Use of Dried Bovine Hemoglobin Powder in the Anson and Mirsky Methods for Pepsin and Trypsin[1]

David Orringer, Frances U. Lauber, and Franklin Hollander

Gastroenterology Research Laboratory, The Mount Sinai Hospital, New York City

As originally reported by Anson and Mirsky (*1*, *2*), the preparation of hemoglobin substrate used in the determination of pepsin and trypsin is a time-consuming, cumbersome procedure. It requires such equipment as a lyophilizer and a freezing unit, which is not available in many laboratories. Although the procedure for preparation of the pepsin substrate was much simplified by Bucher *et al.* in 1945 (*3*), it nevertheless still retains many tedious steps—for example, it requires quantities of fresh beef blood, and large volumes of solution must be centrifuged, dialyzed, and so on. Therefore, after our experiences with preparation of hemoglobin solution from defibrinated beef blood according to Bucher *et al.*, we sought a more convenient and equally reliable method. Through the kindness of Dr. J. B. Lesh, of the Chemical Research and Development Department, Armour and Company, we were provided with samples of lyophilized bovine hemoglobin, which had been prepared as directed by Mirsky. We have been using such preparations[2] since 1946, and have found them entirely satisfactory. The pepsin determinations using this material have been reproducible and reliable, there being no systematic difference between results obtained on the same specimens of human stomach contents with the Bucher substrate and the one herein described. Having had such satisfactory results with the acidified substrate for pepsin determinations, we proceeded to modify the alkaline substrate for trypsin described by Anson (*1*) to utilize this dried hemoglobin preparation. Details for the preparation of the substrate solutions for each of these enzymes are as follows:

Pepsin substrate solution. To prepare 1 l of final solution containing 2.5% hemoglobin, about 30 g of the powder is weighed out in a beaker and made into a smooth paste with a small quantity of water. Then more water is added and thoroughly mixed until the solution is thin enough to be poured readily. The solution is transferred to a 500-ml volumetric flask, diluted to the mark with distilled water, and filtered. The hemoglobin concentration of the filtrate is estimated by the dry-weight assay method described by Anson (*1*). A quantity of this concentrated solution (5%–6%), which contains exactly 25 g of hemoglobin, is transferred to a 1-l volumetric flask, 25 ml of merthiolate (1: 1000) is added as a preservative, and the volume is made up with distilled water. The solution is stored in the refrigerator.

[1] Supported in part by grant from Altman Foundation.

[2] Now commercially available from the Chemical Research and Development Department, Armour and Company, Chicago 9, Illinois, under the designation, Bovine Hemoglobin Enzyme Substrate Powder.

Trypsin substrate solution. In order to prepare 1 l of hemoglobin substrate solution to be used in the trypsin determination, 500 ml of a 5%–6% solution of hemoglobin powder is made up and assayed, following the procedure for the pepsin substrate concentrate. Of this concentrate, a volume calculated to contain exactly 22 g of hemoglobin is introduced into a 1-l volumetric flask together with 80 ml 1 *N* NaOH, 400 g urea, and sufficient water to bring the total volume to about 800 ml. After thorough mixing, the solution is incubated at 25° C for 30–60 min to denature the protein. To the solution is then added 100 ml 1 M potassium dihydrogen phosphate, 20 ml merthiolate (1:1000), and sufficient water to bring the volume up to 1 l. After mixing and filtering, the substrate solution (pH 7.5) is stored in the refrigerator.

In summary, substrate solutions for the determination of pepsin and trypsin according to the methods of Anson and Mirsky have been prepared from dried bovine hemoglobin powder, instead of from fresh blood. Using this modification, the tedious steps in preparation of pure hemoglobin in the laboratory have been eliminated, without effecting the reliability of the analytical results. Small quantities can now be made up at any time, obviating the possibility of deterioration on standing. It is hoped that with this modification these enzyme methods will find wider use in clinical as well as in experimental laboratories.

References

1. Anson, M. L. *J. gen. Physiol.*, 1938, **22**, 79.
2. Anson, M. L. and Mirsky, A. E. *J. gen. Physiol.*, 1932, **16**, 59.
3. Bucher, G. R., Grossman, M. I., and Ivy, A. C. *Gastroenterology*, 1945, **5**, 501.

Response of the Guinea Pig to 200 Roentgens Acute Whole Body X Irradiation[1]

Thomas J. Haley and D. Harriette Harris

Medical School, University of California at Los Angeles

The hematological response of many animal species to ionizing radiations has recently been presented by Jacobson, Marks, and Lorenz (*5*). However, their description of the response of the guinea pig did not present a complete picture of the effect of acute x irradiation on this animal.

The guinea pig has been used in the study of the effect of drugs upon x irradiation mortality (*2*) but no description was given of the effect of either drugs or radiation upon the leukocyte and differential counts, the coagulation time, or the body weight curve. All of these are of importance in determining the response of the guinea pig to x irradiation and can be used as indicators of radiation damage.

[1] This paper is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.