CXLI. HYDROLYSIS OF GLUTATHIONE BY BLOOD SERUM

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THE hydrolytic breakdown of oxidized glutathione (GSSG) by serum from cancer patients was reported recently by Waldschmidt-Leitz [1938]. It was implied that this observation might form the basis of a diagnostic test for cancer, since no activity was found with serum from apparently normal individuals. That hydrolysis occurred was deduced from the increase in free amino-groups observed in GSSG-cancer serum mixtures after incubation. Antiglyoxalase was assumed to be responsible for the cleavage.

Antiglyoxalase, an enzyme (or enzyme system) which destroys the ability of reduced glutathione (GSH) to activate glyoxalase, has been studied extensively in this laboratory [Platt & Schroeder, 1934; Woodward *et al.* 1935; Schroeder *et al.* 1935; Schroeder & Woodward, 1937]. The enzyme was found only in kidney and pancreas extracts, the former containing by far the larger amount. Studies on kidney extracts showed that the enzyme acts by bringing about hydrolysis of both peptide linkages of the GSH molecule, with liberation of the middle amino-acid, cysteine. The oxidized form, GSSG, was attacked similarly, with liberation of cystine.

In view of the possibility that the observation of Waldschmidt-Leitz might be of diagnostic value in cancer, it appeared desirable to investigate further the action of serum on glutathione. In our previous antiglyoxalase work use was made of two highly specific tests to demonstrate glutathione hydrolysis. One of these consisted in incubating GSH with the enzyme for a definite period under standard conditions, then measuring the unchanged GSH by means of the specific glyoxalase method of Woodward [1935]. Any loss of glyoxalase-activating power indicated hydrolysis of the tripeptide. The other test involved the measurement by the Sullivan [1929] method of cysteine or cystine formation in incubated enzyme-glutathione mixtures. Application of these two tests to serum-glutathione mixtures should give a clearer picture of any hydrolytic changes in the glutathione molecule than was provided by amino-acid titration data, and should answer the following questions: (1) Does serum bring about true hydrolysis of glutathione, or is the observed —NH_2 increase due to other factors? (2) If hydrolysis of glutathione actually occurs are both peptide linkages attacked, i.e. is cysteine liberated? (3) Can any differences be observed by these specific methods between the actions of normal and of cancer serum on glutathione?

Since GSH is partially converted into GSSG in the presence of serum, even under anaerobic conditions, use of GSH would involve a lack of uniformity of substrate. For this reason oxidized glutathione, the form used by Waldschmidt-Leitz, was chosen for the present work. In order to apply the glyoxalase method to the measurement of GSSG hydrolysis, it was necessary to reduce to GSH any GSSG remaining after the action of the serum, since this method measures only

GSH. The electrolytic procedure recently developed in this laboratory by Dohan & Woodward [1939] for reducing GSSG to GSH made this measurement possible.

The results obtained in the present investigation show that the ability of serum to hydrolyse GSSG has no value as a diagnostic test for cancer. True hydrolysis of the intact GSSG molecule was produced by all cancer blood sera, but, in disagreement with Waldschmidt-Leitz, hydrolysis of GSSG by normal sera also was observed. There was wide variation between the activities of different sera in each group, but the range of variation was the same for normal as for cancer sera. Other pathological sera likewise produced similar amounts of GSSG cleavage. Cystine was established as a final product of the GSSG hydrolysis, showing that all peptide linkages had been attacked. The enzyme in serum responsible for the hydrolysis is therefore similar to antiglyoxalase from kidney. The average amount found in serum is very small, however, compared with that in rat kidney, being in a ratio of about 1 : 1600.

Method

GSSG was incubated with blood serum in the proportion of 1 mg. GSSG to 1 ml. serum. To 4 ml. serum in a test tube were added 2 ml. 0.2 % GSSG (4 mg.). The tube was stoppered by a cotton-wool plug and placed in a water bath or incubator at 38° for 24 hr. At the end of this time 2 ml. of 0.8 *M* salicylsulphonic acid were added to stop the reaction and precipitate the protein. Free salicylsulphonic acid remained in the filtrate to the extent of approximately 2.5 %, a concentration desirable for the subsequent reduction.

