diameter were found scattered over the epicardium, thymus, and fascial planes of the muscles and tendons. Occasionally petechiae were found on the pleural surfaces of the lungs and on the peritoneal surfaces of the bowel. There were no large hemorrhages.

Microscopic study revealed the usual radiation injuries of the lymphoid tissue, bone marrow, and gonads, while other tissues showed little or no change. We failed to find any increase in the number of mast cells in our birds.

Conclusion. The thrombocytopenia developing after radiation and the associated poor clot and loss of clot retraction would seem to be the factors of importance in the hemorrhagic disease in chickens following P^{32} radiation. Since the blood coagulation time was not significantly altered even after severe radiation injury, it seems that the presence of a heparin-like substance similar to that found in dogs would be unlikely.

Summary. In order to study the hemorrhagic disease resulting from radiation, we produced severe radiation injury by repeated subcutaneous injections of P^{32} into young chicks. Agranulocytosis and thrombocytopenia developed rapidly and during the same period the clotting mechanism of the blood was altered. However, there was no significant increase in coagulation time as found in animals and the hemorrhagic disease which developed was much less severe than that seen in animals. The increased number of mast cells associated with atomic bomb radiation injury in humans was not seen in the chickens.

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16534 P

Inulin Volume of Distribution as a Measure of Extracellular Fluid in Dog and Man.

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(Introduced by Homer W. Smith)

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Current methods for measuring extracellular volume of water utilize substances which enter the tissue cells in a variable proportion (thiocyanate,[1-3] sodium,[4] chloride,[5,6] bromide[7,8]), are very rapidly excreted (sulfate,[1] sucrose[1]), or are partially utilized by the organism (sucrose,[9] mannitol[10,11]).

Inulin has several advantages over any of the above substances: it is not metabolized to

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TABLE I.
Quantitative Urinary Recovery of Injected Inulin in Dog and Man.

		Amt of inulin inj. (mg)	Urine collection		
			Hrs after inj.	% of total amt recovered	% of total amt inj.
Dog	1	187.2	1.25	72	
			2.75	94	
			4.5	100	102
	2	186.0	1	70	
			2.75	94	
			3.75	98.5	
			4.75	100	102
Man	MG	5000	2.5	83	
			3.5	90	
			4.5	93.6	
			6	96.5	
			7	97.7	
			10	99.8	
			12	100	
			19	100	
			30	100	99
	ML	5000	2	77	
			3	86	
			4	90	
			5	92.5	
			6	95	
			7	96.4	
			12	99.5	
			19	100	
			25	100	
			29	100	102

any appreciable degree,[12,13] and the circumstance that it is rapidly and quantitatively recovered in the urine after intravenous injection[10,12] argues against storage in any tissue; it has a large molecular weight (ca.5101)[14] and does not dissociate appreciably in solution,[15] a circumstance which reduces the probability of penetration into cells; it does not penetrate the erythrocyte or escape through the normal renal tubules,[13,16] it is physiologically inert when properly prepared[12,17] and exerts negligible osmotic pressure. It possesses two disadvantages for this purpose: it diffuses slowly[15] and it is rapidly excreted by glomerular filtration,[12,13,18] these two circumstances rendering it unsuitable for use in the single injection method. The volume of distribution of inulin has been measured after ligature of both renal arteries,[19,20] a procedure that permits equilibration but one that has obvious limitations.

The method here described permits the use of inulin for the measurement of that extracellular fluid in active interchange with the plasma water, provided that certain conditions are fulfilled: (1) constant extracellular fluid volume throughout the period of equilibration,

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TABLE II.
Volumes of Distribution of Inulin, Thiocyanate, Radioactive Sodium and Bromide in Dogs.

Exp. No.	Dog No.	Body wt, kg	Inulin				Volume of distribution Percentage of body weight			
			Duration of infusion, hr	Duration of collection, hr	Amt of inulin recovered, mg	% of total amt recovered	Inulin	Thiocyanate	Sodium	Bromide
1	2	19.3	3.5	2	679.7	87				
				3	759.7	97				
				4	783.5	100	20.9			
2	2	18.0	2	2	327.8	90				
				4	360.6	99				
				5	361.9	100	21	32	31	
3	1	17.0	2	3	577.2	86				
				4	650.2	98				
				5.3	662.1	100	22.8	35.5		
4	1	16.4	3.3	2.8	563	88				
				4.5	624.9	98				
				5.5	635.1	100	23.2	34.3	31.4	
5	3	15.0	3.3	1.8	163	81				
				6.8	202	100	21.4	32.5	26.7	28.1
6	3	15.0	3.8	1.8	116	70				
				2.5	161	96				
				4	167	100	21.4	30.7		
7	3	15.8	4	4	220	98				
				5.3	225	100	21.9	28.5		
8	4	13.5	3.3	3.3	177.2	97				
				5.3	182.1	100	21.4	25.8	33	
9	4	13.8	5.8	1.3	149.5	77				
				4.8	193.3	100	21.6			32.7
10	5	12.5	2.8	0.5	97.3	50				
				5	192.6	100	20.3	31.2	30.5	31.5

TABLE III.
Volume of Distribution of Inulin and Thiocyanate in Man.

