thors believe that blood protein studies should be instituted when a nonspecific positive reaction is suspected and that care should be taken in the interpretation of a positive serologic reaction for syphilis in the presence of conditions with hyperproteinemia and hyperglobulinemia. Proven syphilitics may also show[11] a hypergammaglobulinemia and patients with kala-azar, as shown in the present work, and sarcoidosis[27] may have hyperglobulinemia associated with negative serologic reactions for syphilis. The mere presence or absence of hyperproteinemia, hyperglobulinemia or hypergammaglobulinemia is not sufficient to prove or disprove the specificity of positive serologic reactions for syphilis.

Summary. The electrophoretic patterns of 2 human kala-azar sera revealed the presence in high concentration of a unique, abnormal component migrating with the γ globulin of the slowest mobility. The kala-azar sera, negative to serological tests for syphilis, provide instances which show that the presence of hyperproteinemia, hyperglobulinemia or hypergammaglobulinemia cannot be used to prove or disprove the specificity of positive serologic reactions for syphilis.

[27] Cooper, G. R., Rein, C. R., and Beard, J. W., unpublished observations.

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Inactivation of Glutamic-Aspartic Transaminase by Irradiation.*

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Glutamic-aspartic transaminase is an enzyme which catalyzes the reaction: 1 (+) glutamic acid + oxaloacetic acid $\rightleftarrows$ α-ketoglutaric acid + 1 (—) aspartic acid. The prosthetic group of this enzyme is a derivative of vitamin B_6,[1,2] probably identical with codecarboxylase.[3,4] Since pyridoxine, pyridoxal and pyridoxamine are very sensitive to light,[5,6,7] it was expected that transaminase preparations would also be inactivated by irradiation.

It was found that sunlight destroys the enzymatic activity at about the same rate as it inactivates the vitamin B_6 present in the preparations (Table I). The destruction by ultraviolet light is much more rapid (Table II). The effect of X-rays on transaminase was also investigated. Vitamin B_6 has been considered as a remedy for X-ray sickness.[8,9] It was found that X-ray irradiation in therapeutic and higher doses has no effect on transaminase activity. Therefore, the possible therapeutic value of vitamin B_6 in this condition cannot be explained in terms of a restoration of inactivated transaminase.

Experimental Procedures. For the determination of transaminase activity the procedures developed by Cohen[10] were used. The enzyme was purified according to the

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Reported at the Dallas Meeting, Southwestern Section, on October 20, 1945.

[1] Schlenk, F., and Snell, E. E., *J. Biol. Chem.*, 1945, **157**, 425.

[2] Schlenk, F., and Fisher, A., *Arch. Biochem.*, 1945, **8**, 337.

[3] Lichstein, H. C., Gunsalus, I. C., and Umbreit, W. W., *J. Biol. Chem.*, 1945, **161**, 311.

[4] Green, D. E., Leloir, L. F., and Nocito, V., *J. Biol. Chem.*, 1945, **161**, 559.

[5] Hochberg, M., Melnick, D., Siegel, L., and Oser, B. L., *J. Biol. Chem.*, 1943, **148**, 253.

[6] Hochberg, M., Melnick, D., and Oser, B. L., *J. Biol. Chem.*, 1944, **155**, 129.

[7] Cunningham, E., and Snell, E. E., *J. Biol. Chem.*, 1945, **158**, 491.

[8] Maxfield, J. R. Jr., McIlwain, A. J., and Robertson, J. E., *Radiology*, 1943, **41**, 383.

[9] Gendreau, J. E., and Lafleur, L., *Union med. du Canada*, 1944, **73**, 666.

[10] Cohen, P. P., *J. Biol. Chem.*, 1940, **136**, 565.

TABLE I.
Inactivation of Glutamic-Aspartic Transaminase by Sunlight.

Time (hrs)	Irradiated				Dark controls			
	Transaminase activity %	Vitamin B_6 present as determined with*			Transaminase activity %	Vitamin B_6 present as determined with*		
		L. casei γ per g	*S. fecalis* R γ per g	*Sacc. carlsbergensis* γ per g		*L. casei* γ per g	*S. fecalis* R γ per g	*Sacc. carlsbergensis* γ per g
0	30.5	67	305	95	30.5	67	305	95
1	14.9	24	40	31	29.0	47	267	75
2	(1.9)	19	39	25	29.0	56	276	82
3	6.1	11	22	18	31.4	50	305	79

* Pyridoxal was used as standard with *L. casei*, pyridoxamine with *S. fecalis* R. and pyridoxine with *Sacc. carlsbergensis*.

method outlined earlier[2] which yields solutions with no absorption in the visible part of the spectrum.

