will inhibit in albino rats the development of arthritis after injection of formalin into the joints(10). We have previously reported that ACTH lacked a beneficial effect as a preventive in the polyarthritis of rats(4). Since there is no exact counterpart in experimental animals of human rheumatoid arthritis, it is interesting that in these 2 types of experimental arthritis the results with cortisone and ACTH are different. It is also of interest that gold salts will favorably influence the rat polyarthritis as well as many cases of rheumatoid arthritis in man. It would be interesting to check the use of gold salts in the formalin arthritis.

In view of the previous reports of the beneficial effect of aureomycin on pleuropneumonia infections in animals and man (3,7) it is interesting that terramycin is also effective in preventing the development of the rat polyarthritis. This finding suggests that terramycin might be given clinical trial in patients with Reiter's syndrome and in early cases of rheumatoid arthritis.

Summary. Cortisone failed to prevent the development of the polyarthritis of rats when its administration was begun on the day of infection or to improve it when given therapeutically. The animals receiving cortisone gained less weight than the unmedicated controls. Terramycin given in 0.3% in the diet prevented the development, and 0.3% in the drinking water favorably altered the course of the rat polyarthritis.

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Blood and Packed Cell Volume of the Adult Rat as Measured by Tagged Cells.* (18014)

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The blood volume of the rat derived from the dilution of intravenously administered dyes by plasma has been reported frequently. Estimates vary from about 4.3 to 8.0 ml per 100 g body weight(1-8), with a mean of approximately 7.0 ml. Berlin *et al.*(9), on the basis of the dilution of transfused blood containing cells tagged with radioactive iron, phosphorus, or both, reported a mean volume of about 4.6 ml per 100 g, a value considerably lower than most obtained by the plasma-dye method.

It seemed pertinent to measure adult rat blood and packed cell volumes employing both the transfused radio-iron tagged erythrocyte and hemoglobin extraction(10) technics and to compare these data with those of Berlin (9). The tagged cell transfusion method was also applied to the evaluation of the efficiency of perfusion with respect to the total rat as well as individual tissues.

Experimental. The rats used were adult males of the Sprague-Dawley strain bred in this laboratory and maintained on a Purina ration. They appeared in no way unusual.

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TABLE I.
Blood and Packed Cell Volumes of 16 Adult Male Rats as Measured by Transfused* Tagged Cells.

Wt, g	Blood vol., ml/100 g body wt	Hct.	Packed† cell vol., ml/100 g body wt
330	4.64	.46	2.07
340	5.15	.49	2.47
331	4.83	.48	2.25
346	4.88	.51	2.43
340	5.00	.46	2.24
321	4.92	.52	2.49
312	4.90	.48	2.28
330	5.33	.45	2.33
330	4.79	.49	2.28
308	4.77	.49	2.27
260	4.19	.47	1.89
300	5.10	.49	2.43
360	5.37	.50	2.55
342	4.97	.50	2.34
322	5.40	.48	2.44
350	5.30	.48	2.40
Mean	4.98		2.32
Stand. Dev., %	6.3		7.3

* Rats 1-12 inclusive were transfused with 0.5 ml of blood containing Fe^{59} tagged cells; rats 13-16 inclusive with 1.0 ml of blood containing Fe^{55} tagged cells.
† Corrected for transfused cells.

Donors of blood cells labeled with radioactive iron were prepared by intraperitoneal injection of 1-2 ml ferric ammonium citrate solution, pH 7, containing about 0.5 mc Fe^{59} or Fe^{55} per mg iron.† Approximately 10 days later the animals were anesthetized with ether and bled maximally from the abdominal aorta, heparin being used as anticoagulant. The radioactivity of the blood was confined practically completely to the cells and was at a maximum at this time. Before transfusion, the blood of most recipients was tested for compatibility with that of their respective donors. No evidence of incompatibility was observed.

Under ether anesthesia, a group of 16 rats was transfused by way of the exposed femoral or epigastric vein; the first 12 received 0.5 ml of Fe^{59} tagged blood and the remaining four 1.0 ml of Fe^{55} tagged blood. After about 2 hours, again under anesthesia, the recipients were bled from the abdominal aorta or inferior vena cava. The radioactivity of blood from donors and recipients was assayed(11) using argon filled (76 cm) G-M counters that responded to both the Fe^{59} beta rays and Fe^{55} X-rays. The initial sampling of donor blood, carried out immediately following the transfusions, was made with the same syringe and attached needle used for injection of the blood, the volumes measured being the same as those transfused. The maximum difference in radioactivity between any two of quadruplicate samples of donor blood and between duplicate samples of recipient blood was about 2%. The reproducibility of the measurements appears sufficient to rule out any considerable error from these sources. The fraction of packed cells in the samples of blood, *i.e.*, the hematocrit, was determined in duplicate using Wintrobe tubes. Centrifugation was carried out at 3000 rpm for 30 minutes; the distance from the center of the rotor to the bottom of the tube was 22.2 cm.

