CLXXX. ENZYMIC EFFICIENCY IN MALIGNANCY.

I. INFLUENCE OF THE GROWING WALKER CARCINOSARCOMA ON CONCENTRATION OF BLOOD AND TISSUE ENZYMES OF THE ALBINO RAT.¹

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(Received April 5th, 1935.)

SINCE enzymes are the means by which cancer cells as well as normal cells do their work, and since resistance to malignancy seems to be associated with definite changes in concentration of certain blood and tissue enzymes, it is evident that one of the goals of the biochemist in the study of cancer is to make a systematic and intensive study of all possible factors that influence the concentration of such enzymes with the purpose of preventing the transformation of normal epithelial into cancerous tissues.

Recently we have initiated a detailed study of enzymic efficiency in avitaminosis. Being at the same time engaged in an investigation on the therapeutic effect of vitamins on regression of transplantable tumours in the albino rat, we had the necessary equipment, materials and tumour-bearing animals for the study of enzymic efficiency in malignancy.

EXPERIMENTAL.

In first attempts to transplant tumours, we found our Wistar Institute stock quite resistant to the Flexner-Jobling carcinoma, obtaining only about 50 % of takes. We therefore subsequently used a tumour furnished us by Dr F. C. Wood of the Institute of Cancer Research, Columbia University. This tumour was designated as Walker carcino-sarcoma No. 256, and gave us 60-85 % of takes, provided that the tumour used for implantation was not too necrotic.

The transplantations were made under sterile conditions. The tumour tissue, weighing about 20 mg., was inoculated according to the technique of Sugira *et al.* [1921] with a trocar into albino rats subcutaneously in the region of the right axilla. The animals, about equally divided between males and females,

¹ Research Paper No. 321, Journal Series, University of Arkansas. Supported by a fund from the International Cancer Research Foundation.

A preliminary report of the results of this investigation appeared in the *Proc. Soc. Exp. Biol. Med.* 32, 658-9, 1935.

were 3-4 months old and weighed 160-200 g. They were given our regular stock diet No. 1, which is composed of cereal grains fortified with caseinogen, CaCO_3 and NaCl. The animals and food were weighed daily.

The experiments were conducted in pairs; for every animal with an implanted tumour a litter-mate control of the same sex was used, which was restricted to the same plane of nutrition as the tumour-bearing animal [Sure *et al.*, 1935]. The tumours were measured, 3 times weekly, with a caliper in length, width and in some cases in depth. 42 groups were studied in the case of most enzymes. The blood serum phosphatase was included later in the investigation and was studied in 31 groups. The tumours ranged from 15 to 73 mm. in length and from 10 to 65 mm. in width. The age of the tumours of the implanted animals, the blood and tissue enzymes of which were studied, ranged from 3 to 5 weeks in most cases; but some individuals were allowed to proceed to the stage of pronounced ulceration and these tumours were about 7 weeks old at the time the animals were sacrificed.

The tumours were embedded in formalin and examined microscopically by Drs Thatcher and DeGroat of the Department of Medical Pathology, University of Arkansas Medical School, Little Rock. The external observations showed cases varying from non-ulceration and slight ulceration to pronounced ulceration with pus oozing from the exterior. On microscopic examination there were present necrosis and inflammation varying from one-tenth to three-fourths of the entire tumour. The following enzymes were studied, all of which were derived from the blood and tissues of the same animal: blood serum amylase, esterase and phosphatase; pancreatic amylase, lipase and esterase; hepatic lipase and esterase; trypsin and erepsin.

The same technique was used for the sampling of blood and the preparation of tissue extracts as has already been described [Sure *et al.*, 1935].

Methods employed for the determination of the various blood and tissue enzymes.

The trypsin and erepsin were determined according to the alkalimetric method of Willstätter and Persiel [1925].

For the blood serum esterase the method of Green [1934] was employed, with the following modifications. The amount of blood serum was reduced from 0.5 to 0.25 ml., the amount of ethyl butyrate was increased from 0.25 to 0.5 ml. and the time of incubation increased from 2 to 4 hours.

