and is probably responsible for its low isoelectric point. Fraction F contains 9% sialic acid, while Fractions D and E contain 7%.

Fraction G was obtained in a yield of 16 mg. Calculations based on amount of starting material indicate that the active fraction stimulates erythropoiesis when injected in 50 μg quantities. Preliminary dose response data using Fraction G suggest that the injection of 10 μg per day will induce a measurable erythropoietic response.

This separation and isolation of erythropoietin is reproducible, and similar results have been obtained from different lots of starting APF material. Studies are currently underway to further characterize this erythropoietic factor.

Summary. A method for purification of erythropoietin from the filtrate of acidified, boiled plasma prepared from phenylhydrazine anemic rabbits utilizing DEAE-cellulose ion-exchange columns has been described. The active erythropoietic factor thus prepared has been partially characterized and shown to be a low molecular weight, acidic glycoprotein.

TABLE I. Erythropoietic Activity of Effluent Fractions, Procedure I.

Fraction pool	Reticulocytes (%)*			Fe^{59} incorporation in RBC (%)*		
A	2.1	.4	(4)	26.7	4.3	(7)
B	1.6	.5	(4)	27.4	10.4	(4)
C	*6.1*	*1.5*	(4)	*50.5*	*2.2*	(9)
APF	*3.4*	*.6*	(4)	*39.9*	*4.4*	(4)
Saline control	1.8	.3	(4)	21.0	3.6	(7)

* Mean ± S.D.

Figures in parentheses indicate No. of animals in group.

Italicized values statistically significant, P = .01 or less.

TABLE II. Erythropoietic Activity of Effluent Fractions, Procedure II. (Four animals/group.)

Fraction pool	Reticulocytes (%)*		Fe^{59} incorporation in RBC (%)*	
D	2.7	.6	28.3	6.6
E	2.5	.4	26.9	4.5
F	2.6	.4	*29.6*	*1.0*
G	*6.5*	*1.3*	*47.6*	*2.5*
APF	*3.5*	*.5*	*40.7*	*6.9*
NaCl-acetate control	2.3	.4	21.0	2.6

* Mean ± S.D.

Italicized values statistically significant, P = .01 or less.

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Method for Studying Translocation of Some Radioisotopes into Organs from Site of Injection.* (24121)

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Therapeutic application of a radioisotope is outlined by knowledge of its fate in the body. This information is acquired by testing amount of radioactivity in organs of animals

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sacrificed at requisite intervals after radioisotope injection. For pure beta emitters with only a short range of irradiation, the knowledge of their distribution is practically equivalent to knowledge of their action. Thus, therapeutic possibilities of radioactive Yttrium (Y^{90}) were investigated by Kyker(1) in rats serially sacrificed at intervals of 1, 3, 6 and 8 days after injections by various routes. Lahr, Olsen, Gleason and Tabern(2) studied on comparative lines the distribution of pure or prevalently beta emitters (Y^{90} chloride and phosphate, radioactive chromic phosphate and colloidal Au^{198}) 5 to 7 days after subcutaneous or intracavitary administration. Ruth Lewin *et al.*(3) have sacrificed ascites tumor bearing mice on third day after i.p. injection of Y^{90}. It was our purpose to provide additional information by injecting beta emitting isotopes subcutaneously into the mouse tail and by amputating this organ at various intervals between injection and autopsy. Accordingly, we were able to record level of radioactivity at site of injection at various time intervals after administration of an isotope; and, moreover, to correlate these findings with successive changes in amount of radioactivity stored in organs. The results and their interpretation are reported below.