The ratio of the radioactivity transfused to the activity per unit volume of recipient's blood expresses the blood volume of the recipient. This value multiplied by the hematocrit gives the volumes of packed cells. Rat No. 15 (Table I) received the highest ratio of

† Obtained as the chloride from the Isotopes Division, U. S. Atomic Energy Commission. The two radioisotopes of iron were prepared by neutron bombardment of enriched Fe^{54} and Fe^{58} in a nuclear reactor.

TABLE II.
Blood and Packed Cell Volumes of 6 Rats Both by the Transfused Tagged (Fe^{55}) Cells and Hemoglobin Extraction Methods.

	ml of blood per 100 g body wt			ml of packed cells per 100 g body wt	
	Tagged cells	Hb extraction	Hct.	Tagged cells*	Hb extraction*
	5.06	5.10	.49	2.48	2.50
	4.96	4.85	.50	2.48	2.43
	4.90	4.92	.44	2.16	2.16
	4.68	4.61	.46	2.15	2.12
	4.83	4.93	.46	2.22	2.26
	4.90	5.09	.48	2.35	2.44
Mean	4.89	4.92		2.31	2.32

All rats were transfused with 0.75 ml of donor blood.
* Corrected for transfused cells.

transfused blood to body weight. Consequently, the transfused blood might have increased the blood volumes by as much as six percent. The blood volumes reported are those actually found since the fraction of transfused plasma remaining in the circulation after 2 hours is uncertain. On the other hand, the packed cell volumes have been adjusted for the transfused cells. The final blood and packed cell volumes are given in Table I. The volumes found are in good agreement with those previously obtained using the labelled-cell method in another strain of rat(9), the latter values being about 4.6 and 2.2 ml per 100 g for the blood and cell volumes respectively. The agreement is somewhat closer than appears usual when the plasma-dye method has been used in the rat by different workers.

Blood and cell volumes were similarly determined in another group of 6 rats, 0.75 ml of labeled (Fe^{55}) blood being transfused. In these same animals, the volumes were also estimated by comparison of the concentration of hemoglobin in blood removed from the animals with that remaining in the carcasses(10). The volumes found are given in Table II. The results given by the two methods in this group are in good agreement with each other and also agree with those found in the first group (Table I). The mean blood and packed-cell volumes and their standard deviations for all 22 of the rats using labeled-cells are 4.95 $\pm$ .27 and 2.32 $\pm$ .16 ml per 100 g body weight respectively.

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Although the labeled-cell procedure indicates a blood volume in the rat lower than most furnished by the plasma-dye method, it seemed pertinent to test whether significant amounts of the injected blood were removed from the circulation during the interval between injection and final collection of blood from the recipients. Such removal would lead to a higher apparent volume.

Thus a number of rats were transfused as indicated previously, then bled maximally from the inferior vena cava. Ringer's solution was then introduced through the needle used in withdrawing the blood until the heart stopped beating vigorously. To improve heart action at this time the needle was transferred to the right auricle and perfusion continued. The perfusion fluid and blood were permitted to drain from the inferior vena cava about an inch below the needle. Removal of blood from the viscera was aided by occasional massage. At the end of perfusion, individual tissues and the remainder of the carcass were assayed for radioactivity‡ and the presump-

‡ In the determination of radioactivity an appropriate amount of inert iron is mixed with a digest of tissue; the tissue iron, of which some is radioactive, and carrier iron are collected by treatment of the mixture with ammonium hydroxide; the iron is then plated on copper disks(11). The presence of relatively large amounts of salts from tissues interferes with plating. In some cases treatment of the ammoniacal mixture of digest and carrier iron with hydrogen sulfide yields a product that plates more readily. In extreme cases purification of the iron precipitated by ammonia or sulfide by means of cupferron has been necessary.

TABLE III.
Blood Volume of Tissues of 4 Rats Following Perfusion with Ringer's Solution Measured by Transfused Tagged (Fe^{55}) Cells.

