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

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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]

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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.

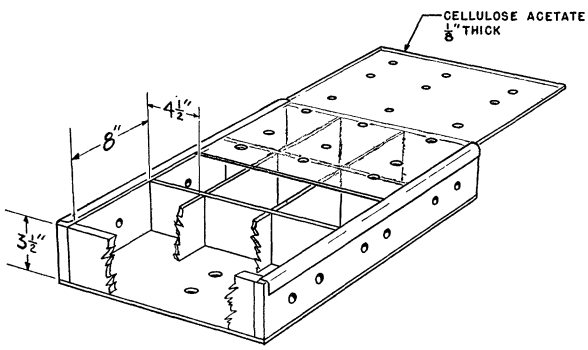


FIG. 1.

We have studied the effect of 200 r acute whole body x irradiation (approximately $LD_{50}$) upon the guinea pig in conjunction with an evaluation of several antihistaminic agents (*4*). The animals were caged in an irradiation chamber, Fig. 1, and the following radiation factors were employed: 250 kv, 15 ma, target subject distance 100 cm, filters 0.21 mm Cu inherent, 0.5 mm Cu parabolic and 1.0 mm Al, half-value layer 1.85 mm Cu, size of field —total body, r/min measured in air 9.48–10.61. Uniformity of dosage was insured by rotating the radiation cage during the treatment. The 250-kv Picker Industrial Unit used was calibrated before each experiment with a Victoreen thimble r-meter. The male guinea pigs, weighing 255–585 g (average 365 g) were divided into equal groups, usually ten animals each. In all, 66 controls and 64 irradiated animals were used. Total leukocyte and differential counts, coagulation time, and body weight were determined twice weekly until the irradiated animals showed signs of recovery. The radiation dosage used varied in lethality from 33% to 90% per group in the seven groups tested, but the average was 62.5%.

Comparison of the values in Table 1 indicates that our normal control animal compared favorably with those reported by Gardner (*3*). Also, comparison of our data with that reported in the literature for other rodents (*5*, *6*) shows that the guinea pig is not different in its response to x irradiation. Absolute values in the differential count are a better comparison of the actual state of the animals than relative ones. This is particularly evident with eosinophils, basophils, and monocytes, where relative values indicate no change but absolute ones show decreases of approximately 50% of preradiation values. The lymphocytopenia and neutropenia begin after the third postirradiation day and recovery is not evident until the 14th postirradiation day. Although the lymphocyte is the least resistant of the leukocytes to x irradiation, we did not observe such a drastic reduction as has been reported for the rabbit (*5*) and the rat (*6*). However, we did not make our first counts until the third postirradiation day, and this may account for our findings. Throughout the period of greatest depression, postirradiation days 5–14, there was a definite shift to the immature forms in the differential count. In all the irradiated animals the lymphocytes showed a faster recovery than the neutrophils. This is identical with the response of other animal species. From the 16th postirradiation day there was a definite increase in the proportion of neutrophils to lymphocytes. The neutrophils showed both higher relative and absolute values than at the beginning of the experiment, while the lymphocytes showed decreased values. This is to be expected because the animal is overproducing its leukocytes and attempting to regain a normal hematological balance.

As we were using multiple determinations, we employed the capillary tube method for determining coagulation time. Other methods are more accurate but they employ cardiac puncture for obtaining the blood specimen and thus would be highly detrimental to irradiated guinea pigs. Coagulation time in normal control animals ranged from 127 to 168 sec (average 146 sec). The irradiated animals began the experiment in this range but by the ninth postirradiation day their coagulation times had in-

TABLE 1

LEUKOCYTES IN THE NORMAL AND X-IRRADIATED GUINEA PIGS

| Cells | | Reported in Literature (*3*) | | Normal animals | | Before irradiation | | Irradiated animals | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 3rd day after | | 9th day after | | 16th day after | |
| | | Range | Avg | Range | Avg | Range | Avg | Range | Avg | Range | Avg | Range | Avg |
| Leukocytes | | 5700 to 17365 | 9800 | 7450 to 10,530 | 9910 | 8895 to 13,431 | 10,353 | 4820 to 8957 | 7867 | 3864 to 6480 | 4680 | 10,350 to 18,100 | 10,755 |
| Lymphocytes | R* | 39.4–63.6 | 53.81 | 55–65 | 61 | 59–67 | 60 | 59–73 | 64 | 77–89 | 85 | 42–60 | 51 |
| | A† | ..... | 5194 | ..... | 6045 | ..... | 6211.8 | ..... | 4934.88 | ..... | 3978 | ..... | 5495.25 |
| Neutrophils | R | 31.1–53 | 40.89 | 33–41 | 37 | 31–39 | 35 | 25–39 | 34 | 12–23 | 17 | 36–56 | 46 |
| | A | ..... | 3920 | ..... | 3666 | ..... | 3623.55 | ..... | 2674.78 | ..... | 795.6 | ..... | 4956.5 |
| Eosinophils | R | 1.99–3.5 | 2.65 | 0–3 | 1.5 | 0–3 | 1.5 | 0–3 | 1.5 | 0–3 | 1.5 | 0–3 | 1.5 |
| | A | ..... | 294 | ..... | 148.65 | ..... | 155.3 | ..... | 118 | ..... | 70.2 | ..... | 161.63 |
| Basophils | R | 0.49–0.7 | 0.45 | 1–3.5 | 2.0 | 1–2 | 1.5 | 1–2 | 1.5 | 1–2 | 1.5 | 1–2 | 1.5 |
| | A | ..... | 44.1 | ..... | 198.2 | ..... | 155.3 | ..... | 118 | ..... | 70.2 | ..... | 161.63 |
| Monocytes | R | 1.5–8.2 | 2.89 | 1–2 | 1.5 | 1–3 | 1.8 | 1–3 | 1.8 | 1–3 | 1.8 | 1–3 | 1.8 |
| | A | ..... | 294 | ..... | 148.65 | ..... | 186.35 | ..... | 141.6 | ..... | 84.24 | ..... | 193.95 |

