

concentrations above equilibrium values, but the HD was less concentrated than that produced by the first method.

Wender, *et al.* (13) have prepared very pure HD by reacting D₂O with lithium aluminum hydride. Data for HD preparation by rectification of liquid H₂-HD-D₂ mixtures are given by Clusius and Starke (3a).

References

1. BELL, R. P. and EMELEUS, H. J. *Quart. Rev. (Chem. Soc., Lond.)*, 1948, 142.
2. BEUTLER, H., BRAUER, G., and JUNGER, H. O. *Naturwiss.*, 1936, **24**, 347.
3. DIBELER, V. H. and MOHLER, F. L. *J. Amer. chem. Soc.*, 1948, **70**, 987.
- 3a. CLUSIUS, K. and STARKE, K. *Z. Naturforsch.*, 1949, **4a**, 549.
4. DIBELER, V. H. *et al.* *Bur. Stand. J. Res.*, 1949, **43**, 97.
5. FARKAS, A. and FARKAS, L. *Proc. roy. Soc. Lond.*, 1934, **A144**, 16.
6. HEDBERG, K. and VERNER, V. Paper presented before 1949 meeting of American Chemical Society.
7. HONIG, R. E. *J. appl. Phys.*, 1945, **16**, 646.
8. ———. *J. chem. Phys.*, 1948, **16**, 837.
9. JONES, W. M. *J. chem. Phys.*, 1949, **17**, 1062.
10. NORTON, F. J. *Phys. Rev.*, 1949, **75**, 1957.
11. ———. *J. Amer. chem. Soc.*, 1949, **71**, 3488.
12. RITTENBERG, D., BLEAKNEY, W., and UREY, H. C. *J. chem. Phys.*, 1934, **2**, 48.
13. WENDER, I., FREIDEL, R. A., and ORCHIN, M. *J. Amer. chem. Soc.*, 1949, **71**, 1140.

A Study of the Albumin and Globulin Content in Postpartum Plasma and Its Use in Rheumatoid Arthritis

Louis W. Granirer,

Arthritis Clinic, Queens General Hospital,
Jamaica, New York

It was first reported from this clinic that a sustained remission can be produced in rheumatoid arthritis by the administration of suitable amounts of postpartum plasma (1). A review of the literature failed to reveal any data on the use of postpartum plasma in rheumatoid arthritis. The remission produced was characterized by an improved sense of well-being, a brighter mental outlook, increased

appetite, a disappearance of joint symptoms, a gain in weight, restoration to normal of the microcytic anemia and albumin globulin ratio. In general, the characteristic response was a striking clinical improvement. There were no toxic effects, and in 320 postpartum plasma transfusions there were no cases of homologous serum hepatitis. The longest remission following cessation of therapy was 16 weeks and the shortest was 3 weeks. Postpartum plasma is an available and comparatively inexpensive form of therapy. During the investigation the author noted that the postpartum plasma had a greenish tint, slightly opalescent and homogeneously distributed.

Table 1 reports the albumin and globulin content of the plasma obtained from 96 mothers after delivery. Each pool represents the pooled plasma of eight mothers.

Despite the hypoproteinemia, the mothers from whom this plasma was taken presented no anemia or edema. The typical average protein value was 4.35 mg % with an albumin globulin ratio of 0.9. The lack of correlation between plasma protein levels and edema was striking. A marked hypoalbuminemia was also observed and yet no impaired hepatic or renal function could be demonstrated. The postpartum plasma proteins reported here apparently resemble the pattern noted in the maternal sera at or near term (3). This similarity may provide an explanation for the efficacy of postpartum plasma in rheumatoid arthritis. The occurrence of pregnancy in patients with rheumatoid arthritis produces a partial or complete remission in a high percentage of cases (2).

At present there is no clear explanation of the ameliorating effect of postpartum plasma in the treatment of rheumatoid arthritis. There is suggestive evidence that this behavior is not due solely to a steroidal factor.

Further study of postpartum plasma will be reported.

References

1. GRANIRER, L. W. *7th int. Cong. rheumatic Dis.*, New York, June, 1949.
2. HENCH, P. S. *Proc. Staff Meet.*, Mayo Clin., 1938, **13**, 161.
3. MOORE, D. H., MARTIN, DUPAN M., and BUXTON, C. L. *Amer. J. Obstet. Gynec.*, 1949, **57**, 312.
4. WEICHELBAUM, T. B. *Amer. J. clin. Path.*, 1946, **10**, 40.

The Action of Pectinase Solutions on Sections of Acetone-fixed Human Tissues: A Preliminary Note

J. F. A. McManus and J. C. Saunders

Department of Pathology,
The Medical College of Alabama, Birmingham

The action of enzymes on tissue sections is a source of chemical information. It has been found that solutions of diastase remove glycogen (5). The usefulness of the procedure is limited by impurity of the enzyme preparations and by complexity of the substrate (2, 6). This paper introduces commercial pectinase as an enzyme active on suitably fixed human tissues, and discusses the validity of the information derived from its use.

Thin pieces of human tissue are fixed in ice-cold ace-

TABLE 1
POSTPARTUM PLASMA PROTEINS (4)

Pool	Albumin mg %	Globulin mg %	Total protein mg %
1	2.1	2.8	4.9
2	2.1	2.2	4.3
3	2.7	1.8	4.5
4	2.4	2.3	4.7
5	2.0	2.1	4.1
6	2.1	2.6	4.7
7	2.1	2.0	4.1
8	2.5	1.6	4.1
9	1.7	2.4	4.1
10	1.9	2.1	4.0
11	2.2	2.3	4.5
12	2.0	2.2	4.2
Average	2.15	2.20	4.35

tone, dehydrated and imbedded in paraffin by the method of Gomori (3). Sections of 4–6 μ thickness are baked on slides with glycerine egg albumin, then washed in toluene, graded alcohols, and finally in water. They are then incubated for 48 hr at 37° C in one of the following solutions, each at 0.4% strength: pectinase (Nutritional Biochemical Company or Rohm and Haas), pectinol O (Rohm and Haas), pectin esterase (Rohm and Haas), polygalacturonase (4), β -glucuronidase (1).