The pancreatic amylase was determined according to the recent procedure of Ross and Shaw [1934], with the following modification. In addition to the slightly varied technique for the preparation of tissue extract, we used 0.25 instead of 0.5 ml. of the enzyme solution of the pancreatic extract of our concentration [Sure *et al.*, 1935]. Employing our modified technique, we found considerably more amylase units per g. tissue for rats on a normal diet than did Ross and Shaw. Whether this is due to more thorough extraction we cannot say.

The blood amylase was determined precisely according to the same technique as the pancreatic amylase, using 0.25 ml. blood serum in duplicate. In this connection it may be mentioned that all determinations were conducted in duplicate unless there was a shortage of blood serum, in which case only single samples were taken for analysis.

The pancreatic and hepatic lipases were determined according to the technique recently described by us [Sure *et al.*, 1935].

The procedure for the determination of pancreatic and hepatic esterases was changed from our previous technique [Sure *et al.*, 1935] to that employed for blood serum esterase, using 0.5 ml. substrate, 2 ml. of the pancreatic extract, and on account of the large amount of tissue esterase present in the liver, the amount of sample in the hepatic extract was reduced to 0.25 ml. The period of incubation was 4 hours.

The method of Jenner and Kay [1932] for the determination of blood plasma phosphatase was found quite suitable for our determination of blood serum phosphatase, with the following modifications. Instead of 0.25 ml. plasma, we used 0.125 ml. serum; also we employed a period of 2 hours' incubation instead of 3 hours' used by Jenner and Kay.

The results of our investigations are submitted in Tables I–III inclusive. In Table I are presented detailed results of our major findings. In Tables II and III are submitted summarised data of the entire investigation.

Table I. *Influence of the growing Walker carcino-sarcoma on concentration of blood and tissue enzymes of the albino rat.*

P = Pathological.						C = Control.		
Exp. No.	Animal marking and sex	Size of tumour		Blood serum amylase units	Blood serum esterase exp. as mg. butyric acid	Hepatic esterase exp. as mg. butyric acid	Trypsin units	Remarks
		L. mm.	W. mm.					
2708	P-D ♀ C- ♀	52	45	4.3 16.0	3.0 25.9	— —	20 20	Ulcerated with much pus; necrosis; 1/2 of section with inflammation
2705	P-D ♀ C-R ♀	57	40	10.9 23.0	8.3 29.6	— —	12 40	Dry slight ulceration; marked necrosis and inflammation
2704	P-DL ♀ C-L ♀	55	45	10.1 25.3	7.6 27.9	26.4 41.3	48 66	Ulcerated with much pus; necrosis; 1/2 of section with inflammation
2699	P-D ♀ C-DR ♀	55	50	10.3 19.0	11.0 27.2	35.6 44.8	32 48	Pronounced ulceration; necrosis; 1/2 of section with much inflammation
2699	P- ♂ C-D ♂	64	55	5.7 17.9	7.4 28.6	35.3 47.5	— —	Pronounced ulceration which had dried; slight necrosis; inflammation
2705	P- ♂ C-R ♂	53	46	8.3 17.3	12.7 33.4	31.2 43.2	38 32	Small ulceration; slight necrosis with inflammation
2720	P-R ♂ C-DR ♂	72	65	46.3 30.5	15.8 23.7	44.8 51.4	32 36	Pronounced ulceration; necrosis with inflammation
2779	P-L ♂ C-LDR ♂	39	19	14.7 29.4	19.4 21.6	46.6 48.0	32 40	Ulcerated; necrosis; 3/4 of section with much ulceration
2793	P- ♂ C-L ♂	23	23	25.3 24.5	24.5 24.2	50.9 50.9	23 47	Ulcerated; centre necrotic with much inflammation; cancer extended into muscle
2776	P-R ♀ C-D ♀	38	38	16.3 16.9	17.9 20.9	49.5 47.5	20 54	Ulcerated; centre necrotic with inflammation
2799	P- ♂ C-L ♂	43	42	12.2 20.7	— —	— —	19 34	Ulcerated with pus; necrosis and 1/2 degeneration
2720	P-DL ♀ C-R ♀	63	40	2.7 22.9	12.3 27.7	32.5 42.2	22 34	Non-ulcerated; necrosis 1/2 section; much karyolysis
2717	P-DL ♀ C-L ♀	65	37	7.0 19.6	10.1 32.1	31.2 42.6	20 40	Non-ulcerated; necrosis 1/3 section; much karyorrhexis
2721	P-L ♂ C-D ♂	44	38	20.7 19.6	17.7 29.9	33.4 43.7	40 46	Non-ulcerated; 3/4 necrosis; much inflammation; many polymorphonuclear leucocytes
2702	P-DL ♀ C- ♀	59	51	9.8 4.3	22.8 14.5	25.7 23.3	30 47	Non-ulcerated
2750	P-DL ♂ C-DR ♂	24	24	18.5 22.8	25.5 34.7	47.5 50.6	26 26	Non-ulcerated; 1/10 section necrotic; with inflammation

