haptoglobin hemoglobin complex, when small amounts are administered, suggests that a minor component, possibly the bone marrow, may be primarily involved. A final answer to these questions will have to await studies by which the complex can be localized in tissues.

Summary. 1. Haptoglobin hemoglobin complex labelled with Fe^{59} or I^{131} injected into 13 rabbits was removed from the blood with half times of 110 minutes and 78 minutes respectively. 2. The complex did not significantly interfere with phagocytosis of carbon particles by the reticuloendothelial system and was not selectively removed by the liver during a single passage.

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Incorporation of Selenium-75 into Dog Hair. (26048)

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With the proposal that selenium be included among the essential dietary elements (1), interest has been focused on many unanswered questions about the metabolism and fate of selenium in the mammalian organism, especially the chemical nature of selenium compounds in animal tissues. The apparent incorporation of trace amounts of selenium in various tissue proteins has been discussed(2, 3). Continuing these studies, hair which is high in cystine content was examined for selenium incorporation. Hair from Se^{75}-injected dogs was fractionated and assayed for Se^{75}, which was found when cystine-rich protein keratin was isolated as the thiosulfate, S-sulfokerateine. The cystine fraction isolated from the hair by conventional methods contained Se^{75}.

Methods and procedures. The method of Swan(4) was used for the specific and symmetrical fission of disulfide bonds in keratinous tissue. The cystine disulfide bonds in dog hair containing Se^{75} were broken by the reaction with cupric-ammonium sulfite, as illustrated in the equation:

$$RSSR + 2\,Cu^{++} + 2\,SO_3^{=} \rightarrow 2\,RSSO_3^{-} + 2\,Cu^{+}$$

The protein S-sulfokerateine was precipitated from the copper reagent extracts of hair containing Se^{75} by acidification and dilution(4). The protein was then washed repeatedly with water, lyophilized, weighed, and assayed for Se^{75}. A small fraction of the hair that was insoluble in the copper reagent was separated by centrifugation, washed thoroughly with water, lyophilized and counted. The uniformity of the S-sulfokerateine preparations was established by paper electrophoresis analysis. Samples of S-sulfokerateine were dissolved in Veronal buffer (pH 8.6; μ 0.075) and applied to Schleicher and Schuell 2043-A mgl. electrophoresis paper. A constant current of 7 ma was applied for 6 hours. The protein fractions were developed using the standard alcoholic bromphenol blue method. In another experiment, cystine from hair containing Se^{75} was isolated by the method of Gortner and

TABLE I. Selenium Administration.*

Dog	Sex	Wt, kg	Se^{75}, mc	Selenium, mg
1	♂	19.5	.61	.37
2	♂	26.8	.61	.37
3	♀	19.5	1.97	2.50
4	♂	20.4	.84	2.03
5	♂	30.3	1.40	2.14
6	♂	20.4	1.12	.41

* Se^{75} was given subcut. as $H_2Se^{75}O_3$, except that Dog 5 received $Se^{75}Cl_4$.

Hoffman(5), and assayed for Se^{75}. The cystine fraction was recrystallized twice, and qualitative tests indicated that tyrosine was not present. The cystine preparations were chromatographed in both one and two dimensions. Two solvent systems were employed: a *tert*-butyl alcohol-formic acid-water mixture (Solvent I); and a single phase phenol-ammonia-water mixture (Solvent II)(6). Whatman filter paper No. 3 MM was used, and the chromatograms were developed with the ninhydrin reagent of Underwood and Rockland(7).

In a time-distribution study, dogs were injected with trace amounts of Se^{75} and the hair was assayed for Se^{75} at various times after injection. The hair was washed with detergent, rinsed thoroughly with water followed by 95% alcohol and ether. The air-dried hair samples were weighed and assayed for Se^{75} in duplicate. In another experiment, 4 dogs were given single injections of selenium, and after several months comparisons were made of concentration of Se^{75} in hair and in the several tissues. Table I shows amounts of Se^{75}, selenium, and the form of selenium administered to the various dogs.