The pH of the solution is adjusted to 4.0 by acetate-acetic acid buffer and checked with a Beckman pH meter. A crystal of thymol is added to each solution to inhibit bacterial growth.

After the period of incubation the slides are washed in running tap water for 5 min. The sections are stained with hematoxylin and eosin, or with the acid orcein stain for elastic tissue, or colored by the periodic acid-Schiff's reagent (PAS) method (7), with and without a counterstain of hematoxylin. Usually, each set of sections has been stained or colored by the three methods.

There is a loss of PAS-positive materials—mucin, glycogen, reticulin of spleen and lymph node, ground substance of cartilage, hyaline, etc.—with pectinase solutions. With pectinol O the removal is not so complete as with pectinase although qualitatively similar. Polygalacturonase removes about the same amount as pectinase. Pectin esterase does not remove PAS-positive material but enhances the coloration. β -Glucuronidase does not remove PAS-positive material. Diastase in 1% solution at pH 6.8 removes everything, and usually the section from the slide with 48-hr incubation at 37° C. Pectinase solution does not remove nuclear material or elastic tissue.

Two effects of pectinase solutions are to be differentiated—one morphological and the other histochemical. The removal of hyaline, while leaving nuclear material and elastica, allows something like a microdissection on the slide. Elastic fibers of blood vessels become traceable in their finest ramifications. The hyaline in the glomeruli in Kimmelstiel-Wilson intercapillary glomerulosclerosis is seen to contain nuclear material. It can be completely removed, as can tubular basement membrane, whereas glomerular basement membrane is preserved.

The evidence that PAS-positive materials may be carbohydrates is enhanced by their removal by pectinase and especially by polygalacturonase. The data are not taken to be conclusive for the reasons mentioned earlier—complexity of enzyme and substrate. A pure enzyme is difficult to prove and a pure substrate is difficult to find, especially in nature. The chemical information should be considered conditional until pure enzymes, electrophoretically homogeneous and crystalline, have been used in a large series.

Techniques and results will be described in full in later publications. For the present, morphological information of definite value can be derived from the action of pectinase solutions on acetone-fixed human tissues. In the future, enzymes of the pectinase group may give chemical information about tissue structures composed of or containing the appropriate substrate.

References

1. ASTWOOD, E. B. Unpublished observations, in Talbot, N. B., et al., *J. biol. Chem.*, 1948, **151**, 607.
2. DANIELLI, J. F. *Symposia of the society for experimental biology no. 1*. Cambridge: Cambridge Univ. Press, 1947. P. 101.
3. GOMORI, G. *Proc. Soc. exp. Biol. Med.*, 1945, **58**, 362.
4. JANSEN, E. F. and MACDONNELL, L. R. *Arch. Biochem.*, 1945, **8**, 97.
5. LILLIE, R. D. and GRECO, J. *Stain Technology*, 1947, **22**, 67.
6. LISON, L. *Histochemie animale: méthodes et problèmes*. Paris: Gauthier-Villars, 1936.
7. MCMANUS, J. F. A. *Stain Technology*, 1948, **23**, 99.

The Action of Radioactive Phosphorus in *Drosophila*¹

Johanna Blumel^{2, 3}

Genetics Laboratory, The University of Texas, Austin

Since Muller's (2) classical discovery that x-rays produce mutations in *Drosophila melanogaster*, much attention has been directed to the study of this action. More recently ultraviolet light, radium, and chemicals have also been employed to induce mutations. Law (1) attempted to influence the lethal mutation rate of *Drosophila melanogaster* by the use of radioactive phosphorus. However, no lethals were found after injecting various concentrations of radioactive Na₂HPO₄ into 4-day-old larvae of the Oregon-R strain. In the present investigation, radioactive P³² was used to study the action of beta rays on *Drosophila melanogaster* and *Drosophila virilis*.

The stock of *Drosophila virilis* Sturtevant used is a lethal-free and fertile strain from Pasadena. The Muller-5 of *D. melanogaster* used has an X-chromosome marked by the dominant gene Bar (B), the recessive gene apricot (w^a), and the scute (sc^s) inversion.

In each case, pairs of mature flies from stock bottles were placed in shell vials containing a radioactive medium. This culture medium was prepared by adding approximately 3.2 ml of radioactive H₃PO₄ (containing about 1.54 mc/ml at the time of its use) to 300 ml of the standard *Drosophila* culture medium. The radioactivity of the original volume was determined with a Geiger counter, and was found to be 265,000 cpm/ml. The medium was distributed among 50 vials, each containing approximately 6 ml. Twenty-five vials were used to test *D. melanogaster*, and 25 for *D. virilis*.

Twelve days after exposure, the distribution of radioactivity in the various tissues of *D. virilis* and *D. melanogaster* was determined. The results are summarized in Table 1. The determinations on distributions of radioactivity on *D. virilis* were made on the original flies, the treated larvae, and the brains, gonads, and salivary glands dissected from treated larvae. No treated adult *D. virilis*

¹ This investigation was supported partially by a Rosalie B. Hite predoctoral fellowship.

² Present address, St. Joseph's Infirmary School of Nursing, Houston, Texas.

³ I wish to thank Dr. G. S. Rabideau, Department of Botany, University of Texas, for his assistance and use of equipment.