* R—relative number.

† A—absolute number.

creased to an average of 233 sec. However, in the animals that recovered, the final value varied between 136 and 140 sec. This prolonged coagulation time was probably one of the contributing factors in the skin hemorrhages seen in all irradiated animals. However, although we only estimated the total number of platelets in our differential counts, the decreased number of platelets found was probably a factor in the increased coagulation time and should be considered, as should the possibility of hyperheparinemia (*1*).

The normal control animals showed a progressive weight gain throughout the experiment, whereas the irradiated animals lost weight (up to 100 g/week) beginning on the fifth postirradiation day and continuing until the 16th day, after which time those that survived began gaining weight faster than the controls.

In 13 irradiated animals, autopsy showed varying degrees of intestinal damage, from rupture to complete dissolution of parts of the small intestine. Intestinal damage is a general finding after x irradiation (*7*) and was probably one of the reasons for the weight loss observed in the irradiated animals.

It is concluded that the guinea pig responds similarly to other animals subjected to x irradiation and that for many purposes is much more suitable for such studies than the mouse or rat. Dependable results can be obtained using the guinea pig because its size and general temperament are suitable for studies employing large numbers of animals. However, all hematological studies should be reported in both relative and absolute terms to avoid a misinterpretation of the results observed.

### References


1. ALLEN, J. G. and JACOBSON, L. O. *Science*, 1947, **105**, 388.
2. CLARK, W. G., UNCAPHER, R. P., and JORDAN, M. L. *Science*, 1948, **108**, 629.
3. GARDNER, M. V. *J. Franklin Inst.*, 1947, **243**, 498.
4. HALEY, T. J. and HARRIS, D. H. *UCLA-28*, June 1949.
5. JACOBSON, L. O., MARKS, E. K., and LORENZ, E. *Radiology*, 1949, **52**, 371.
6. LAWRENCE, J. S., DOWDY, A. H., and VALENTINE, W. N. *Radiology*, 1948, **51**, 400.
7. WARREN, S. L. *Biological effects of radiation.* New York and London: McGraw-Hill, 1936. Pp. 473–539.


## Persistence of 2,4-D in Plant Tissues


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The persistence of 2,4-D in plant tissues of seedlings produced from plants which exhibited 2,4-D injury has been reported by several writers. Pridham (*3*) found that, if bean plants were sprayed with 2,4-D while the pods were still green, seedlings from seeds from these pods developed malformations characteristic of those produced by 2,4-D. Brown, Holdeman, and Hagood (*1*) reported that no abnormalities were found in cotton seedlings from seed collected in Louisiana in cotton fields affected by 2,4-D. Dunlap (*2*) reported that abnormal root development and deformed leaves were produced by seed collected from cotton plants that exhibited 2,4-D symptoms the year before. No other reports, to the writers' knowledge, have been published that would indicate any persistence of 2,4-D in plant tissues from one growing season to the next other than in seeds.

FIG. 1. On right, 2,4-D injury to shoot of *Stillingia sebifera*. Healthy shoot on left.

In the spring of 1949, trees of the Chinese Tallow tree, *Stillingia sebifera* Michx., growing in the vicinity of Beaumont, Texas, were observed to be producing shoots with symptoms characteristic of 2,4-D injury (Fig. 1). These trees had been accidentally injured with 2,4-D during the summer of 1948. Other trees of this variety were also observed that were purposely sprayed in 1948 in an attempt to kill them. Of 100 trees in this group that were examined, 14 were dead, and all of the rest showed characteristic symptoms of 2,4-D injury. No 2,4-D had been used in 1949, and the symptoms of injury appeared on the earliest growth. This indicates that the 2,4-D had persisted in the buds and other vegetative tissues of this plant from the time of injury in 1948. Some chinaberry trees, *Melia Azedarach* L., were also severely injured in the vicinity of Beaumont, Texas, in 1948, but no symptoms of 2,4-D injury were found on them in 1949, which indicates that 2,4-D does not persist in the vegetative tissues of this plant.

### References


1. BROWN, C. A., HOLDEMAN, Q. L., and HAGOOD, E. S. *La. Bull.* No. 426, 1948.
2. DUNLAP, A. A. *Phytopath.*, 1948, **38**, 638.
3. PRIDHAM, A. M. S. *Science*, 1947, **105**, 412.


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