Table I (cont.).

Exp. No.	Animal marking and sex	Size of tumour		Blood serum amylase units	Blood serum esterase	Hepatic esterase	Trypsin units	Remarks
		L. mm.	W. mm.		Exp. as mg. butyric acid	Exp. as mg. butyric acid		
2754	P- C-L	♂ ♂	34 33	34.3 37.1	15.4 15.4	48.8 54.6	24 24	Non-ulcerated; 1/4 necrosis with inflammation
2749	P-DL C-DR	♀ ♀	32 34	14.2 20.9	15.4 28.6	45.3 49.3	— —	
2753	P- C-L	♀ ♀	46 42	17.4 26.7	15.8 26.0	42.7 57.2	— —	Non-ulcerated; 1/2 necrosis with inflammation
2748	P-R C-D	♀ ♀	45 42	19.8 28.6	18.3 29.0	36.3 49.5	30 28	
2751	P-R C-D	♀ ♀	53 41	16.4 16.4	20.5 27.3	44.0 55.8	24 43	Non-ulcerated; 1/2 necrosis with inflammation
2754	P-R C-D	♀ ♀	32 30	11.7 20.7	27.1 28.2	34.5 52.1	20 30	
2775	P-DL C-R	♂ ♂	33 20	22.3 28.7	27.3 26.4	44.3 43.0	12 34	Non-ulcerated; centre necrosis; much inflammation
2776	P-D C-L	♂ ♂	33 22	21.3 31.9	23.3 26.2	— —	42 36	
2773	P- C-L	♂ ♂	27 24	23.4 40.9	31.9 30.4	41.8 52.4	46 56	Non-ulcerated; slight necrosis; slight inflammation
2774	P-R C-D	♂ ♂	28 27	26.7 32.7	23.8 24.5	47.1 59.2	36 42	
2778	P- C-L	♀ ♀	33 23	17.4 28.9	28.6 26.7	46.5 50.8	36 54	Non-ulcerated; necrosis; 1/3 of section with inflammation
2774	P-DL C-DR	♀ ♀	29 25	16.6 27.8	16.7 23.8	38.6 57.0	20 48	
2706	P-D C-R	♂ ♂	44 29	11.2 17.2	21.2 27.0	— —	32 26	Slightly necrotic; small abscess in centre
2726	P-DLR C-D	♂ ♂	73 42	12.5 13.6	13.6 31.2	28.6 38.7	44 60	
2724	P-DR C-D	♂ ♂	59 45	9.2 20.1	13.8 21.2	33.0 46.6	30 40	Non-ulcerated; necrosis; 1/2 with much karyorrhexis
2725	P-R C-DL	♂ ♂	45 36	15.2 20.1	18.6 29.0	47.9 47.0	26 30	
2779	P-D C-R	♀ ♀	27 25	19.1 18.0	33.3 26.8	47.3 52.2	38 60	Non-ulcerated; 1/3 necrosis; slight inflammation
2752	P-R C-D	♂ ♂	60 41	12.0 27.2	22.4 31.7	44.9 52.4	34 52	
2753	P-DL C-DR	♂ ♂	48 44	14.7 27.5	22.6 27.5	42.7 51.9	24 38	Non-ulcerated; 1/2 necrosis with inflammation
2794	P-DL C-DR	♀ ♀	41 24	16.1 42.3	18.0 24.5	39.1 44.7	42 42	
2798	P-DL C-DR	♂ ♂	24 24	24.5 22.0	28.9 30.4	45.3 49.5	56 56	Non-ulcerated; slight necrosis with inflammation
2796	P-R C-D	♀ ♀	30 26	15.8 15.5	20.6 35.9	44.4 49.7	44 62	
2797	P-DL C-DR	♀ ♀	32 28	17.6 39.2	20.7 35.2	45.8 57.6	20 38	Non-ulcerated; 1/3 necrosis with inflammation
2797	P-R C-D	♂ ♂	34 25	21.8 31.5	20.6 35.9	57.0 44.0	32 54	
2796	P-RDL C-L	♂ ♂	37 30	24.5 28.0	18.5 20.7	— —	38 48	Non-ulcerated; 1/2 necrotic with inflammation
2794	P-R C-D	♂ ♂	41 35	17.0 39.2	26.4 42.5	— —	36 44	

