creased endotoxin is in hepatic cells rather than Kupffer cells.

The relatively constant percentages of radioactivity remaining in liver and spleen suggest a number of possibilities. First, radioactivity in liver represents cleaved Cr^{51}. This seems unlikely because it has been shown that free Cr^{51} does not tend to accumulate in liver or spleen. A second possibility is that the Cr^{51} is attached to some degraded or detoxified endotoxin in these organs. The possibility of a liver "endotoxinase" must be considered. A third possibility is that the cells of the reticuloendothelial system protect the animal by holding the endotoxin and releasing it very slowly over long periods. One finding in this study that is difficult to relate to reticuloendothelial system saturation as a factor in death from endotoxin is the pattern of distribution following intramuscular administration of endotoxin. Amount and distribution of radioactivity, and presumably endotoxin, found in the reticuloendothelial system following intramuscular injection is compatible with life as demonstrated by intravenous injection of sublethal amounts of endotoxin, yet the animals injected intramuscularly, died. One possible explanation is that the tag is cleaved in muscle tissue with the Cr^{51} being excreted or more generally distributed and the Cr^{51}-free endotoxin is present but undetectable in liver. Another possible explanation is that a lethal mechanism of endotoxin is its persistence in the blood. Initially blood levels of endotoxin following intramuscular injection are low, but, in contrast to the pattern seen when other routes of injection were used, levels continued to rise and persist for at least 24 hours. The lethal mechanism of endotoxin may be vascular in this instance. Some evidence for such a time-dose type of theory is that mice rarely die from a barely lethal dose of endotoxin within the first 8 hours after injection and most are dead within 24 hours after injection.

Summary. 1. Intravenous injection of Cr^{51} tagged endotoxin into mice resulted in marked increase of circulating endotoxin as dosages reached the lethal range. 2. Distribution pattern of endotoxin in mice injected intracranially was similar to that of mice injected intravenously. 3. Distribution pattern of endotoxin in mice injected intramuscularly differed markedly from those found in mice injected intravenously or intracranially. 4. Premedication of mice with trypan blue resulted in greater susceptibility to endotoxin and increased amounts of circulating endotoxin in mice injected intramuscularly or intravenously. 5. Using the technique described, lethality of endotoxin for mice could not be related to any one distribution pattern.

1. Braude, A. I., Corey, F. J., Sutherland, D., Zalesky, M., *J. Clin. Invest.*, 1955, v34, 850.

2. Noyes, H. E., Balch, H. H., Pulaski, E. J., *Ann. Surg.*, 1956, v144, 51.

3. Braude, A. I., Corey, F. J., Zalesky, M., *J. Clin. Invest.*, 1955, v34, 858.

4. Corey, F. J., Braude, A. I., Zalesky, M., *ibid.*, 1958, v37, 441.

5. Rowley, D., Howard, J. G., Jenken, C. R., *Lancet*, 1956, v1, 366.

6. Cremer, N., Watson, D. W., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 510.

Received September 29, 1958. P.S.E.B.M., 1959, v100.

Chromatography of McCaslan Pole Bean Hemagglutinin on DEAE-Cellulose. (24526)

MORTON D. PRAGER AND R. J. SPEER

J. K. and Susie L. Wadley Research Inst. and Blood Center, Dallas, Texas

Phytohemagglutinin of McCaslan Pole Bean (*Phaseolus vulgaris*) has been a material of interest since discovery of its ability to agglutinate human erythrocytes (rbc). Boyd (1) observed that it agglutinates cells of blood groups A, B, and O (all Rh positive). Part of the interest in this material stems from its utility in preparing high purity leukocyte sus-

