters of ethyl butyrase.

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