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.

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

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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-

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TABLE I.
Literature Data on Red Cell Volumes by Different Technics and Relation of Average Body Hematocrit to Venous Hematocrit.

		Red-cell volume		
		^{32}P	^{59}Fe	Avg body hematocrit
Investigator	Species	T-1824	T-1824	Venous hematocrit
Nachman (6)	Man	.80		.88
Gibson (7)	"		.85	.91
Gibson (7)	Dog		.82	.91
Meneely (8)	Man	.	.80	.89
Reeve (9)	"	.87		.95
Mayerson (10)	"	.99		.915*
Gregerson (11)	"			.96*

* A correction factor for plasma trapped in hematocrit determination.

1824 dye. The discrepancy is, in their opinion, due in part to differences between hematocrit values in peripheral veins and smaller vessels, and in part to the intrinsic error of the centrifuge hematocrit. They estimate that the body hematocrit is on the average 0.88 times that of the venous hematocrit. Earlier data of others(7-11) are summarized in Table I.

Material and methods. The Evans-blue (T-1824 dye) technic for the determination of plasma volumes in mice has already been reported(1,3). The red-cell volumes were obtained by the injection of ^{32}P-labeled homologous erythrocytes according to the method of Hevesy and Zerahn(12) with a modification by Nieset *et al.*(13). Plasma labeled with ^{131}I was prepared by the procedure of Fine and Seligman(14) with the following modification. The unbound inorganic iodide was removed by passing the iodinated plasma repeatedly through an Amberlite IR-400 anion-exchange resin column, thereby eliminating the 48- to 72-hour dialysis. The organ-blood volumes were obtained as follows: The desired substance was injected in volumes of 0.1 to 0.2 ml into the tail vein of the animal under nembutal anesthesia. The blood was withdrawn from the heart in 5 to 6 minutes, the animal sacrificed, and the hematocrit measured by the capillary technic of Parpart and Ballentine(15). An autoscaler with automatic sample changer (Tracerlab Co., Boston, Massachusetts) was used to determine the radioactivity of the ^{32}P samples and a gamma ionization chamber(16) for that of ^{131}I. The ^{131}I activity was measured by inserting the entire organ in the chamber, whereas to measure ^{32}P activity the organ was dissolved in hot concentrated nitric acid and the resulting solution diluted to a known volume. An aliquot was evaporated to dryness in a cup and counted in the autoscaler. Care was taken to avoid any significant self-absorption by taking aliquots with very little total solid content.

Results. Simultaneous determinations of

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TABLE II.
Plasma Volumes in Normal and Hypervolemic Mice Determined Simultaneously with T-1824 Dye and Plasma Labelled with ^{131}I. Derivation of conversion value 1.2.

Procedure	Normal mice					Tumor-bearing mice: Granulosa				Lut.	Ca.
T-1824	5.5	5.5	5.6	5.8	6.3	8.2	8.4	9.5	9.6	6.1	12.9
^{131}I plasma	4.1	4.9	5.4	4.9	5.3	6.9	7.2	8.1	7.5	5.4	10.6
T-1824 / ^{131}I plasma	1.3	1.1	1.0	1.2	1.2	1.2	1.2	1.2	1.3	1.1	1.2

Plasma volumes are given as % body wt. Lut. = Transplantable luteoma, Strain IX(17). Ca = Transplantable carcinoma of ovarian origin (to be published).

TABLE III.
Plasma Volumes in Normal Rabbits Determined with T-1824 Dye and ^{131}I-Labeled Plasma.

Procedure	Females			Males		
	No.	Range % wt	Avg % wt	No.	Range % wt	Avg % wt
T-1824	9	3.8-5.1	4.3	14	3.5-4.8	4.1
^{131}I-plasma	12	3.0-4.9	4.0	9	2.7-4.4	3.8
T-1824/^{131}I			1.08			1.08

Evans-blue and ^{131}I-plasma volumes were made by introducing these two materials in immediate succession intravenously into the mice and rabbits. The results are shown in Tables II and III. These data indicate that the Evans-blue values, calculated on the basis of dye concentration after the conventional mixing time of 5 to 6 minutes, are higher than the iodinated plasma values. It is noteworthy that the concentration of ^{131}I plasma does not drop significantly within 2 to 5 minutes after injection as does Evans blue, indicating that during the so-called mixing time the dye is lost from the circulation. No difference was found in the values when either Evans blue or ^{131}I plasma was injected first.