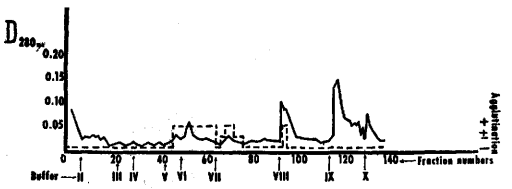

FIG. 1. Chromatogram of phytohemagglutinin. Buffer system (Roman numerals refer to individual buffers and their point of addition to dropping bottle): constant NaCl, 0.0125M; in following sequence M PO_4 and pH are given respectively, I, 0.005, 9.3; II, 0.01, 8.5; III, 0.02, 8.0; IV, 0.04, 7.5; V, 0.08, 7; VI, 0.16, 6; VII, 0.20, 5; VIII, 0.50, 5; IX, 1, 4.5; X, same as IX with NaCl increased to 0.50M. Flow rate, 15 ml/hr. Eluate vol, 2400 ml. D_{280} line ———; agglutination – – – – –.

pensions from peripheral blood samples(2,3). In a study of its purification and properties, Rigas and Osgood(4) found that phytohemagglutinin occurs as a mucoprotein and that hemagglutinating ability remains with protein after removal of polysaccharide. This report is concerned with further characterization of this substance by ion-exchange chromatography on the DEAE-cellulose of Sober and Peterson(5,6) under conditions with which we have studied a number of protein systems. Hemagglutinin distribution was determined by reacting column fractions with a panel of human rbc.

Methods. DEAE-cellulose (Brown Co., type 20, 100-300 mesh) with exchange capacity of 0.4 meq/g was prepared by serially washing 3 times with 0.1N NaOH, water, and 0.1N H_3PO_4, followed by a final wash with 0.1N NaOH. About 10 g in hydroxide form was brought to pH 9.3 with 0.005M H_3PO_4 containing 0.0125M NaCl. Equilibration was completed by washing twice by decantation with 21 buffer I (Fig. 1). Exchanger, poured into the column (10 mm i.d.) as a slurry, was packed under N_2 pressure (15-20 p.s.i.) to a height of 15 cm. Gradient elution was conducted by adding each buffer from an inverted bottle with a glass tube dipping beneath the liquid surface of a 500 ml Wolff bottle, which served as mixing chamber. The latter, fitted tightly to the column, was agitated with magnetic stirrer. A crude preparation of phytohemagglutinin was made by the method of Boyd(1) and was thoroughly dialyzed *vs.* buffer I before being added to the column. Chromatography was conducted on 3 ml of this material characterized by a titer of 1/512 with fresh rbc of varying blood group and Rh types. Chromatogram evaluation (Fig. 1) was by optical density measurement on individual fractions at 280 mμ (Beckman DU) and by agglutination tests with a panel of rbc from 5 donors, selected for a variety of blood group combinations. Rbc were typed for the following factors: A, B, O, C, D, E, c, e, C^w, D^u, E^u, M, N, P, Fy^a, Jk^a. These tests were performed by adding one drop of 2% rbc suspension (in saline) to one drop of chromatographic fraction in 10 x 75 mm test tube; tests were incubated at 25°C, 1 hr, and read microscopically. After overnight refrigeration, they were re-read microscopically. Fractions that gave agglutinates at first reading were scored +, those that were positive only after prolonged standing were scored ±.

Results. Fig. 1 reveals fractionation of the protein charge, as evidenced by several optical density peaks. Protein recovery by this chromatographic technic has been consistently 85-90%. Agglutinin was found in fractions 46-63, 66-75, and 93-94; several fractions on either side of 70 and 71 gave positive reactions only after overnight refrigeration. Each of the 5 cell types used in testing reacted equally well in each instance that positive reaction was observed. Titers were obtained for the 3 different groups of chromatographic fractions in which agglutinin was demonstrable; for fractions 52, 70 and 93, titers were 1/4, 1/1, 1/2, respectively. Titrations were identical with each of the 5 cells used. Because of dilution of activity by the large eluate volume, no fraction had a titer as high as that of the charge despite the significant protein concentration in fractions with no hemagglutinating activity.

