TABLE I. Effect of Increasing Time Between Injections of PMS and HCG upon Number of Ova Induced to Ovulate in Immature Mice.

No. of animals	Time interval between injections (hr)	No. of ova induced	Mean No. ova/animal	% Ovulating
3	24	114	38.0	100
3	30	144	48.0	100
6	50	104	17.3	83
12	60	199	16.6	83
18	100	89	4.9	89
9	120	25	2.7	77
4	141	3	.75	50
3	148	2	.66	33
14	152	0	0	0

tered gonadotrophin is not of longer duration.

Summary. Increasing the time interval between PMS and HCG administration in immature mice indicates that the biological life of small concentrations of PMS is approximately 6 days.

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Angiotensinase Activity of Fractionated Human Plasma.* (29620)

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One mechanism which may be important in the understanding of angiotensin metabolism is that of the angiotensinase activity of plasma. Our experiments designed for isolation and purification of this plasma factor have reached a point where a source of an angiotensinase active fraction is now readily available. The active fraction is easily produced, and retains its activity during storage which permits its use over a period of time as a standard of reference.

Methods and materials. The precise measurement of plasma angiotensinase activity was by a standard bioassay(1). In principle, this test consists of mixing equal volumes of the plasma or its subfractions and a solution of synthetic angiotensin standard (1 μg per ml of val^5 angiotensin II amide, Ciba). After a period of incubation the destruction of the standard was determined by observing the loss of pressure response on intravenous injection of this mixture into a standard bioassay rat, as compared to the response of the standard alone. The term % angiotensinase activity was adopted and is by definition the % destruction of the angiotensin standard when reacted for 60 minutes at 37°C with the plasma fractions, as compared to the

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vasopressor response of the standard alone. Replicate determinations gave a standard deviation of ± 4%, as an index of the accuracy of the method(1). Complete destruction of this enzymatic action was achieved by boiling the mixture for 5 minutes at the end of the reaction period.

Preparative electrophoretic fractionation of plasma was made on a Pevikon block(2). Pevikon is a plastic material consisting of a copolymer of polyvinyl chloride and polyvinyl acetate. The 30 cm wide, by 50 cm long, by 0.6 cm thick block was formed by pouring a slurry of Pevikon and a pH 8.4 buffer mixture of 0.025 M NaCl and 0.025 M Na phosphate into a trough, whose open ends were sealed by sponges forming an open box. A 4 mm wide slot was cut into the formed block, 15 cm from the cathodal end, its bottom sealed with Pevikon, and the slot filled with 10 ml of heparinized plasma from a normal male donor, mixed with Pevikon. Electrophoresis was at 5 volts per cm for 20 hours at 2°C.

Two cm cuts were taken and the fluid extracted by putting the cuts in individual plastic cups whose perforated bottoms were covered with Millipore HA filter membranes having a pore size of 0.45 μ. These cups were then placed in plastic centrifuge tubes and the fluid centrifuged free of the Pevikon at 3000 *g*.

Each cut, at plasma concentration, was analyzed for angiotensinase activity, the protein content determined by microkjeldahl analysis, characterized by immunoelectrophoresis by the microtechnique, and by electrophoresis on Millipore cellulose acetate. The electrophoretic cuts which showed angiotensinase activity were also analyzed by Tiselius electrophoresis.

Subfractions of human plasma were made by a modification of Method XII of Surgenor *et al*(3), in which blood was collected through a Dowex-50 resin column, cooled quickly in ice water, twice centrifuged at 4000 *g* for 10 minutes, and the greater part of the plasma globulins precipitated with zinc glycinate reagent at a final concentration of 20 mM. This was completed within 30 to 40 minutes from

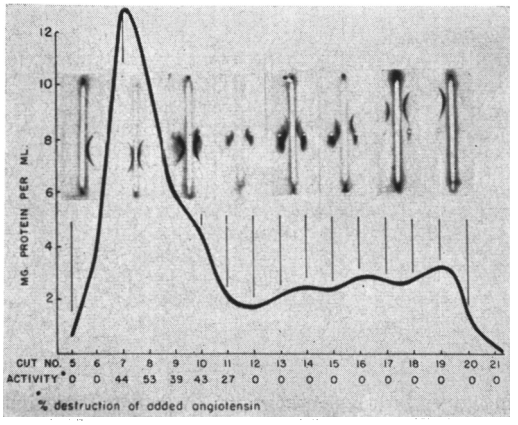

FIG. 1. Analysis of fractionated plasma electrophoretically separated on a preparative Pevikon block, for angiotensinase activity, nitrogen content, and plasma profile.