Simultaneous direct cell and plasma volume determinations were made by the injection of ^{32}P-tagged homologous cells and Evans blue. The results in normal and in tumor-bearing mice are shown in Table IV. The Evans-blue values were divided by a factor of 1.2 to obtain the presumably correct plasma-volume concentration. These data indicate that the cell volumes, as calculated by the conventional Evans blue-hematocrit technic, are above those obtained by direct cell determinations by a factor of 1.54. Since the disappearance curves indicate thorough mixing of both plasma and cells at time of sampling it follows that blood in smaller vessels contains a smaller percent of cells than in the large vessels.

The direct determination of plasma and cell volumes enables a calculation of the average body hematocrit. This ratio of average body hematocrit to the venous hematocrit is 0.88 (Table IV). Thus all data indicate that there is a greater concentration of plasma in capillary than in venous blood by an unknown factor which is smaller than 0.88.

Data on three types of neoplasms are presented in Table IV: (a) an estrogenizing granulosa tumor, (b) a masculinizing luteoma, and (c) ovarian carcinoma causing no secondary changes in sex organs. The granulosa tumor causes a marked plasma-volume rise without a drop in red-cell volume, the luteoma a marked rise in cell volume with a slight but distinct rise in plasma volume and the ovarian carcinoma a moderate plasma-volume rise. These observations confirm those made earlier with T-1824(1) although

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TABLE IV.
Simultaneous Red-Cell and Plasma-Volume Determination in Mice.

Mice	No. in group	Red cell vol.			Plasma vol.		Total blood vol.			Hematocrit		
		^{32}P	T-1824	T-1824 ^{32}P	^{32}P	T-1824 1.2	^{32}P	T-1824	^{32}P + T-1824 1.2	Ven.	Avg body	Avg Ven.
Normal Rf/Ak ♂	3	3.31	5.10	1.54	4.20	5.14	7.52	11.27	8.45	44.2	39.2	.89
,, ♀	2	3.00	5.03	1.68	4.18	5.51	7.18	11.64	8.51	42.1	35.2	.84
Normal Rf ♂*	6	2.66	3.92	1.47	3.74	4.54	6.39	9.36	7.20	41.5	37.0	.89
Granulosa ♂†	2	3.55	4.93	1.39	9.00	10.34	12.55	17.35	13.89	28.2	25.6	.91
,, ♀†	3	3.45	5.80	1.68	5.75	8.17	9.21	15.59	11.62	37.6	30.2	.80
Ov. carcinoma ♂†	2	2.80	3.67	1.31	5.93	7.47	8.74	12.63	10.28	29.0	27.2	.94
Luteoma ♂†	2	4.35	7.25	1.67	4.23	5.65	8.58	14.04	10.0	50.5	43.3	.86
Avg				1.54								.88

The figures represent averages. *These males were fighting in the cage and may have been dehydrated. † Tumor-bearing mice.

the values published require corrections as indicated in Fig. 1.

The organ-blood volume values (Table V) are given here chiefly to indicate the usefulness of this procedure in such studies as hypervolemia, polycythemia and shock. These determinations yielded unexpectedly low blood-volume values in tumors. In sections, the tumors are rich in giant thin-walled vessels and, on the basis of fixed and stained microscopic sections, the tumors would appear to have a relatively high blood volume. It is possible that the circulation in tumors is sluggish; if so, tumor cells live in a state of relative anoxia. Experiments are in progress to ascertain the facts concerning circulation in tumors in order to determine the correctness of this assumption.