Discussion. The several absorbance peaks in the chromatogram give evidence of significant protein fractionation. The elution system (previously described for serum protein chromatography(7)) differs from that utilized by Sober *et al.*(6) and Fahey *et al.*(8), who have made extensive studies with DEAE-cellulose, in that higher initial pH and ionic

strength are used. We found that these eluting conditions prevent break-through of serum gamma globulins. Like human anti-A, anti-B, and anti-D(7), phytohemagglutinin is heterogeneous under these chromatographic conditions. In fractions exhibiting activity, agglutination was observed with each test rbc. When titrated *vs.* the rbc panel, crude bean extract and fractions from the 3 activity peaks gave the same titers with each rbc used. Thus, despite fractionation of hemagglutinating activity, it appears that panagglutinin character is preserved qualitatively and quantitatively in each segment of the chromatogram.

Summary. A crude preparation of McCaslan Pole Bean phytohemagglutinin has been fractionated chromatographically on DEAE-cellulose. Hemagglutinating activity, followed by testing each fraction with an rbc panel, was found in 3 groups of fractions. In each of these groups the activity was panagglutinating and gave the same titers with each rbc used in testing.

1. Boyd, W. C., Reguera, R. M., *J. Immunol.*, 1949, v62, 333.
2. Li, J. G., Osgood, E. E., *Blood*, 1949, v4, 670.
3. Prager, M. D., Goerner, M., Matthews, N., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 726.
4. Rigas, D. A., Osgood, E. E., *J. Biol. Chem.*, 1955, v212, 607.
5. Peterson, E. A., Sober, H. A., *J. Am. Chem., Soc.*, 1956, v78, 751.
6. Sober, H. A., Gutter, F. J., Wyckoff, M. M., Peterson, E. A., *ibid.*, 1956, v78, 756.
7. Speer, R. J., Prager, M. D., Kelley, T. F., Hill, J. M., *Abst. Am. Chem. Soc.*, Sept. 1957, p80C.
8. Fahey, J. L., McCoy, P. F., Goulian, M., *J. Clin. Invest.*, 1958, v37, 272.

Received September 29, 1958. P.S.E.B.M., 1959, v100.

Effect of Growth Hormone Upon Thyroid Secretion Rate in the Rat.* (24527)

CLARK E. GROSVENOR† AND CHARLES W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia

It has been shown that thyroid hormone output increases during growth(1) and lactation(2) in the rat. Growth hormone (GH), in addition to its well known ability to cause an increase in body weight(3), has been demonstrated to cause an increase in milk secretion in this species(4). It seemed of interest, therefore, to determine if these effects of GH were associated with an alteration in thyroid activity.

Materials and methods. Adult virgin female rats of Sprague-Dawley-Rolfsmeyer strain weighing 240-300 g were kept under conditions of uniform temperature (78 ± 1° F) in room artificially illuminated during normal daylight hours for 5-6 weeks prior to experiments. Some were bred with male rats of same strain during this time. Each of 29 litters obtained was reduced to 6 young shortly after birth and, on 4th day postpartum, each mother was injected i.p. with 2 μc carrier-free I^{131}. Thirty-five non-lactating rats were similarly injected. Forty-eight hours were allowed for fixation of I^{131} by the thyroid and for urinary elimination of excess isotope. External thyroid counts were taken at this time and at either 24 or 48 hour intervals thereafter by first anaesthetizing each animal with ether, then placing it on a lead plate with its thyroid region over a scintillation probe. Measurements of thyroidal radioactivity were made with scintillation counter, Nuclear-Chicago (N.C. Model DS 5) connected to pulse height analyzer (N.C. Model 1810) which, in turn, was connected to a rate meter (N.C. Model 1620A). Conventional corrections were made for radioactive decay and background. *Measurement of thyroid secretion rate (TSR).* Twenty-four lactating and 15 non-lactating rats were injected subc.

* Contribution from Mo. Agr. Exp. Sta., Journal Series No. 1917. Approved by Director.

† Postdoctoral Fellow of N.I.H. This investigation was supported in part by grants from P.H.S.