time of collection. After 2 hours of stirring, the mixture was centrifuged, and EDTA at pH 7.2 was added to the supernatant to 20 mM concentration. This was then dialyzed overnight at 2°C against large amounts of distilled water. Following this the supernatant, called Stable Plasma Protein Solution, or SPPS, was frozen with dry ice and alcohol, and then lyophilized and stored. Several lots had been stored at —15°C for over 2 years.

Further fractionation of the subfractions was made on a Sephadex G-100 column in isotonic saline at pH of 5.5. The eluates were brought to pH 7.4 using 0.1 N sodium bicarbonate, concentrated by ultrafiltration, read in a Cary 14 Spectrophotometer at 280 mμ and tested in the bioassay described.

Results. Fig. 1 shows the fractionation of plasma on the Pevikon block. The curved line represents the microkjeldahl nitrogen analysis of each cut with its per cent angiotensinase activity listed below the abscissa. Immunoelectrophoretic analysis of each cut showed that while cut 6 had no activity, albumin was clearly present. Cut 7 was the first cut to show angiotensinase activity. This cut was taken just below the albumin peak. This cut, when analyzed by immunoelectrophoresis, showed in addition to albumin, orosomucoid and alpha-1 lipoprotein.

Subfractions of SPPS. Three different stored lots of SPPS when tested for angio-

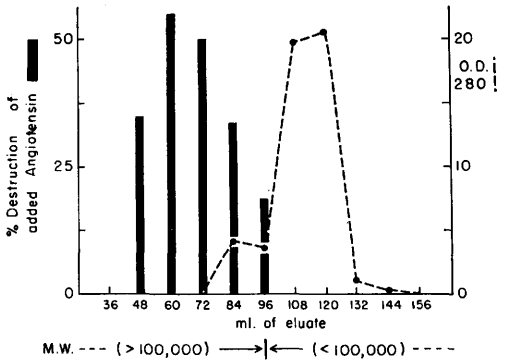

FIG. 2. Chromatographic separation of the angiotensinase active fraction from albumin on a Sephadex G-100 column. The optical density (O.D.) plotted on the ordinate represents readings at a wavelength of 280 mμ in a Cary 14.

tensinase activity had activities of 42%, 0% and 67%. The lot having no angiotensinase activity was different from the other 2 only in that a comparatively long period (45 minutes) elapsed from time of collection to time centrifugation was started.

After two years the SPPS (having 67% angiotensinase activity) was brought into solution with isotonic saline, placed on the preparative Pevikon block and electrophoresis performed. The albumin fraction was found to represent 85% of the total protein of SPPS and to have 67% angiotensinase activity. Three identical runs over a 2-month period with 3 individual tests gave angiotensinase activities of 70 ± 7%.

Fig. 2 shows a run in which this albumin peak electrophoretically separated on the Pevikon block and equivalent to that coming from an estimated 3 ml of resin collected plasma was placed on a Sephadex G-100 column (57 cm × 2 cm). Readings of the effluent cuts in the Cary 14 Spectrophotometer at 280 mμ showed the angiotensinase activity in cuts having little protein and separated from the major protein peak, which was albumin. Cuts 48, 60 and 72 had no detectable protein in the analytical procedures used, while cut 84 had approximately 8 mg protein per ml. These active cuts were also scanned from 200 to 400 mμ, and no adsorption was observed. Angiotensinase activity was found in an estimated 0.05% of the original plasma proteins and in that column fraction associated with molecules greater than 100,000 molecular weight. Exclusion of the latter part of this column fraction eliminates more of the contaminating proteins.

Discussion. The angiotensinase activity of stored lyophilized SPPS, SPPS that was reconstituted and placed in the refrigerator for one week, and Sephadex separated SPPS, shows that the enzyme activity in this fraction is stable under many conditions of temperature, pH and ionic strength.