Material and methods. a) Swiss Albino male mice of about 25 g weight were used. b) Radioactive yttrium chloride ($Y^{90}Cl_3$) was obtained from Abbott Laboratories, Oak Ridge, Tenn.; radioactive chromic phosphate ($CrP^{32}O_4$) from the same, North Chicago, Ill. Doses of 0.05 mc in 0.1 ml were used for injection. c) *Technics of injections and amputations.* Doses of 0.05 mc of $Y^{90}Cl_3$ solution and of $CrP^{32}O_4$ colloidal suspension in 0.05 or 0.1 ml were injected into ventral surface of tail of mice. At intervals of 1 minute to 3 days after injection, the tails were serially amputated and preserved until the 3rd day when every mouse was sacrificed. Their organs (liver, lung, spleen, kidney), tails, and heart blood (0.3 ml) were emulsified and tested for radioactivity content. The material was injected into a thin layer of connective tissue on ventral side of tail, carefully avoiding blood vessels. The tail was amputated with sharp surgical scissors at its junction with the sacral spine. It was refrigerated until the animal was sacrificed for autopsy. d) *Autopsies.* The tail, blood (0.3 ml of unclotted blood from heart), and the most important visceral organs (liver, spleen, both kidneys and the lung) were ground and suspended in detergent solution, placed into tin cups, and dried. Whole organs were used, since their weight was significantly constant in mice of the same batch. e) *Radioactivity counts.* Counting was performed with a Geiger tube and scaler. Specimens from all mice of the same group (sacrificed after same interval) were counted on same day within 4 to 5 hours. It is realized that the decay between the first and last count may amount to 5% for isotopes of short half life. To avoid this error we count, at the present time, all specimens within 1 hour by working with less active material. f) *Method of calculation.* To obtain strictly comparable data, we calculated the individual pattern of radioactivity for each mouse, using radioactivity in standard amount of heart blood (0.3 ml) as a unit. For each group of animals, the average pattern and extreme variations were recorded in the Tables as ratios of radioactivity in blood at various stages of distribution. This method of expressing results as count ratios instead of average activity of tissue was used in our previous publications(5). For comparison of radioactivity ratio "organ/blood" in various groups, the average blood radioactivity in group 2 (amputation after 10 minutes) was also used as standard in the other groups. The last column of each Table was calculated upon this standard.

Results. The calculated ratios of radioactivity were tabulated separately for each isotope (Tables I and II) according to source of material (various organs) and interval of time elapsing between injection of isotope and removal of injected tissue, *i.e.* tail amputation. Thus, in each Table, horizontal lines show patterns of radioisotope distribution in the body at various intervals, while vertical columns illustrate the fate of the isotope in each surveyed organ.

Both Tables present consistently a common feature, *i.e.*, absence of wide variations in radioactivity content of heart blood (last

TABLE I. Spread of Y^{90} from Site of Injection (Tail) into Blood and Organs at Various Intervals before Removal of Injected Tissue (Tail Amputation).

Group	Time between Y^{90} inj. into tail and tail amputation	Radioactivity content of inj. tissue (tail) and of visceral organs expressed as products of avg blood radioactivity in the same group					Comparison of blood radioactivity in different groups, with group 2 as 1
		Tail	Lung	Kidney	Spleen	Liver	
1	1–5 min.	82.0 (80.2-100.3)	1.0 (.9-1.2)	1.7 (1.3-2.2)	2.5 (1.1-2.7)	4.4 (1.9-6.2)	1.1 (.9-1.3)
2	10	86.5 (78.7-96.1)	1.8 (1.1-2.3)	1.3 (1.2-1.6)	2.7 (1.0-2.3)	3.9 (3.4-4.2)	1.0 (1.0-1.1)
3	15–20	86.1 (80.8-92.5)	1.7 (1.2-2.2)	1.2 (1.0-1.8)	2.6 (1.3-3.3)	3.8 (3.7-4.1)	1.1 (1.0-1.1)
4	30	53.4 (42.6-59.9)	1.0 (.9-1.2)	1.3 (1.0-1.6)	1.5 (1.2-1.9)	5.1 (3.1-5.4)	1.1 (.9-1.2)
5	45	54.1 (44.9-75.2)	1.3 (.9-1.5)	1.9 (.9-2.9)	1.3 (1.0-1.5)	4.6 (3.9-5.2)	1.0 (1.0-1.1)
6	1 hr	59.7 (55.1-72.1)	1.2 (1.0-1.5)	1.7 (.9-2.7)	1.2 (1.0-1.4)	5.9 (5.0-7.2)	1.3 (1.1-1.4)
7	3	69.8 (60.1-75.4)	1.1 (1.0-1.7)	1.9 (1.1-2.4)	1.9 (1.3-2.2)	5.3 (4.4-6.1)	1.2 (1.1-1.2)
8	24	57.9 (55.5-62.0)	1.7 (1.4-1.8)	1.7 (1.3-2.2)	1.7 (1.4-2.0)	4.9 (4.1-5.0)	1.2 (1.0-1.3)
9	48	43.5 (37.6-62.4)	1.9 (1.7-2.3)	1.6 (1.2-1.7)	1.6 (1.2-1.7)	5.2 (3.8-6.1)	1.1 (1.0-1.1)
10	72	40.8 (36.7-43.3)	2.3 (2.1-2.6)	1.9 (1.5-2.3)	1.9 (1.5-2.3)	5.1 (4.2-5.8)	1.1 (1.1-1.2)

Ratio for each tissue is avg of 15 mice in each group; range of variation is shown by minimum-maximum values in parentheses.