* Animal in dying condition.

Table II. *Influence of the growing Walker carcino-sarcoma on concentration of blood and tissue enzymes of the albino rat.*

Enzyme	P = Pathological.		C = Control.					
	No. of groups studied	No. of pathological groups showing decrease	Average for all groups		% decrease in pathological	% animal groups showing decrease	% animal groups showing increase	% animal groups showing no change
			P	C				
Blood serum amylase (units)	42	35	16.5	24.5	48.5	83.3	14.3	2.4
Blood serum esterase (mg. butyric acid)	41	34	18.7	27.6	47.0	82.9	14.6	2.5
Blood serum phosphatase (units)	26	17	41.4	47.3	11.1	65.4	34.6	0.0
Trypsin (units)	38	29	31.3	43.5	39.0	76.3	10.5	13.2
Erepsin (units)	38	21	26.9	28.3	5.2	55.3	36.8	7.9
Pancreatic amylase (units)	34	30	26.7	30.3	13.5	88.2	5.9	5.9
Pancreatic lipase (mg. oleic acid)	39	27	15.7	17.0	8.3	69.2	25.6	5.2
Hepatic lipase (mg. oleic acid)	35	16	29.9	32.5	8.7	45.7	48.5	4.0
Pancreatic esterase (mg. butyric acid)	37	21	16.5	17.7	7.3	56.8	35.1	8.1
Hepatic esterase (mg. butyric acid)	35	30	40.5	47.2	16.5	85.7	11.4	2.9

Table III. *Percentage decrease in concentration of various blood and tissue enzymes of tumour-bearing albino rats compared with litter-mate controls of the same sex restricted to the same diet and same plane of nutrition.*

Enzyme	Groups of animals	Enzyme	Groups of animals		
Blood serum amylase	48.5	42	Blood serum phosphatase	11.1	26
Blood serum esterase	47.0	41	Hepatic lipase	8.7	35
Trypsin	39.0	38	Pancreatic lipase	8.3	39
Hepatic esterase	16.5	35	Pancreatic esterase	7.3	37
Pancreatic amylase	13.5	34	Erepsin	5.2	38

ANALYSIS OF EXPERIMENTAL DATA.

Table I shows that inflammation was found in the majority of the tumours on microscopic examination. We should like to emphasise in this connection that the implantations of tumour tissue were conducted under the most rigid sterile conditions possible. If an infectious organism invaded the tumour-bearing animals, this must have been the result of loss of resistance to infection by virtue of the development of this rapid-growing and necrosing carcino-sarcoma.

The most marked changes were found in the concentrations of blood serum amylase, blood serum esterase and trypsin, showing a reduction percentage in the tumour-bearing animals compared with their litter-mate controls of the same sex and restricted to the same plane of nutrition, of 48.5, 47.0 and 39.0 respectively.

While this work was in progress the paper of Green [1934] appeared, showing the marked decrease in blood serum esterase content of rats inoculated with the Jensen sarcoma. Our results of the blood serum esterase of animals implanted with the Walker carcino-sarcoma No. 256, therefore, concur with those of Green. We did not, however, find such a marked reduction, either in the blood serum or hepatic esterases. The average results of Green expressed as $N/100$ NaOH for the blood serum esterase are: cancer group, 4.4; normal group, 18.1—a difference of about 400 %. For the liver esterase, the average results of Green are: cancer group, 20.6; normal group, 56.4—a difference of about 175 %. Our tumour-bearing animals showed a reduction in blood serum and hepatic esterases of 47.0 and 16.5 % respectively. The difference in degree of

reduction of these esterases is probably due to the fact that the authors and Green worked with different types of tumours.

