peritoneal injection of 0.2 to 0.44 mc of colloidal radioactive gold. Control mice remained untreated or were treated with inactive (decayed) gold preparations.

2. On 3 consecutive days after treatment, specimens of peritoneal fluid were withdrawn by exploratory puncture from treated and control mice. Stained smears from these specimens revealed the absence of tumor cells or cytological abnormalities therein in gold treated mice, while in controls the tumor cells were numerous and showed high proportion of mitoses. The non-viability of morphologically alterated cells was demonstrated by their inability to multiply or even to survive after their transfer into new mice.

3. Ultramicroscopic colloidal gold particles mixed immediately after injection with the peritoneal fluid became condensed, after 24 to 48 hours, into dot-like particles in the cytoplasm of macrophages and during the following days into coarse clumps. The macrophages loaded with gold particles maintained their structural and functional integrity; lymphocytes and polymorphonuclears were partially and temporarily destroyed by radiation effect.

4. It is concluded that in the cellular peritoneal exudate from mice inoculated *i.p.* with S-37 cells, the tumor cells were highly sensitive to small amounts of radiation well tolerated by the mice themselves while macrophages showed very high resistance to the same agent. Thus, the complete destruction of free tumor cells in the peritoneal cavity of the mice may be described as a selective radiotherapeutic effect of radioactive colloidal gold.

Received May 26, 1950. P.S.E.B.M., 1950, v74.

A Simple Chromatographic Procedure for the Separation of Angiotonin from Crude Mixtures. (18002)

O. M. HELMER

From the Lilly Laboratory for Clinical Research, General Hospital, Indianapolis, Ind.

The purification of angiotonin (hypertensin) has been hampered not only by its lability but by its relatively high solubility in water and low solubility in ordinary organic solvents. In preliminary chromatographic experiments with the capillary-ascent test tube method of Rockland and Dunn(1), using 7% aqueous solution of phenol as the developing agent, it was found that angiotonin could be separated from materials that gave a positive ninhydrin color reaction. Although a satisfactory separation could be obtained with small quantities by this technic, as much as 800 to 4000 units of angiotonin could be isolated on a column prepared from No. 1 Whatman paper macerated in a Waring blendor with 7% aqueous phenol solution.

Methods. Preparation of column. Whatman No. 1 filter paper was macerated in a Waring blendor with 5% aqueous phenol (7 g reagent grade phenol in 100 cc distilled water) and transferred to a sulfur absorption tube of 30 mm diameter (Corning 39620) the fritted glass disc of which had been covered with a layer of glass beads. The tube was fitted into a suction flask by means of a one-hole rubber stopper. Suction was applied to the tube while the pulped paper was added in small portions, a wide-footed glass rod being used to aid in forming a tightly packed column. The column was washed with 7% phenol solution to remove any material possibly soluble in the solution.

Preparation of angiotonin. The angiotonin was prepared by Plentl and Page's modification(2) of the method of Page and Helmer(3) from renin substrate processed from hog serum and an angiotonase-free hog renin.

1. Rockland, L. B., and Dunn, M. S., *Science*, 1949, v109, 539.

2. Plentl, A. A., and Page, I. H., *J. Biol. Chem.*, 1945, v158, 49.

3. Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, v71, 29.

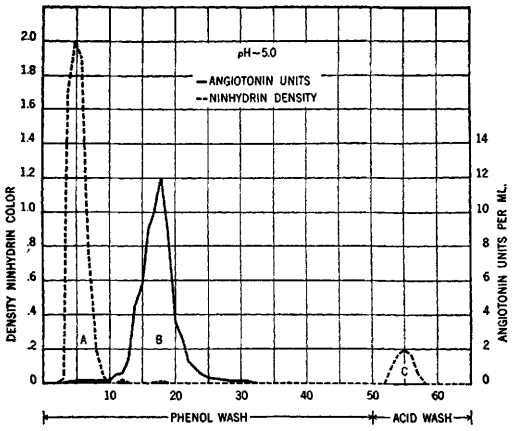

FIG. 1.

Chromatogram of a crude angiotonin preparation at pH 5.0. The number of 10 cc aliquots of effluent are shown by the abscissa.

Angiotonin assay. The angiotonin was tested in pithed cats prepared as described by Shipley, Helmer, and Kohlstaedt(4). The various samples were compared to a lyophilized laboratory standard of angiotonin, 30 μg of which is designated as 1 unit.

Ninhydrin color reaction. The photometric ninhydrin method of Moore and Stein(5) was used to determine the amount of ninhydrin color produced in the aliquots of effluent. The values were expressed in density units per ml of effluent.

Procedure. The crude angiotonin preparations to be fractionated were adjusted in different experiments at pH 4.6 to 7.0. In Fig. 1 is shown an experiment at pH 5.0 in which the paper column was 70 mm in height. Ten ml of angiotonin containing 80 units per ml were poured onto the top of the paper. Then 1 ml of 80% phenol solution was added to bring the mixture to a concentration of approximately 7% phenol. Gentle suction was applied to pull the solution into the paper. The column was then washed with 7% phenol until 50 aliquots of 10 ml of effluent had been collected. Then 0.1% acetic acid was substituted for the phenol solution and another 15 samples were obtained. Each aliquot was extracted with ether to remove phenol and assayed for pressor activity and ninhydrin reacting components.