TABLE II. Spread of $CrP^{32}O_4$ from Site of Injection (Tail) into Blood and Organs at Various Intervals before Removal of Injected Tissue (Tail Amputation).

Group	Time between $CrP^{32}O_4$ inj. into tail and tail amputation	Radioactivity content of inj. tissue (tail) and of visceral organs expressed as products of avg blood radioactivity in the same group					Comparison of blood radioactivity in different groups, with group 2 as 1
		Tail	Lung	Kidney	Spleen	Liver	
1	1–5 min.	56.4 (52.2-57.1)	2.8 (1.9-3.1)	1.6 (1.2-2.1)	1.4 (1.1-1.7)	3.3 (2.2-4.1)	1.2 (1.0-1.4)
2	10	52.1 (51.4-54.3)	1.2 (.9-2.9)	1.7 (1.2-2.2)	1.2 (.8-1.3)	7.5 (5.1-8.9)	1.0 (.9-1.2)
3	15–20	62.1 (47.2-71.9)	1.6 (.9-2.2)	1.8 (1.2-1.5)	1.4 (1.2-1.9)	6.7 (5.3-7.2)	1.2 (1.0-1.2)
4	30	64.4 (62.2-71.6)	1.6 (1.1-1.3)	1.4 (.9-1.2)	1.4 (.9-2.0)	12.6 (10.2-15.6)	1.0 (1.0-1.1)
5	45	69.2 (53.3-77.5)	1.2 (1.1-1.3)	1.3 (.9-1.5)	1.3 (.9-1.5)	17.3 (16.2-18.2)	1.4 (1.2-1.6)
6	1 hr	43.9 (41.3-47.8)	1.1 (.9-1.1)	1.3 (.7-1.5)	1.1 (.9-1.3)	16.9 (15.3-17.4)	1.2 (1.1-1.3)
7	3	45.1 (39.3-47.7)	1.8 (1.0-2.1)	1.9 (1.2-2.2)	1.4 (1.1-1.8)	16.4 (15.8-16.9)	1.3 (1.2-1.4)
8	24	44.7 (38.2-47.3)	2.4 (1.7-2.5)	2.2 (1.7-2.8)	1.7 (1.5-1.9)	27.0 (25.1-29.0)	1.5 (1.1-2.0)
9	48	41.1 (37.3-46.1)	2.5 (1.8-2.2)	2.7 (1.6-3.5)	2.5 (2.1-3.5)	23.3 (21.4-24.4)	1.4 (1.1-1.7)
10	72	43.1 (41.5-46.7)	2.2 (1.8-2.6)	2.6 (2.2-3.9)	2.2 (1.8-3.2)	29.3 (28.2-30.1)	1.2 (1.0-1.3)

Ratio for each tissue is avg of 15 mice in each group; range of variation is shown by minimum-maximum values in parentheses.

column); thus, changes in ratios reflected variations of radioactivity in the organs. Both isotopes showed a common trend to be partially dislocated from the injected tissue, but for each isotope timing and amount of translocation were different: Y^{90} was accumulated in the tail during the first 20 minutes after injection in a much larger amount than $CrP^{32}O_4$, but it was found to be less after 30 minutes and remained at the same level for 24 hours and then dropped again. For $CrP^{32}O_4$ the initial storage was more limited and after a transient relative increase (15 to 45 minutes) it dropped below the initial level and remained at the same level during 3 days of observation.

Of visceral organs only the liver showed considerable radioactivity content which for Y^{90} did not vary significantly during the experiment. On the contrary, $CrP^{32}O_4$ pick-up by liver increased progressively during period of observation.

In other organs (spleen, kidney, lung), the organ/blood ratio of radioactivity was mostly within 1 to 2.8 range and only occasionally below 1; it was above 2 only for Y^{90} in the spleen during the first 30 minutes, and for chromic phosphate in all organs after 24 hours.

Accordingly, the pattern of radioisotopes distribution at various intervals after injection into the tail was outlined by radioactivity level in the tail and in the liver.

Discussion. The steady fall of radioactivity in the tail during the first hours after injection obviously resulted from removal of injected material by circulating blood. On the other hand, transient increase of radioactivity level 30 to 45 minutes after $CrP^{32}O_4$ injection and 1 to 3 hours after Y^{90} administration can be attributed to temporary obstacles of blood passage in tail vessels (blood clots, compression by swelling, etc.). This tentative explanation of the difference of the radioactivity in liver following administration of the two preparations would require dialysis as crucial evidence of the chromic phosphate preparation and a statement of the chemical dose of yttrium.