Exp. No.	Subject	Body wt, kg	Inulin: Duration of infusion, hr	Inulin: Urine collection: Duration of collection, hr	Inulin: Urine collection: Amt recovered, mg	Inulin: Urine collection: % of total amt recovered	Volume of distribution % of body wt: Inulin	Volume of distribution % of body wt: Thiocyanate
1	MG	76.5	3.5	5.5	564	80		
				10	698	100		
				19	698	100		
				24	698	100	9.5	20.8
2	ML	74.0	6	.75	293	28		
				3.5	805	78		
				5.3	922	89		
				15.3	1036	100	15.0	24
3	IS	86.8	8	2	766	56		
				4	1049	77		
				13	1354	99		
				16.5	1365	100	15.4	
4	MG	77.0	20.5	9	1261	99		
				17.5	1284	100	16.0	19.2

(2) uniform distribution of inulin between all compartments of the extracellular space. To attain (2), a priming injection of inulin is followed by a constant rate of infusion adequate to compensate for the rate of excretion, the infusion being maintained for a period sufficient to insure uniform distribution. The minimal equilibration time is 1 to 2 hours in dogs and about 6 hours in man.§

Prior to the inulin injection a control blood and timed urine sample are collected for the determination of B_o and U_oV, the latter being subtracted from the total urine inulin collected in the post-infusion period.

At the end of the infusion a blood sample is taken for determination of the plasma concentration of inulin. Simultaneously, the bladder is emptied by catheter and rinsed, and the infusion abruptly discontinued. The urine is then collected for 4 to 6 hours in dogs and 10 to 18 hours in man, which periods are necessary for excretion of at least 98% of the inulin in the body. In man urine collection was effected by spontaneous voiding. The quantity of inulin (in mg) recovered divided by the plasma concentration (in mg per cc) equals the volume of distribution in cc.

In some cases thiocyanate, radioactive sodium (Na^{22} and Na^{24}) and bromide spaces were determined simultaneously by the single injection method. Blood samples for determinations of these substances were taken after 1 hour for thiocyanate, and after 3 hours for sodium and bromide. Chemical analyses were made by the method of Harrison for inulin,[21] Crandall and Anderson for thiocyanate,[22] and Friedman for bromide.[23] Radioactive sodium was measured with a Geiger-Müller counter.

Results and discussion. Complete recovery of a single injection of inulin in dogs is obtained in a period of 4 to 6 hours (Table I). In man similar recovery is obtained in about 10 hours (Table I), confirming the observations of others.[10,13]

The volume of distribution of inulin as determined by the above method in dogs is shown in Table II. It ranges from 20.9 to 23.2% of the body weight (average 21.6).

§ Renal function tests can be performed during this period.

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This value is constant in the same animal in measurements made 15 to 30 days apart and with different periods of equilibration. These figures are substantially less than the volume of distribution of thiocyanate, bromide and radioactive sodium (Table II).

The volume of distribution of inulin in man (Table III) ranges from 15 to 16% of body weight and is not increased if the equilibration time is prolonged from 6 to 20 hours. This equilibration time was confirmed in a completely anuric patient whose inulin space after a single injection became constant after 6 hours. As in the dog, the simultaneous thiocyanate space was substantially larger than the inulin space.

Summary. The volume of distribution of inulin has been determined by a priming dose and a constant intravenous infusion to insure uniform distribution throughout the extracellular space, followed by collection of the total inulin excreted in the urine after the infusion is discontinued. The inulin space in the dogs ranges from 20.9 to 23.2% of the body weight (average 21.6) and in man from 15 to 16%, as compared with larger spaces obtained with thiocyanate, bromide and sodium.

16535

A Comparative Study of Blood Volume in Dogs.

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Although Evans Blue Dye (T-1824) is the most commonly used method for the determination of blood volume, there has been much controversy concerning the accuracy of the values obtained. While Gregersen[1] and others who have used the method for a long time uphold its validity, Hempden *et al.*[2] recently have offered experimental evidence to indicate inherent sources of error. The following experiments were carried out as part of a survey for a more accurate and rapid method of obtaining plasma and blood volumes.

In order to utilize constituents of the circulating blood which are known to remain in the vascular bed for relatively long periods, and to obtain a high degree of sensitivity, it was decided to use red cells and plasma protein tagged with radioactive elements. The red cells were labelled with P^{32} and the protein with I^{131}. Fe^{59} or Fe^{55} would undoubtedly be superior to P^{32}, but phosphorus has the advantage of permitting *in vitro* labelling without the necessity of metabolic incorporation into the cell. The methods for these studies were those of Hevesy,[3] who first described the use of red cells tagged with P^{32} to study blood volume, and those of Fine and Seligman,[4] who first reported blood volume studies employing plasma iodinated with I^{131}. Minor modifications were introduced by us.

The radioactive isotopes were obtained from the Isotope Division of the Atomic Energy Commission at Oak Ridge. Radioactive phosphorus has a half life of 14.3 days and emits a beta particle with a peak energy of 1.69 M.E.V. Radioactive iodine has a half life of 8.0 days. It emits a beta particle of .60 M.E.V. and two gamma rays of .367 and .08 M.E.V.

Healthy, full grown dogs weighing 9-22 kg were used. The tests were carried out in the

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