

The results of this investigation demonstrate that antibodies, prepared from rabbit antisera, to highly purified preparations of human growth hormone and sheep ICSH fix complement when allowed to react with the respective antigens. On a quantitative basis, the complement fixation reaction will detect about one-hundredth of the quantity of hormone measured by precipitin reactions. The close agreement of the values obtained by the technique of complement fixation with those obtained by quantitative precipitin tests indicates that complement fixation can be used as a quantitative immunochemical technique for measuring minute quantities of HGH and sheep ICSH.

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<sup>1</sup> Hayashida, T., and Li, C. H., *Science*, **128**, 1276 (1958).

<sup>2</sup> Li, C. H., Moudgal, N. R., and Papkoff, H., *J. Biol. Chem.*, **235**, 1038 (1960).

<sup>3</sup> Moudgal, N. R., and Li, C. H., *Arch. Biochem. and Biophys.* (in press).

<sup>4</sup> Read, C. H., and Bryan, G. T., in *Ciba Foundation Colloquia on Endocrinology*, **13**, 68 (1960).

<sup>5</sup> Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry*, 97 (Charles Thomas, Springfield, Illinois, 1948).

<sup>6</sup> Greenspan, F. S., Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinol.*, **45**, 455 (1949).

### Effect of Riboflavin on Liver Histomucoid

Isenberg and Szent-Györgyi<sup>1</sup> studied the reaction between tryptophan and tryptophan metabolites and riboflavin. It was observed that free tryptophan or this amino-acid in proteins formed a complex with riboflavin. The vitamin molecule takes up one electron from tryptophan and a semiquinoid riboflavin free radical is formed. Those authors speculated that this complex occurs *in vivo*. Since it was established by Winzler *et al.*<sup>2</sup> that seromucoid contains tryptophan, and in view of the hepatic origin of that fraction<sup>3</sup>, experiments were conducted by us to investigate a possible effect of riboflavin on liver histomucoid *in vivo*.

Male Wistar rats with mean body weight of 135 gm. were injected intraperitoneally with a freshly prepared riboflavin solution (20 mgm./100 ml. of physiological saline). The dose used was 0.2 mgm. of riboflavin per 100 gm. of body weight. One group of animals received only one injection and the other was treated with a daily dose for 7 days. Control rats were injected with the same volume of the saline solution. All the animals were fed *ad libitum* a standard balanced diet, and liver histomucoid determinations were carried out, by the method previously reported<sup>4</sup>, 24 hr. after the last injection. A summary of the results is shown in Table 1.

Table 1 clearly shows that in both riboflavin groups lowered levels of liver histomucoids were observed. The differences between riboflavin-injected rats and the controls were statistically significant (1 treatment:  $t = 3.733$ ,  $P < 0.01$ ; 7 treatments:  $t = 4.711$ ,

Table 1. LIVER HISTOMUCOID IN RATS TREATED WITH RIBOFLAVIN

Treatment	No. of injections	No. of rats	Histomucoid	
			Range	Mean $\pm$ S.D.
Saline	1	6	105-191	152 $\pm$ 36
Riboflavin	1	6	89-108	96 $\pm$ 7
Saline	7	8	110-187	146 $\pm$ 24
Riboflavin	7	11	67-127	97 $\pm$ 20

$P < 0.001$ ). From the results presented it is obvious that one dose of riboflavin is sufficient for the production of the full effect.

Seromucoid determinations<sup>5</sup> in the same animals were carried out and no differences were disclosed between control and riboflavin-treated rats. This result warrants the conclusion that the lowered liver histomucoid observed was not produced by a mobilization of mucoproteins from that organ to the plasma.

On the other hand, incubation of extracts from liver acetone powders<sup>4</sup> or liver homogenates in physiological saline with riboflavin at 37° C. up to 3 hr. gave no change in the histomucoid-level.

Further investigations are being undertaken to elucidate the mode of the action of riboflavin on liver histomucoids.

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<sup>1</sup> Isenberg, I., and Szent-Györgyi, A., *Proc. U.S. Nat. Acad. Sci.*, **44**, 857 (1958).

<sup>2</sup> Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M., *J. Clin. Invest.*, **27**, 609 (1948).

<sup>3</sup> Winzler, R. J., *Methods Biochem. Anal.*, **2**, 279, edit. by Glick, D. (Interscience, 1955).

<sup>4</sup> Abreu, L. A., and Abreu, R. R., *Nature*, **184**, 2016 (1959).

### D-Penicillamine as an Antidote to 8-Hydroxyquinoline and Alloxan

OWING to the chelate-forming effect of its —SH and NH<sub>2</sub> radicals, DL-penicillamine has hitherto been used for removing copper deposited in brain tissues in cases of Wilson's disease and for preventing intoxication caused by heavy metals<sup>1</sup>. It is, however, undesirable to use L-penicillamine as a remedy or a preventive, because of its strong toxicity which causes acute and chronic deficiency of vitamin B<sub>6</sub> (refs. 2-4).

D-penicillamine, on the other hand, has no toxicity. We have examined its preventive and remedial effects on intoxication by 8-hydroxyquinoline and alloxan, which are diabetogenic agents.

0.03 mgm./gm. of body-weight was determined as the lethal dose of 8-hydroxyquinoline for mice 10 hr. after intramuscular injection. 0.2 mgm./gm. of D-penicillamine, injected 5 min. before the injection of lethal doses of 8-hydroxyquinoline, prevented death of 60 per cent of the experimental animals or showed effects in retarding death. Injection of 0.5 mgm./gm. of D-penicillamine prevented death completely but could not prevent toxic symptoms

Table 1

No. of Mice	10	10	10	10	10
D-Penicillamine mgm./body-weight (gm.)	1.0	0	0.2	0.5	1.0
8-Hydroxyquinoline mgm./body-weight (gm.)	0	0.03	0.03	0.03	0.03
Toxic symptoms	—	+++	++	+	—
No. of mice dead	0	10	4	0	0