Whether this angiotensinase activity is due to one or several angiotensinases is not resolved. Recent work has shown the principal angiotensinase activity of plasma to be that of one or more aminopeptidases(4).

On the basis of recovery of added nitrogen the conditions under which the Sephadex column was run permitted little if any adsorption of the plasma constituents. Further, the column readily accomplished (1) a separation of the fraction having angiotensinase activity from the albumin, and (2) tentatively characterized the active molecules as having molecular weights of greater than 100,000.

The simplicity of the method of preparation lends itself to including the stable angiotensinase preparation in clinical and research tests. The growing body of work pertaining to the possible role of angiotensin in hypertensive and edematous states has clearly indicated the need for more information regarding its *in vivo* metabolism. The techniques described, permitting isolation of a stable angiotensinase active fraction should permit more sophisticated investigations of this important mechanism.

Summary. Electrophoresis and column fractionation of plasma, SPPS, and the albumin peak of SPPS after electrophoresis, demonstrated that the described angiotensinase activity is normally present in a fraction distinct from albumin, alpha-2, beta and gamma globulins. Its physical characteristics generally tend to classify it as an alpha-1 globulin of a single species or of several species having very similar characteristics. That it is a protein is strongly supported by the fact that protein precipitation techniques were used in its purification, that it was non-

dialyzable, and that it was completely destroyed by heating to 100°C.

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Intestinal Absorption of D-glucose (*in vitro*) in Hamster: Effects of a Large Dose of Reserpine.* (29621)

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The sympatho-adrenal system has been shown to be influential in intestinal absorption. Soulairac(1) reported that sympathectomy in the rat diminished intestinal absorption and reduces the "Coefficient of absorption" (*i.e.* mg sugar absorbed/100 g of rat/hour). Intestinal absorption of sugar is reduced, *in vivo*, after adrenalectomy(1,2) and thyroidectomy(1,3). The mechanism by which surgical removal of these endocrine organs results in a reduction of intestinal sugar absorption is not known. It therefore seemed pertinent to study sugar absorption and transport in the intestine of animals in which the humoral background was altered with a large dose of reserpine. An *in vitro* technique, utilizing isolated intestinal sacs, was selected for measurement of sugar absorption.

Materials and methods. A colony of hamsters, *Cricetus auratus*, each weighing from 100 to 125 g was maintained on an *ad libitum* diet of commercial Purina lab chow and intermittent feedings of fresh vegetable matter. Animals for reserpine treatment and controls were selected at random from the colony. Reserpine 2.5 mg/kg was administered intraperitoneally.

Animals were sacrificed at various periods, 6, 14, 20, and 24 hours after treatment with reserpine. Each animal was spinalectomized and the gut was removed. Two segments of intestine were used. Segment I, 1 to 7 cm from the pylorus, and Segment II, 8 to 14 cm from the pylorus, represented duodenal and jejunal tissue respectively. Histological examination confirmed morphological differences between the two areas.

Absorption and transport of D-glucose was measured *in vitro* by means of everted intestinal sac preparations; detailed descriptions have been published(4,5,6). Isolated segments were everted and suspended in Krebs-Ringer sodium bicarbonate buffered solution containing 100 mg % D-glucose. The everted intestinal sac was suspended in 8 ml of this solution and filled with 1 ml of the same solution. Thus the initial concentration in each side of the intestinal wall was identical. Uptake from the musocal side and concomitant increase on the serosal side was indicative of active transport. (The sugar moved against an apparent concentration gradient.) D-glucose concentrations in the mucosal and serosal compartments were analyzed colorimetrically using a glucose oxidase reaction (Glucostat from Worthington Biochemical Corp.). Dry weights of tissue were determined by analytical weighing and drying in an oven at 105°C for 24 hours. The results were calculated in terms of micromoles of sugar decreased in the mucosal compartment and the amount increased in the serosal compartment after 30 minutes of incubation.

The remaining pieces of gut, which had been flushed with cold Krebs-Ringer bicarbonate, were cut open, blotted, and quickly frozen at the temperature of dry ice. Using

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