Discussion. Iodinated homologous plasma circulates in the blood for a period of 10 to 20 minutes at close to the level reached within a minute or two after injection and falls very slowly thereafter, whereas the Evans-blue values drop rapidly for 1 to 5 minutes following injection. Therefore, it can be presumed that this disappearance of Evans blue is due, not to mixing, but to withdrawal of dye from the circulation during this period. Evans blue like ^{131}I circulates in the blood in a form bound to blood proteins. The recent studies of Kruse and McMaster(18) indicate that protein-bound Evans blue is taken up primarily by reticuloendothelial cells (macrophages). The rapid withdrawal from the blood of various materials by the macrophage system parallels the rapid drop of Evans-blue tagged protein. Thus it is probable that Evans blue is actually well mixed within a minute or two, that some dye is withdrawn during this mixing period and that its subsequent drop is due to withdrawal mainly by macrophages. However, postmortem examinations of animals at a later time indicate widespread blue discoloration of organs that are not part of this system. The possible use of the rate of disappearance of Evans blue from the blood as a measure of reticuloendothelial function requires further study. In

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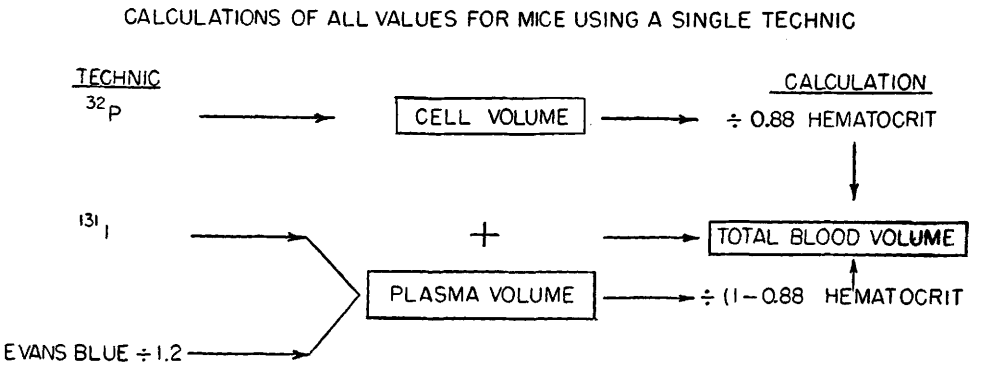

FIG. 1.

TABLE V.
Organ-Blood Volumes by the 131I-Tagged Plasma Technic.

Mice	No. in group	Pl. vol. % body wt	Plasma volume (ml) per g of tissue				
			Spleen	Lung	Kidney	Liver	Tumor
Normal	9	5.1	.11	.23	.16	.20	—
Tumor bearing:							
Granulosa	5	7.5	.11	.30	.21	.24	.03
Ovarian ca.	2	8.5	.09	.22	.28	.35	.01
Luteoma	1	5.4	.06	.24	.19	.23	.02

The figures are averages. Most tumors in this series were of small or medium size.

both normal and hypervolemic mice there was a ratio of approximately 1.2 between Evans-blue and ^{131}I-plasma values. Thus the Evans-blue determinations are useful and nearly accurate if such a correction value is applied. It deserves emphasis that blood volumes calculated with the aid of the conversion values here given are only approximate and exact values can be obtained only by direct determinations. The conversion figures are based on estimated average body hematocrits; but the capillary hematocrits are expected to vary in different physiological and pathological states introducing an error of unknown magnitude.

Summary and conclusion. Direct single and combined cell- and plasma-volume determinations were made on normal and polycythemic mice with ^{32}P-tagged red cells and ^{131}I-tagged plasma. The data indicate that: (a) The Evans-blue plasma volumes in mice are above the probably more correct ^{131}I-plasma volumes by a factor of 1.2 and in rabbits by a factor of 1.08. (b) The so-called mixing time of Evans blue is a withdrawal time of this dye by Evans blue-affin cells, most of which are probably macrophages. (c) The cell volume calculations based on Evans-blue values are higher than those obtained by direct determinations with the ^{32}P technic by a factor of 1.56 in normal and 1.51 in tumor-bearing mice tested. (d) The average body hematocrit in mice is approximately 0.88 times the venous hematocrit. (e) On the basis of these conversion values a calculation of all values can be made if only one technic is employed (Fig. 1). (f) Hypervolemia caused by estrogen-secreting tumors is attributable to an almost selective plasma-volume rise while that with luteoma is due primarily to polycythemia and, to a lesser extent, to a plasma volume rise.

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