The electrolytic reduction procedure of Dohan & Woodward [1939] was applied to 4–5 ml. of the filtrate, whereby GSSG is reduced to GSH and cystine is reduced to cysteine. The amount of GSH in the reduced filtrate was determined in 0.2 ml. portions by the manometric glyoxalase method of Woodward [1935]. Iodimetric titration could not be used for the GSH analysis since it does not distinguish between the intact GSH tripeptide and the SH-compounds which might be split from it. Cysteine analyses were carried out by the Sullivan method [1929] on either 1 ml. of the electrolytically reduced filtrate with 1/5 quantities of his reagents, or 2.5 ml. with 1/2 quantities.

If the serum showed more than a trace of haemolysis, correction for the possible cystine already present was made by a separate blank determination. Since serum itself contains no GSSG or cystine, a blank determination was not otherwise necessary. Most of the sera were used within 7 days, although samples were kept for much longer periods (71 days) with no change in activity.

Results

Tables I–III present the data obtained on the destruction of GSSG by serum and the resulting cystine formation. The destruction of GSSG was found to be from 16 to 99% with 10 normal sera, 11 to 81% with 10 cancer sera, and 11 to 72% with sera of 10 individuals with other pathological conditions. There is wide variation within each group, and each group covers approximately the same range. The conclusion is, therefore, that there is no correlation between the condition of an individual, diseased or otherwise, and the ability of his serum to destroy GSSG.

The amount of cystine formation resulting from the hydrolysis of the GSSG leads to the same conclusion. In general, the cystine formation parallels the GSSG loss. Except with a few sera which cause the highest amounts of GSSG destruction, the amount of cystine found entirely accounts for the loss of GSSG.

Table I. *Normal individuals*

Subject	Cleavage of 1 mg. GSSG by 1 ml. serum	
	GSSG lost %	Cystine formed %
1	99	62
2	97	61
3	74	46
4	56	51
5	30	33
6	28	36
7	24	29
8	19	25
9	17	—
10	16	16

Table II. *Cancer cases*

Case	Cleavage of 1 mg. GSSG by 1 ml. serum	
	GSSG lost %	Cystine formed %
1. Multiple carcinoma of pelvis and spine	81	69
2. Adenocarcinoma of breast—after operation and radiotherapy	53	45
3. Carcinoma of breast and lung—after radiotherapy	49	46
4. Carcinoma of breast, recurrent, with metastases—after radiotherapy	41	38
5. Lymphosarcoma of abdomen, untreated	44	41
6. Reticulum cell sarcoma, multiple	38	44
7. Multiple fibrosarcomata	40	43
8. Carcinoma of breast removed by operation 2 yr. previously. No recurrence apparent	38	32
9. Carcinoma of appendix with metastases—1 day after exploratory operation	37	31
10. Carcinoma of stomach—4 days after exploratory operation	19	—
	18	24
	11	24

Table III. *Miscellaneous pathological cases*

Case	Cleavage of 1 mg. GSSG by 1 ml. serum	
	GSSG lost %	Cystine formed %
1. Splenomyelogenous leukemia	72	63
2. Hypertensive arteriosclerosis with cerebral haemorrhage	60	59
3. Hypertensive arteriosclerosis	60	57
4. Hypopituitarism	51	39
	54	52
5. Phlebosclerosis and atherosclerosis	36	26
	36	27
6. Hypertension and atherosclerosis	25	35
7. Hypertension and atherosclerosis	21	21
8. Diabetes, insulin treated	17	24
9. Arteriosclerosis	13	23
10. Marked hypertension and arteriosclerosis	11	28

In cases where the cystine formation appears greater than the GSSG loss, explanation may be found in the tendency of the Sullivan method to yield values which are too high when the cysteine concentration of the solution being analysed is very low. In all cases cystine was an end-product of the hydrolysis of GSSG by serum.

Reduced glutathione, incubated 24 hr. with blood serum anaerobically in N_2 or in H_2 , was similarly hydrolysed. Considerable amounts of cysteine were found in unreduced filtrates from the incubation mixtures. The experiments were not of a quantitative nature, however, since there was always considerable loss of GSH and cysteine by oxidation.

SUMMARY

Blood serum was found to contain a small amount of an enzyme which hydrolyses oxidized glutathione with liberation of cystine. This hydrolysis is similar to that produced by antiglyoxalase from kidney, but the activity of serum is exceedingly small compared with that of kidney. The degree of hydrolytic activity of serum towards oxidized glutathione cannot be used as a diagnostic test for cancer, since there was no significant difference between the action of the normal and the cancer or other pathological sera examined.

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