The abundant storage of Y^{90} in the injected tissue(1,2,4) is usually attributed to its fixation on tissue proteins. Considering conditions within the tail (highly vascularized narrow area with a minimum amount of loose connective tissue), an early release of yttrium-protein complex into the blood may be postulated for an explanation of the sharp drop in radioactivity after 30 minutes. The residual Y^{90} remained attached to tissue and underwent only a moderate loss afterwards.

The parallelism between decrease of $CrP^{32}O_4$ in the tail and its increase in liver suggested considerable translocation of material from site of injection into the liver. This process was excluded by the consistently stationary and low level of Y^{90} in the liver. It may be presumed that the Y^{90} released from the tail was taken by liver and spleen (increase of spleen radioactivity during its initial fall in the tail), but mostly by some tissue unrecorded in our experiment (bone(2)) or by excretion(1).

In these experiments, translocation of Y^{90} and colloidal $CrP^{32}O_4$ from site of injection was considerably more significant than in previously reported results(1,2,4) of intramuscular and subcutaneous administration. The pick-up of these isotopes by liver and on a small scale by other organs resembled the distribution pattern after their intravenous use. In fact, it could be roughly characterized as a delayed distribution pattern of intravenously injected $CrP^{32}O_4$ and Y^{90}. It should be remembered that in the ventral tail area the thin skin layer over the bone and cartilage frame encloses relatively large blood vessels with only a minimum of connective tissue between them. It is conceivable that in this area the injected material or its complex with proteins reached the blood stream more easily and more abundantly than from the dense muscle and the connective tissue provided only with small vessels.

Summary and conclusions. (1) Doses of 0.05 mc of $Y^{90}Cl^3$ solution and of $CrP^{32}O_4$ colloidal suspension in 0.05 or 0.1 ml were injected into ventral surface of tails in mice. At intervals of 1 minute to 3 days after injection, tails were serially amputated and preserved until 3rd day when every mouse was sacrificed; their organs (liver, lung, spleen, kidney), tails and heart blood (0.3 ml) were

emulsified, dried and tested for radioactivity content with a Geiger Tube Detector. (2) Results showed only a narrow range of radioactivity for each organ specimen calculated as a product of the average blood radioactivity in the same group of mice (amputated at the same time). (3) The calculated and tabulated data show that a significant proportion of $CrP^{32}O_4$ was translocated into the liver from the tail; for Y^{90} the initial storage in the tail was very high, but some translocation occurred, presumably by excretion. (4) The pattern of Y^{90} and $CrP^{32}O_4$ distribution in the body after injection into the tail was comparable to delayed results of intravenous injection; this resemblance was interpreted by high vascularization of the tail. (5) It was concluded that the method of serial tail amputation may be useful for outlining the dynamics of isotopes spread in bodies of experimental animals.

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Effects of Ethyl Alcohol on Cardiac Output and Its Distribution in the Rat.* (24122)

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Ethyl alcohol is generally considered to increase peripheral blood flow. Particular interest has been directed to its possible effects as a coronary and cerebral vasodilator. The present studies were designed to determine whether alcohol influences the cardiac output and its distribution in the albino rat.

Methods employed in determination of cardiac output and regional uptake of indicators have been described(1,2). The conditions in which the fractional distribution of an indicator and the fractional distribution of the cardiac output correspond with each other have also been indicated(1,2). The cardiac output and the uptake fraction of all organs other than the brain were measured with Rb^{86}. Iodoantipyrine (I^{131}) was used in the brain studies. Female rats of Sprague-Dawley strain were used unless otherwise noted. Animals weighed 200-250 g after an 18 hour fast during which they were allowed free access to water. Each animal received 5 ml 20% alcohol by intraperitoneal injection. This amount was sufficient to produce light anesthesia. Five to 10 minutes after administration of the alcohol, either $Rb^{86}Cl$ or iodoantipyrine (I^{131}) was given in 0.4 ml by injection into an exposed femoral vein. Determination of cardiac output was made with Rb^{86} in animals in which the carotid artery had been catheterized with a short length of polyethylene tubing. Blood samples were collected at 0.67 second intervals, using a sample collector described elsewhere(3), and the arterial concentration curve of indicator constructed. Cardiac output was calculated as described(1). Other animals were employed for determination of Rb^{86} or iodoantipyrine (I^{131}) uptake by the organs at 15 or 30 seconds after administration. At these times, all organs other than brain have the same extraction ratio for Rb^{86} as the whole body; the fraction of Rb^{86} appearing in an organ, therefore, describes the fraction of cardiac output which perfuses it. Cerebral blood flow, which cannot be meas-

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