Tables II and III show that the reductions in the concentrations of pancreatic amylase, blood serum phosphatase and pancreatic esterase in the tumour-bearing animals are small but definite.

The reduction of 8.7 % in hepatic lipase in the pathological animal is of no significance, since a large percentage of the groups showed increase in this enzyme.

It is of interest to note (as shown in Table II) that considerable reduction in the blood and tissue enzymes of the pathological animals occurred in 76–83 % of the groups.

The changes in the concentration of hepatic lipase, pancreatic esterase and erepsin in the pathological animals are too small to be considered of any value.

It is evident from a careful examination of Table I that there is no absolute relationship between the growth of the tumour, the extent of ulceration, necrosis and inflammation, and the extent of reduction of the concentration of blood and tissue enzymes of the tumour-bearing animals. Because, however, there is a great deal of individual variation in concentration of enzymes in various animals, we have followed up the influence of this growing tumour on the blood serum amylase and blood serum esterase in the living animal, taking two blood samples weekly from the tail of the rat, the purpose being to determine when during the development of the malignancy of this carcino-sarcoma the first enzymic changes begin. Six blood serum amylase and 14 blood serum esterase¹ groups were studied. The results show that pronounced reduction in the concentrations of these enzymes takes place only after the tumour has developed to approximately the 20 mm. size.

The interpretation of our findings will become clearer by a consideration of the recent results of Baló and Lovas [1933]. These investigators studied the enzymes trypsin, lipase and amylase from the pancreas of 70 people who died from different diseases, such as pulmonary tuberculosis, peritonitis purulenta, diabetes, chronic gastric ulcer, haemorrhage of the brain, meningitis, Addison's disease, Basedow's disease and carcinoma. They found in these diseases that the concentrations of the pancreatic enzymes show a parallelism, *i.e.* high lipase content accompanies high trypsin and amylase values; and, in general, when a decrease takes place, all these enzymes are involved. Infectious diseases constitute an exception. The tryptic activity is decreased in the majority of these cases and the lipase and amylase contents remained normal. In diseases where cachexia occurred, a significant decrease of all three enzymes was observed. This decrease was the most pronounced in the tuberculous patients whose conditions led to cachexia. High lipase content was shown to be related to pancreatic fat necrosis.

In our experimental study of rats inoculated with the Walker carcino-sarcoma No. 256, there was no cachexia, since the animals showed no noticeable reduction in daily food intake and since the controls were restricted to the same plane of nutrition as the tumour-bearing animals, the problem of malnutrition was ruled out. The fact, however, that there was a marked reduction in trypsin and only small changes in pancreatic lipase and amylase would indicate that no organism was involved. This agrees with our histological findings of inflammation in most of the tumours examined.

¹ The work on the blood serum esterase was carried out by Miss Thelma James, the results of which constituted partial fulfillment of the requirements for the degree of Master of Science at the University of Arkansas.

The reduction of blood serum amylase would suggest atrophy of the acini of the pancreas, since Elman, using the quantitative viscosimetric method of Elman and McCaughan [1927], has found this to occur in a number of tumour cases [Elman *et al.*, 1929; Elman, 1931]. Our histopathological examinations, however, disclosed only minor changes in the pancreatic tissues.

Our findings of marked reduction of blood serum esterase in the tumour-bearing animals is in accordance with Green's observations in human cancer [1934]. Green, however, found that, where the general health was good, the serum esterase content was on the average slightly higher than in non-cancerous "normal persons", whereas in the terminal stages of cancer it was well below normal, reaching the level observed in acute and subacute bacterial disease.

SUMMARY.

Inoculations of the Walker carcino-sarcoma No. 256 subcutaneously into the albino rat produced the following changes in the concentration of blood and tissue enzymes. A marked reduction was observed in the contents of the blood serum amylase and blood serum esterase and trypsin. Small but definite decreases were found in the concentrations of hepatic esterase, pancreatic amylase and blood serum phosphatase. The decreases in hepatic and pancreatic lipase, in pancreatic esterase and in erepsin were too small to be considered of any significance.

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