Irradiation by Sunlight. For irradiation by sunlight large open Petri dishes were placed in an icebath. The light path was 0.4 cm; irradiation was from 1 P.M. to 4 P.M., May 14, 1945; 29° North latitude. At the times given in Table I samples were withdrawn for the determination of transaminase and for the microbiological assay of the B_6 vitamins.[11]

Microbiological assay was carried out following 15 minutes autoclaving of the sample with N/10 sulfuric acid. No attempt at differential assay[12] for various members of the vitamin B_6 group was made, since the relative activity of the coenzyme for the various organisms and the conditions which effect its complete hydrolysis and release from the protein molecule with which it is associated are at present unknown.[4] The figures given in Table I represent the total vitamin B_6 per gram of protein as indicated by assay with each organism against the appropriate standard.

Irradiation by Ultraviolet Light. For ultraviolet irradiation a quartz mercury lamp (General Electric Co., Model F; 400 watt) was used. The distance between the lamp and the solution was 45 cm. Otherwise the procedure outlined above was followed. For comparison solutions of pyridoxine, pyridoxal, and pyridoxamine (10 γ per ml) were irradiated under the same conditions. The destruction was determined using the colorimetric reaction of Swaminathan[13] in the modification of Bina, Thomas, and Brown.[14]

The inactivation as recorded in Tables I and II is not due to hydrogen peroxide. The hydrogen peroxide concentration in distilled water irradiated by ultraviolet light under the same conditions for 30 minutes remained below 6×10^{-5} moles per liter. We found

[11] Snell, E. E., and Rannefeld, A. N., *J. Biol. Chem.*, 1945, **157**, 475.

[12] Snell, E. E., *J. Biol. Chem.*, 1945, **157**, 491.

[13] Swaminathan, M., *Nature* (London), 1940, **145**, 780.

[14] Bina, A. F., Thomas, J. M., and Brown, E. B., *J. Biol. Chem.*, 1943, **148**, 111.

TABLE II.
Inactivation of Transaminase and of Vitamin B_6 by Ultraviolet Light.

Irradiation (min.)	Transaminase activity %	Vitamin B_6 compounds		
		Pyridoxal γ	Pyridoxamine γ	Pyridoxine γ
0	37.2	40.0	40.0	40.0
5	10.3	8.0	18.5	14.3
10	4.3	2.1	9.8	8.9
15	1.9	1.2	5.9	4.3
30	<1	0.6	2.9	0.8

that transaminase is rather resistant to hydrogen peroxide. The stability of the members of the vitamin B_6 group has been reported earlier.[7] The inactivated enzyme was not reactivated by addition of pyridoxine, pyridoxal, pyridoxamine, or boiled tissue extract.

Irradiation by X-rays. The conditions were as follow:† 200 K.V.; 16 milliamp; 50 cm distance; filter, 2.5 mm. Al inherent in tube, none added. Since no appreciable inactivation of transaminase (Table III) was observed, no vitamin B_6 determinations were carried out.

TABLE III.
X-Ray Irradiation of Transaminase.

Irradiation r units	Transaminase activity %
0	39.7
500	36.2
1,500	38.4
4,000	35.8
7,000	39.1
10,000	34.5

Summary. Glutamic-aspartic transaminase is inactivated by sunlight and by ultraviolet light. Destruction of enzymatic activity by these agents parallels the destruction of vitamin B_6, a derivative of which is present as the prosthetic group of the enzyme.[1-4] The irradiated enzyme is not reactivated by addition of pyridoxine, pyridoxal, pyridoxamine or boiled tissue extracts. Irradiation with X-rays leaves the enzyme intact.

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Biological Activity and Metabolism of d,l-O-Heterobiotin in the Chick.*

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O-Heterobiotin, an oxygen analog of biotin, has biotin-like activity for the chick[1] and for rats fed egg white.[1,2] Hofmann *et al.*[1] found the d,l analog 10% as active as d-biotin in curing egg-white toxicity in rats and approximately 6 and 20% as active in promoting growth in chicks when fed at different levels on a biotin deficient ration. Rubin *et al.*[2] found d,l-O-heterobiotin 5% as active as d-biotin in counteracting egg-white toxicity in the rat.

Since levels of 40 γ of d,l-O-heterobiotin

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