Results. As can be seen from Fig. 1, there was a fairly sharp separation of angiotonin from the components giving a positive ninhydrin reaction. There was also considerable purification. The starting material required 765 μg to give a pressor response equivalent to 1 angiotonin unit, whereas 38 μg of fraction B were equal to 1 unit. Consequently one passage through the column produced a 20-fold purification. The recovery from such experiments before lyophilization has been from 79 to 98%. Fractions A and C were without significant pressor activity when injected in quantities of 200 μg. Sample A, given in greater concentration, did have pressor properties, but, as demonstrated by its resistance to angiotonase, the pressor activity was not due to angiotonin. Of the pH adjustments of the original sample to be fractionated, pH 5.0 seems to be the most satisfactory. At pH 4.6 the separation of fractions A and B was not as sharp as at pH 5.0, while at pH 7.0 fraction B (angiotonin) was spread out over a wide area, all of the angiotonin not being recovered even when fifty 10 ml aliquots were collected.

One of the drawbacks of the use of alkaline silver in the purification of angiotonin has been the possible concomitant precipitation of histidine(2). In the present studies, histidine, along with salts and other impurities, was removed in fraction A which was found to give a strong color reaction with Ehrlich's diazo reagent. Edman(6) eliminaed histidine by electrodialysis. It appears that histidine can be easily removed under the conditions of the experiments reported in this paper.

Preparations of various degrees of purity have been run through the column at pH 5.0. One which assayed 130 μg per unit was increased in activity to 30 μg per unit; another assaying 50 μg per unit, after chromatography was equivalent to 27 μg per unit. The latter is the purest material separated by one passage through the column at pH 5.0, and its activity compares favorably with the best preparations made by the laborious technics

4. Shipley, R. E., Helmer, O. M., and Kohlstaedt, K. G., *Am. J. Physiol.,* 1947, v149, 708.

5. Moore, S., and Stein, W. H., *J. Biol. Chem.,* 1948, v176, 367.

6. Edman, P., *Arkiv. f. Kemi, Mineralogi och Geologi,* 1945, v22, 1.

of Plentl and Page(2) using metallic salt precipitations. Furthermore, a very high recovery of the angiotonin activity of the starting material can be obtained.

It has been possible to separate angiotonin in a salt-free condition from saturated sodium chloride solutions and from solutions containing ammonium sulfate.

By means of the column angiotonin has been demonstrated to be present in the blood stream of cats after the injection of renin. In these experiments the blood of the animal was collected directly into alcohol to prevent possible *in vitro* formation of angiotonin.

Discussion. Since each laboratory has its own standard for testing angiotonin activity it is difficult to compare the purity of the preparations described in this paper to that reported by Edman and by Plentl and Page. Edman's angiotonin gave a ninhydrin color reaction whereas ours did not. The activity in terms of weight may not necessarily be a criterion of purity since angiotonin may be a mixture of polypeptides. The latter question will be investigated.

Summary. A method has been described for the purification of angiotonin by means of a paper pulp column. By using an aqueous solution of phenol as a developing medium, the polypeptide (angiotonin) has been separated from free amino acids and salts and possibly from other inactive polypeptides.

The author wishes to gratefully acknowledge the technical assistance of R. M. Sanders, R. C. Powers, C. L. Goodman, and O. A. Harvey.

Received May 26, 1950. P.S.E.B.M., 1950, v74.

Direct Determinations of Plasma, Cell, and Organ-Blood Volumes in Normal and Hypervolemic Mice. (18003)

LEON WISH, JACOB FURTH, AND ROBERT H. STOREY

From the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Transplantation studies of X-ray induced ovarian tumors have shown that all animals bearing estrogen - secreting transplanted growths have a marked increase of plasma volume(1,2), while those bearing transplanted masculinizing growths of lutein cell origin have an associated polycythemia(3). In these earlier studies, the blood volume determinations were made with Evans blue (T-1824) and their accuracy, particularly that of the cell volume, is open to doubt. The duration of the correct mixing time is uncertain and there is some disappearance of dye from the blood during the period now commonly believed to be the "mixing" time(4,5). Furthermore, the venous hematocrit differs from the capillary hematocrit and the two may not parallel each other under abnormal conditions. For these reasons the radioisotope technics have been adapted to the direct determination of cell and plasma volumes in mice and procedures were worked out to obtain approximate information of organ volumes. Changes in organ volumes may aid in detecting the primary site of the plasma-volume rise which in turn may give a clue to the site of albumin production.

Comparative studies of red-cell and plasma volumes using ^{32}P-tagged red cells and T-1824, respectively, have been made recently in man and dogs. Nachman and associates (6) found that in man the red-cell volume as measured with ^{32}P-labeled red cells is about 20% less than the values obtained with T-

1. Furth, J., and Sobel, H., *J. Natl. Cancer Inst.*, 1946, v7, 103.

2. Bali, T., and Furth, J., *Cancer Res.*, 1949, v9, 449.

3. Gottschalk, R., and Furth, J., in preparation.

4. Lawson, H. C., Overbey, D. T., Moore, J. C., and Shadle, O. W., *Am. J. Physiol.*, 1947, v151, 282.

5. Krieger, H., Storaasli, J. P., Friedell, H. L., and Holden, W. D., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 511.