

Serum Haptoglobin in Rabbits after Subcutaneous Injection of Freund's Adjuvant or Turpentine.* (27863)

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Murray and Connell(1) have reported an increase in serum haptoglobin concentration in rabbits following one injection of 1 ml of turpentine subcutaneously. The increase was noted on starch gel electrophoresis but was not accurately quantitated.

During studies on the antigenicity of hemoglobin-haptoglobin complexes we have used single and multiple injections of turpentine to produce haptoglobin-rich rabbit serum and injections of Freund's adjuvant to encourage antibody response in the animals challenged with hemoglobin and hemoglobin-haptoglobin complex. Because the rate of clearance of hemoglobin from the blood is considerably faster than the clearance of hemoglobin-complex(2) the haptoglobin concentration could affect the antigenic activity of hemoglobin. It was necessary, therefore, to determine accurately the normal concentration of serum haptoglobin in rabbits as well as the haptoglobin response to single or multiple injections of turpentine or Freund's adjuvant.

Materials and methods. White female chinchilla rabbits weighing about 2.5 kg, obtained from a commercial breeder, were maintained in air conditioned quarters on a diet of Purina guinea-pig chow *ad lib*. All blood was obtained by cardiac puncture without anesthesia and with care to avoid hemolysis. Turpentine or Freund's adjuvant (Difco Complete Adjuvant) was injected subcutaneously into the clipped dorso-lumbar region; the injection and bleeding schedule is shown in Fig. 2 and 3.

Blood was allowed to clot, centrifuged, and serum was harvested. Haptoglobin concentration was determined by scanning and integration from filter paper electrophoresis strips (3). Duplicate determinations on the same serum have shown the accuracy of the method to be ± 9 mg % at a concentration of 150

mg %. Simple inspection of starch-gels(4) was used as a check on the filter paper method. Determination of serum hemoglobin concentration was by the method of Dacie (5).

Results. The normal concentration of serum haptoglobin in the rabbit is 0 to 50 mg % (Fig. 1). The distribution curve is roughly symmetrical. A single subcutaneous injection of turpentine (1 ml) causes a significant rise of serum haptoglobin concentration, the peak of the response occurring during the 1st to 4th day (Fig. 2). By the 7th day 5 of 6 rabbits had returned to normal. Multiple subcutaneous injections of turpentine produce a sustained response (Fig. 3) which tends to diminish over a period of 26 days. Multiple injections of turpentine produce higher haptoglobin levels than do single injections. Single or multiple subcutaneous injections of Freund's adjuvant produce no significant elevation of the serum haptoglobin concentration, since no value higher than 70 mg% was encountered even after 16 days of multiple injections.

Discussion. Murray and Connell(1) gave daily injections of turpentine to one rabbit and found that the maximum level of haptoglobin and the rate of disappearance were within the limits observed when only a single injection was given. Our data show instead that the haptoglobin response to multiple injections is both more intense and longer lasting. Robert *et al.*(6) also found the haptoglobin level to be elevated after 3 months of biweekly turpentine injections. There is no support, therefore, for the suggestion(1) that there exists a pool of tissue haptoglobin which can be readily used up as a result of a single response to inflammation. The multiple-stimulated rabbit can serve as a long term source of haptoglobin-rich serum.

Serum haptoglobin concentration is in-

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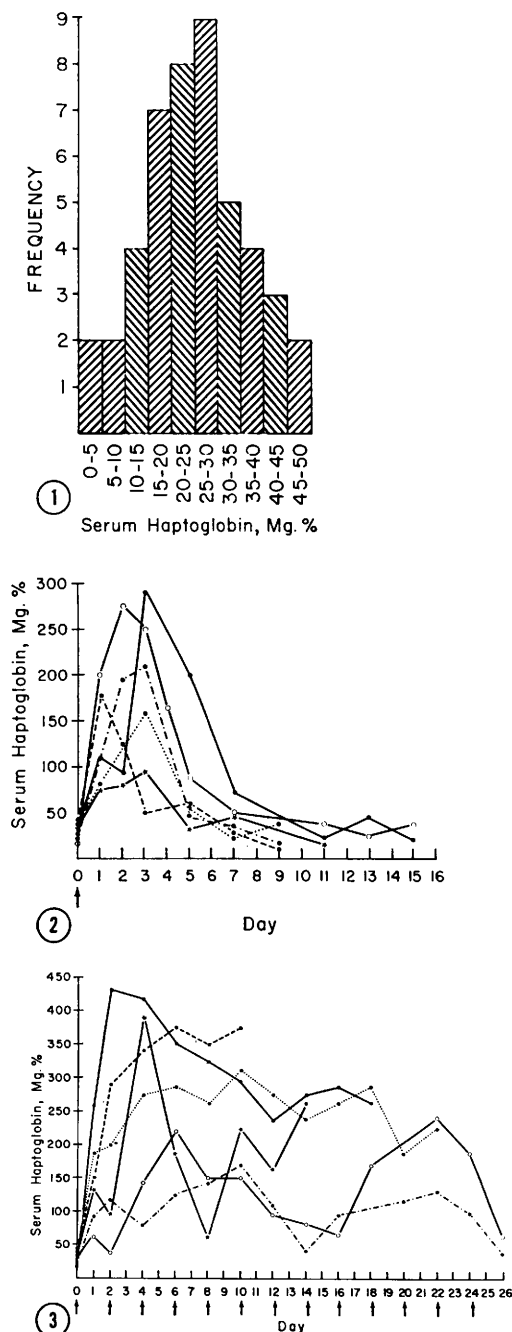


FIG. 1. Frequency distribution of serum haptoglobin.

globin concentration in 46 normal untreated rabbits.

FIG. 2. Serial determinations of serum haptoglobin concentration in 6 rabbits after a single injection of 1 ml of turpentine subcutaneously on day 0.

FIG. 3. Serial determinations of serum haptoglobin concentration in 6 rabbits given multiple injections at different sites of 1 ml of turpentine subcutaneously every other day.

creased in a wide variety of diseases in the human(7). In the rabbit, hyperhaptoglobinemia can be produced by injecting turpentine(1,6) and various enzymes(6). Chemical inflammation produced by injecting 2N HCl does not result in hyperhaptoglobinemia. The failure to increase the serum concentration of haptoglobin by single or multiple injections of Freund's adjuvant may be of interest to those investigating the immunology of hemoglobin.

Summary. The concentration of serum haptoglobin in normal and stimulated rabbits was studied. Hyperhaptoglobinemia could be produced by both single and multiple subcutaneous injections of turpentine; multiple injections resulted in more striking and longer lasting hyperhaptoglobinemia. Single or multiple subcutaneous injections of Freund's adjuvant caused no significant elevation of the serum haptoglobin concentration.

1. Murray, R. K., Connell, G. E., *Nature*, 1960, v186, 86.
2. Murray, R. K., Connell, G. E., Pert, J. H., *Blood*, 1961, v17, 45.
3. Miale, J. B., *Laboratory Medicine-Hematology*, C. V. Mosby Co., St. Louis, p852.
4. Smithies, O., *Adv. in Protein Chem.*, 1959, v14, 65.
5. Dacie, J. V., *Practical Haematology*, Chemical Publishing Co., Inc., New York, 1956, p139.
6. Robert, L., Mombelloni, P., Crosti, P., *Proc. Soc. Exp. Biol. and Med.*, 1961, v107, 499.
7. Nyman, M., *Scand. J. Clin. and Lab. Invest.*, 1959, v2, Suppl. 39.

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