Estimate of perfusion	ml of blood per 100 g of tissue						
	Heart	Kidney	Brain	Spleen	Leg muscle	Liver	Carcass
Excellent	.062	.208	.014	11.5	.081	.910	.172
"	.040	.095	.024	11.1	.082	.296	.244
"	.132	.132	.008	10.8	.049	.456	.231
"	.031	.089	.007	9.94	.046	.347	.264
Mean	.066	.131	.013	10.8	.065	.502	.228

TABLE IV.
Blood Volume of Tissues of 7 Rats Following Maximal Bleeding, Measured by Transfused Tagged (Fe^{59}) Cells.

	ml of blood per 100 g of tissue					
	Heart	Kidney	Brain	Spleen	Leg muscle	Liver
	4.0	9.0	1.0	10.3	0.6	7.7
	3.9	5.8	0.7	16.0	0.4	4.1
	5.3	2.5	0.8	12.8	0.3	4.4
	4.1	3.0	0.8	11.7	—	4.0
	3.8	5.1	0.7	27.7	0.3	4.5
	3.9	3.6	0.6	16.3	0.3	3.4
	4.1	5.4	0.7	21.6	0.4	5.1
Mean	4.2	4.9	0.8	16.6	0.4	4.7

tive blood volumes calculated.

The data for individual tissues are given in Table III; the total rat blood volumes, before perfusion, are given in Table I (Rats 13-16). The apparent volume of blood remaining in the whole animal after perfusion was about five percent of the volume found in the same non-perfused rat. Thus, the apparent blood volume indicated by the labeled-cell procedure may be as much as 5% too high.

For comparative purposes and to evaluate the efficiency of the perfusion, the blood volumes of individual tissues from rats bled maximally were determined by means of transfused labeled cells. The data are given in Table IV. It appears that the perfusion removed an appreciably smaller amount of the radioactivity, about 30%, from spleen than from other tissues. The radioactivity apparently represented blood and not deposited iron since the average blood volume of four spleens, taken from perfused rats and assayed by the hemoglobin method, was 11.7 ml per 100 g of body weight.

Discussion. Tagged cell methods employed by us measure packed cell volumes. If the average body hematocrit is known, these methods may be used to measure blood volumes. The large vessel hematocrit may differ from that of the average body hematocrit although such a difference has not been reported for the rat. Gibson(12) found the average body-hematocrit to be 9 percent lower than that of the large vessels in humans and dogs. Mayerson(13), using humans, concluded that the difference between the two hematocrits is negligible. If one assumes this difference to be 9% in the rat, then the blood volumes reported here are 9% too low. The hemoglobin assays are subject to a further correction for extracted myoglobin that may be of the order of 3%(14).

In view of the wide variation in the re-

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ported rat blood volumes obtained by the dye method, it is not possible to draw any conclusions as to the factors responsible for the difference between these data and that yielded by transfused tagged cells.

Summary. 1. The mean blood and packed cell volumes of 22 adult male rats, as measured by transfused tagged cells, was found to be 4.95 and 2.32 ml per 100 g of body weight respectively.

2. The mean blood and packed cell volumes of six of the 22 rats, assayed by the hemoglobin extraction method, were found to be 4.92 and 2.32 ml per 100 g of body weight.

3. The efficiency of perfusion for the total rat was 95%. This corresponds to a possible error in blood volume, introduced by removal of tagged transfused cells from the recipients circulation, no greater than 5%.

4. Comparison of the blood volume of various tissues taken from maximally bled rats with the blood volumes of similar tissues taken from perfused rats indicates that perfusion is least effective for spleen and most effective for kidney and brain.

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Isoimmunization in the Pig Following Multiple Transfusions of Incompatible Blood.* (18015)

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The existence of 4 distinct blood group systems in the pig has been described(1). These have been conveniently designated as Pi groups 1, 2, 3 and 4 (Pi_1, Pi_2, Pi_3, Pi_4). The Pi_1 group gave the consistently strongest reactions and was dominant in this respect over the other 3 groups. Members of Pi_1 possessed either the homologous antigen or antibody, or, in 15% of animals studied, carried neither antigen nor antibody (O_1). Members of Pi_2 were similar in this respect except that (O_2) was more common than the homologous antigen or antibody (77.9%). There was a reciprocity between the Pi_1 and Pi_2 antigen-antibody components when they occurred together. Occasionally an animal was O for both groups. Further isoagglutination and absorption experiments demonstrated the existence of two additional reciprocally related systems (Pi_3 and Pi_4) which were somewhat analogous to the A and B blood groups in man. On the basis of these groups, it was possible to predict the existence of fourteen blood group combinations.

In the present report, 2 pigs of anti-Pi_1 specificity were injected with serial blood transfusions from one pig of Pi_1 specificity over a period of 43 days in order to determine the immunizability of the recipient pigs against this blood group antigen. This was done with a view to utilization of the pig as an experimental animal in the production of experimental hemolytic syndromes. Young(2) has reported similar procedures in the dog.

Methods. The schedule of isoimmunization was so arranged that citrated whole blood from the donor pig (Pi_1) was given intravenously by syringe to 2 recipient pigs (anti-Pi_1) in amounts which increased progressively from 7.5 ml to 15.0 ml every 2 to 3 days over a period of 26 days. Following this time, the quantity of blood given at any one time was

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