Results. Radioactive assay of the hair protein, S-sulfokerateine, obtained from 2 dogs at various time intervals after administration of Se^{75} is presented in Table II. About 42% of the original weight of the hair was obtained as the thiosulfate, S-sulfokerateine. About 18% (Dog I) and 16% (Dog II) of the original hair Se^{75} activity were present in the organic thiosulfates. The percent of total hair Se^{75} activity in the insoluble fraction (in copper reagent) for 12 determinations was 10.9 ± 3.1%. Paper electrophoresis studies of S-sulfokerateine showed one component which had a mobility approximately the same as that of dog serum albumin. Results from paper chromatographic studies of the cystine fraction isolated from hair revealed that only one spot was present, with no other detectable amino acids. The fact that specific activity of the cystine fraction showed no significant change between first and second crystallizations indicated high purity of the sample. Se^{75} activity in the cystine fractions of hair containing Se^{75} from 4 dogs is shown in Table III. The hair in each experiment was obtained from different dogs, and was taken at different time intervals after Se^{75} administration. Se^{75} activity ex-

TABLE II. S-sulfokerateine Containing Se^{75} from Dog Hair.

Dog 1					Dog 2				
		S-sulfokerateine					S-sulfokerateine		
Wk after inj.	Specific activity of hair, cpm/g	% of hair Wt	% of hair Se^{75}	Specific activity, cpm/g	Wk after inj.	Specific activity of hair, cpm/g	% of hair Wt	% of hair Se^{75}	Specific activity, cpm/g
3	4800	36.0	20.3	2750	3	2030	30.9	22.6	1070
4	1790	29.5	23.2	1410	7	21940	37.1	10.4	6090
8	12380	41.7	12.9	3890	9	14950	50.8	8.1	2480
9	10420	48.1	10.5	2270	10	7260	46.9	8.8	1370
10	8040	57.6	14.3	2010	11	43760	51.6	16.8	14230
11	21400	55.1	18.8	7300	12	50480	61.8	14.2	11580
12	20600	59.4	19.5	6780	14	21240	37.7	18.9	10670
13	15490	45.4	20.0	6840	15	26810	42.3	13.6	8650
14	12410	35.8	20.3	7030	16	15230	46.8	20.2	6560
15	15330	42.5	23.2	8370	17	8220	34.4	20.9	4990
17	9250	37.4	19.6	4850	18	8500	31.7	18.8	1560
18	9950	31.6	11.9	3770	19	4650	35.1	12.9	3560
19	12670	30.3	19.7	8210					
Avg		42.3 ±8.3	18.0 ±3.5		Avg		42.3 ±7.8	15.5 ±4.2	

TABLE III. Cystine Fraction from Dog Hair Containing Se^{75}.

Exp.	Hair: Wt, g	Hair: Total activity, cpm	Specific activ. cystine fraction: 1st crystallization, cpm/g	Specific activ. cystine fraction: 2nd crystallization, cpm/g	cpm Se^{75} cystine fraction / cpm Se^{75} hair, %
1	200	330,000	2187	2220	1.41
2	100	119,000	1408	1440	2.70
3	50	104,000		2270	2.79
4	108	425,000		6720	7.72

pressed as c.p.m. per g of cystine varied from 1.4 to 6.7 x 10^3 c.p.m./g and 1.4 to 7.7% of the original hair Se^{75} activity was found in the cystine fraction.

Time-distribution studies of Se^{75} in dog hair are shown in Table II, columns 2 and 7, where specific activity of hair (c.p.m. g) is expressed against time. Concentration of Se^{75} in hair gradually reached a maximum around the 12th week, then decreased. Table IV indicates that amounts of Se^{75} in hair, on a dry-weight basis, were as high or higher than in other tissues examined on a wet-weight basis.

Discussion. The long life span, the coherent hard keratinous structure of hair, and absence of apparent metabolic turnover in the hair shaft permitted incorporated selenium to be retained for long periods, as demonstrated in time-distribution studies. Significant amounts of selenium were deposited and retained in hair when compared with other tissues of the animal organism. Keratin, the principal protein of hair, when isolated as the thiosulfate, S-sulfokerateine, contained about 17% of original hair Se^{75} activity. The values 1.4 to 7.7% of the original hair Se^{75} in the cystine fraction represent minimum values, since our experience has been that losses of cystine and Se^{75} occur in hydrolysis, norit treatment, and recrystallization procedures (2). The concept that the cystine fraction of tissues isolated from animals treated with Se^{75} contains an isomorphic mixture of cystine and the selenium analogue, selenocystine, has been discussed elsewhere(3), and presumably applies in the present hair experiments.

TABLE IV. Distribution of Se^{75} in Various Tissues.

Dog	Days after inj.	Hair	Toenails	Kidney	Liver	Spleen
		cpm/g				
3	141	250,000	57,300	28,700	20,200	14,260
4	267	7,000	2,700	2,810	9,560	2,270
5	310	3,560	4,500	1,110	5,320	361
6	316	2,250	1,500	5,300	3,460	2,340

Because of the great reactivity of certain elements with sulfhydryl groups of the hair follicular proteins, the elements have a special affinity for hair(8). These may combine with the sulfhydryl groups of the proteins of the matrix cells as in the case of selenium, copper and arsenic. However, selenium may be converted into either or both selenocystine and selenomethionine in tissues other than hair by some unknown reaction, then incorporated into the protein of hair. The possibility exists that organoselenium from serum proteins may be utilized for hair protein formation, since it has been established that selenium is incorporated in serum proteins(9, 10). Related experiments by Fleischer *et al.*(11) indicate that utilization of C^{14}- and S^{35}-tagged plasma proteins for hair protein formation involves breakdown of plasma proteins to amino acids or small peptides. In view of the affinity of selenium for sulfhydryl compounds and cystine, it has been assumed by workers in the field that selenium enters into chemical combination with keratin(12, 13,14,15). Further elucidation of this concept is presented in this report which shows first, that Se^{75} is incorporated into keratin when this protein is isolated as the thiosulfate, S-sulfokerateine; and second, that Se^{75} is found in the cystine fraction of hair. The above experimental data are presented as additional evidence to that already reported(2, 3) that selenium may exist as selenocystine in tissue proteins.

Summary. Trace amounts of Se^{75} injected subcutaneously into dogs as either $Se^{75}Cl_4$ or $H_2Se^{75}O_3$ were deposited and retained in hair for as long as 316 days. Se^{75} was found

in the cystine-rich protein keratin when the latter was isolated as the thiosulfate, S-sulfokerateine, and in the cystine fraction isolated from hair. The source of selenium which is incorporated into hair protein is discussed.

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Effect of Bladder Distension on the Venous System of Man.* (26049)

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Forty years ago Wernöe(1) observed that with certain painful visceral diseases "anemic zones" developed in the skin overlying the affected organ. He attributed the pallor to "ischemia" secondary to vasoconstriction. Stürup(2) described areas of hyperalgesic pallor in the skin of patients with acute appendicitis. Using a photometer Adams-Ray and Nórlen and Adams-Ray(3,4) observed that upon distension of the bladder, pallor developed in the cutaneous ventral segments of T_{11} and T_{12}. They believed the pallor to be due to a spinal reflex with the afferent fibers passing through sacral nerves S_2, S_3 and S_4 and efferent fibers traveling with the sympathetics producing vasoconstriction in the subcapillary venous plexus.

The present study was undertaken to learn the extent of this reflex by studying the effect of bladder distension on an intact isolated venous segment of the forearm of normal and diseased subjects.

Materials and methods. Twelve subjects ranging in age from 27 to 58 years (mean, 36 years) were studied; 5 were male and 7 were female. The cardiovascular system was normal in all but 2 subjects. One subject had luetic heart disease and chronic congestive heart failure and another had transection of the spinal cord at T_6 secondary to metastases from pulmonary carcinoma.

Venous pressure was measured in an isolated intact venous segment and in an adjacent vein open to the systemic venous system of the forearm as previously described(5,6). The pressures were recorded simultaneously and continuously by means of Statham strain gauge pressure transducers and a suitable multichannel direct writing recorder.

A straight French catheter was inserted into the bladder of the subject and was connected by means of rubber tubing, which led out of the subject's room, to a calibrated glass

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